

# The Multifaceted Nature of Food and Nutrition Insecurity around the World and Foodservice Business

Edited by António Raposo and Heesup Han Printed Edition of the Special Issue Published in *Sustainability* 



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# The Multifaceted Nature of Food and Nutrition Insecurity around the World and Foodservice Business

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Editors

António Raposo Heesup Han

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*Editors* António Raposo CBIOS (Research Center for Biosciences and Health Technologies) Universidade Lusófona de Humanidades e Tecnologias Lisboa Portugal

Heesup Han College of Hospitality and Tourism Management Sejong University Seoul Korea

*Editorial Office* MDPI St. Alban-Anlage 66 4052 Basel, Switzerland

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# Contents

About the Editors	
Preface to "The Multifaceted Nature of Food and Nutrition Insecurity around the World and Foodservice Business"	
António Raposo and Heesup Han	
The Multifaceted Nature of Food and Nutrition Insecurity around the World and Foodservice Business	
Reprinted from: <i>Sustainability</i> <b>2022</b> , 14, 7905, doi:10.3390/su14137905	
Anna Brytek-Matera	
Negative Affect and Maladaptive Eating Behavior as a Regulation Strategy in Normal-Weight Individuals: A Narrative Review	
Reprinted from: <i>Sustainability</i> <b>2021</b> , <i>13</i> , 13704, doi:10.3390/su132413704	;
Sergei Andronov, Andrey Lobanov, Elena Bogdanova, Andrei Popov, Alexander Yuzhakov	
and Olga Shaduyko et al. The Relationships among Microelement Composition of Reindeer Meat ( <i>Rangifer tarandus</i> ) and	
Adaptation: A Systematic Review and Meta-Analysis	
Reprinted from: <i>Sustainability</i> <b>2022</b> , 14, 1173, doi:10.3390/su14031173 <b>21</b>	
Sueny Andrade Batista, Elke Stedefeldt, Eduardo Yoshio Nakano, Mariana de Oliveira Cortes, Raquel Braz Assunção Botelho and Renata Puppin Zandonadi et al. Design and Development of an Instrument on Knowledge of Food Safety, Practices, and Risk Perception Addressed to Children and Adolescents from Low-Income Families	
Reprinted from: <i>Sustainability</i> <b>2021</b> , <i>13</i> , 2324, doi:10.3390/su13042324	1
Elena Bogdanova, Sergei Andronov, Andrei Soromotin, Gennady Detter, Oleg Sizov and	
<b>Kamrul Hossain et al.</b> The Impact of Climate Change on the Food (In)security of the Siberian Indigenous Peoples in the Arctic: Environmental and Health Risks	
Reprinted from: <i>Sustainability</i> <b>2021</b> , <i>13</i> , 2561, doi:10.3390/su13052561 69	)
Mursleen Yasin, Adnan Younis, Fahad Ramzan, Talha Javed, Rubab Shabbir and Hamza	
Armghan Noushahi et al. Extraction of Essential Oil from River Tea Tree ( <i>Melaleuca bracteata</i> F. Muell.): Antioxidant and	
Antimicrobial Properties	
Reprinted from: <i>Sustainability</i> <b>2021</b> , <i>13</i> , 4827, doi:10.3390/su13094827	
Abdullah Abdullah, Shujaul Mulk Khan, Andrea Pieroni, Aminul Haq, Zahoor Ul Haq and Zeeshan Ahmad et al.	
A Comprehensive Appraisal of the Wild Food Plants and Food System of Tribal Cultures in the Hindu Kush Mountain Range; a Way Forward for Balancing Human Nutrition and Food Security	
Reprinted from: <i>Sustainability</i> <b>2021</b> , <i>13</i> , 5258, doi:10.3390/su13095258	
Sapna Langyan, Zahoor A. Dar, D. P. Chaudhary, J. C. Shekhar, Susila Herlambang and	
Hesham El Enshasy et al. Analysis of Nutritional Quality Attributes and Their Inter-Relationship in Maize Inbred Lines	

Analysis of Nutritional Quality Attributes and Their Inter-Relationship in Maize Inbred Lines for Sustainable Livelihood

Anisa Zahwa Akbara, Bee-Lia Chua, Heesup Han and António Raposo
Investigating International Students' Perception of Foodservice Attributes in Malaysian
Research Universities
Reprinted from: <i>Sustainability</i> <b>2021</b> , <i>13</i> , 8190, doi:10.3390/su13158190
Margherita Paola Poto and Arianna Porrone
A Co-Created Methodological Approach to Address the Relational Dimension of
Environmental Challenges: When Critical Legal Analysis Meets Illustrated Storytelling
Reprinted from: <i>Sustainability</i> <b>2021</b> , <i>13</i> , 13212, doi:10.3390/su132313212
Sangmook Lee, Gumkwang Bae and Hyojin Kim
A Study on the Sustainable Use Intention of Restaurant Companies Using the Information
Attributes of SNS: The Dual Process Theory
Reprinted from: <i>Sustainability</i> <b>2021</b> , <i>13</i> , 12922, doi:
Gabriella Vindigni, Iuri Peri, Federica Consentino, Roberta Selvaggi and Daniela Spina
Exploring Consumers' Attitudes towards Food Products Derived by New Plant Breeding
Techniques
Reprinted from: <i>Sustainability</i> <b>2022</b> , <i>14</i> , 5995, doi:10.3390/su14105995
Elena Bogdanova, Konstantin Filant, Ekaterina Sukhova, Maria Zabolotnikova, Praskovia
Filant and Dele Raheem et al.
The Impact of Environmental and Anthropogenic Factors on the Migration of the Rural Arctic
Population of Western Siberia
Reprinted from: <i>Sustainability</i> <b>2022</b> , <i>14</i> , 7436, doi:10.3390/su14127436
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### **About the Editors**

#### António Raposo

António Raposo is an Assistant Professor at Universidade Lusófona de Humanidades e Tecnologias and he is an Integrated Member of CBIOS (Research Center for Biosciences and Health Technologies).

He graduated in Nutritional Sciences from Instituto Superior de Ciências da Saúde Egas Moniz, Portugal, 2009, and obtained his Ph.D. with European Mention in Animal Health and Food Safety from the University Institute of Animal Health and Food Safety, University of Las Palmas de Gran Canaria, Spain, 2013.

His main research interests are: studies on the utilization of Catostylus tagi jellyfish in health sciences, particularly as a food ingredient; food habits; food safety evaluation; food innovation; natural food products; food security; and sustainability.

He is a member of the editorial boards and an invited reviewer of relevant international peer-reviewed journals in his research field. He has published more than 70 papers in indexed JCR journals, as well as scientific book chapters and a patent model, and co-authored a book in English with a special focus on Nutrition, Food Security, and Food Safety Sciences. He has been a Guest Editor for several Special Issues published in high-impact JCR journals such as *Foods, Sustainability* and *International Journal of Environmental Research and Public Health*. He has collaborated as a Visiting Professor in Portuguese, Spanish, Chilean, and Vietnamese Universities.

#### Heesup Han

Heesup Han is a Professor in the College of Hospitality and Tourism Management at Sejong University, Korea. His research interests include sustainable tourism, green hotel, cruise, airline, medical tourism, digital currency, the Fourth Industrial Revolution, and hospitality and tourism marketing. Heesup Han is a highly cited researcher (HCR) (2019, 2020, and 2021) of the world in social science (identified by the Web of Science Group—Clarivate).

# Preface to "The Multifaceted Nature of Food and Nutrition Insecurity around the World and Foodservice Business"

Food and nutrition are undoubtedly the core aspects of human life. This book introduces the multifaceted nature of food, such as food security, nutrition, and food quality and service. (1) Food security is an international concept as all individuals across the world should have physical, social, and economic access at all times to sufficient, safe, and nutritious foods that meet their dietary needs and food preferences for an active and healthy life. Four parameters, namely availability, access, utilization, and stability, should accordingly be measured to determine food security status. In the past, the food security term has been the issue of food availability and accessibility, and the utilization aspect has been identified as essential more recently. (2) Nutrition, on the other hand, is centered on consuming adequate diversified meals and nutrient absorption that could contribute to other forms of malnutrition, such as hidden hunger and obesity. (3) Food quality is another critical issue across the globe. In their daily life, an individual eats at a restaurant/café/hotel and seeks better quality food and service. The quality performance of food and service at a foodservice operation contributes to making one's consumption happier and his/her life healthier. A total of 13 research works are included in this book. The authors are from diverse countries and address the critical issues of food.

The editors are very grateful to their families and friends for all of the support they provided. We would also like to extend a very special thanks to all researchers who published their works herein and the entire MDPI team for their commitment and dedication. Only in this way was it possible to carry out this successful project.

António Raposo and Heesup Han Editors





### **Editorial The Multifaceted Nature of Food and Nutrition Insecurity around the World and Foodservice Business**

António Raposo <sup>1,\*</sup> and Heesup Han <sup>2,\*</sup>

- <sup>1</sup> CBIOS (Research Center for Biosciences and Health Technologies), Universidade Lusófona de Humanidades e Tecnologias, Campo Grande 376, 1749-024 Lisboa, Portugal
- <sup>2</sup> College of Hospitality and Tourism Management, Sejong University, 98 Gunja-Dong, Gwanjin-Gu, Seoul 143-747, Korea
- \* Correspondence: antonio.raposo@ulusofona.pt (A.R.); heesup.han@gmail.com (H.H.)

**Keywords:** agriculture; consumer behavior; food habits; food industry and technology; food policy; food safety and quality; food security; nutritional diseases; tourism; service quality at restaurants/cafés/hotels

Food security is more than a basic requirement for survival; it is a human right that has implications for global safety, economic strength, security, and sustainability [1,2]. The international concept of food security is a situation where all people have physical, social, and economic access at all times to sufficient, safe, and nutritious food that meets their dietary needs and food preferences for an active and healthy life. All four parameters (availability, access, utilization, and stability) should therefore be measured to determine food security status [3,4].

The matter of hunger, which is generally equated with access to inadequate amounts of food and compromised food quality to reach the required daily intake, is addressed by both food and nutrient insecurity. In the past, the food security term has been the issue of food availability and accessibility, and the utilization aspect has more recently been identified as essential [5,6]. Nutrition, on the other hand, centered on consuming adequate diversified meals and nutrient absorption that could contribute to other forms of malnutrition, such as hidden hunger and obesity [7].

Food quality is another critical issue across the globe. In their daily life, individuals eat at restaurants/cafés/hotels and seek better quality food and service. The quality performance of food and service at a foodservice operation contributes to making one's consumption more satisfying and his/her life healthier [8].

Taking into account these premises and the multifaceted nature of food and nutrition insecurity around the world and foodservice business, this Special Issue presents 12 papers published by researchers from 19 different countries all over the world, including Brazil, China, Czech Republic, Egypt, Finland, India, Indonesia, Iraq, Italy, Korea, Malaysia, Norway, Pakistan, Poland, Portugal, Russia, Saudi Arabia, Slovakia, and the USA.

Regarding the review papers included in this Special Issue, two investigations can be found that address the following themes: the evaluation of the relationship between negative affect and maladaptive eating behavior as a regulation strategy in normal-weight individuals [9] and a summary of the data on the chemical composition of reindeer meat depending on the region of the *Rangifer tarandus*—a systematic review and meta-analysis [10].

In terms of original articles, we can mention 10 relevant works that focus on different areas common to the objectives of this Special Issue, namely: the study by Batista et al. [11], which constructed and validated an instrument containing three questionnaires to identify the level of knowledge, practices, and risk perception of food safety by low-income students between 11 and 14 years old; the application of a multidisciplinary approach based on ecological and medical research methods with the inclusion of socioeconomic

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**Copyright:** © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). analysis to investigate the impact of climate change on the food (in)security of the Siberian indigenous peoples in the Arctic, focused on the environmental and health risks [12]; and the evaluation of the yield of Melaleuca bracteata essential oil together with its antioxidant and antimicrobial properties under local prevailing conditions of the subtropics [13]. Furthermore, on 8 May 2021, Abdullah et al. [14] published the significant work entitled "A Comprehensive Appraisal of the Wild Food Plants and Food System of Tribal Cultures in the Hindu Kush Mountain Range; a Way Forward for Balancing Human Nutrition and Food Security" dedicated to the memory of Habib Ahmad (TI), Emeritus of Hazara University, Pakistan, and Fellow of the Pakistan Academy of Sciences who passed away on 7 April 2021. Habib was an extraordinary scholar and great human being, and he represented an irreplaceable academic guide for generations of young botanists, plant ecologists, and agricultural scientists across the globe. Langyan et al. [15] presented an investigation planned to understand the variability and inter-relationships among various nutritional quality attributes of maize kernels to identify potential donors of the respective traits for future hybridization programs. Akbara et al. [16] conducted an importance-performance analysis to examine international students' perceived importance and perceived performance of university foodservice attributes. Lee et al. [17] conducted a study to define detailed factors by combining the factors of social network services information attributes and dual processing process theory and to investigate the relationship between customer satisfaction, brand attitude, and sustainable use intention. Poto and Porrone [18] promoted a co-created methodological approach to address the relational dimension of environmental challenges: where critical legal analysis meets illustrated storytelling. Vindigni et al. [19] presented the results of an online survey carried out in Italy with 700 randomly selected participants on consumer attitudes towards food obtained by new plant breeding techniques. Bogdanova et al. [20] developed a study that aimed to reflect on appropriate policies for strengthening resilience and reducing migration outflows in the Arctic Siberian population.

The issues raised in this Special Issue are thought-provoking, and researchers, academics, policymakers, food processors, indigenous peoples, and other stakeholders should reflect on them.

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## Negative Affect and Maladaptive Eating Behavior as a Regulation Strategy in Normal-Weight Individuals: A Narrative Review

Anna Brytek-Matera 匝

Review

Katowice Faculty of Psychology, SWPS University of Social Sciences and Humanities, 40-326 Katowice, Poland; abrytek-matera@swps.edu.pl

**Abstract:** Emotions have a powerful influence on eating behavior, and eating behavior can have a powerful effect on emotions. The objective of the present narrative review was to evaluate the relationship between negative affect and maladaptive eating behavior as a regulation strategy in normal-weight individuals. A search of the literature within PubMed<sup>®</sup>, MEDLINE<sup>®</sup> and PsycINFO was conducted using a combination of the following terms: "affect", "negative affect", "affect regulation" and "maladaptive eating behavior". A total of 106 papers were identified for full text review and were included in the final set of literature. The manuscript presents an overview of the literature on negative affect and maladaptive eating behavior. It offers a brief overview of restrained, uncontrolled and emotional eating in normal-weight individuals and looks at maladaptive eating behavior used to regulate their affect. Based on the previous research findings, we argue that using more adaptive strategies for emotion regulation (cognitive reappraisal) might result in downregulating integral negative affect to food and in improving eating behavior.

Keywords: negative affect; affect regulation; maladaptive eating behavior; normal weight

#### 1. Introduction

Maladaptive eating behavior is a serious problem for health and psychological wellbeing. In eating psychology, two main maladaptive eating behaviors have been defined: restrained eating (persistent and conscious food intake restriction) [1] and disinhibited eating (an incapacity to restrain food intake once begun) [2] divided into emotional eating (overeating in answer to internal cues, e.g., emotions, affect, mood state) and external eating (overeating in response to external cues, e.g., seeing or smelling food) [3]. Maladaptive eating behavior is related to unhealthy attitudes and behaviors regarding food and could also be defined as eating unhealthy food (having less nutritional value and increased intake of high-sugar and high-fat foods) and not eating healthy food.

People are exposed to a multiplicity of external environmental cues in their daily lives, which have an impact on eating or not eating different foods. Human eating behavior is guided by response to food-related cues rather than by a physiological need [4]. Previous studies have suggested that both external and internal cues influence eating behavior [5–7]. Researchers are interested in internal states as, apparently, external environmental cues are unable to explain all of the observed maladaptive eating behavior. One of these internal cues is affect. This term is mainly used in referring to any state that represents how a situation affects a person [8]. Affect is used to describe the physiological, conscious or behavioral components of emotion. It can be described as the superordinate category for emotion episodes, moods, dispositional states and traits [9].

Experiencing negative affect has strong effects on unhealthy eating behavior (e.g., increased food intake in reaction to negative emotions, more palatable and less healthy meals) and poor food choices (e.g., more snacking behavior, a decrease in fruit and vegetable consumption) in both normal and overweight individuals [10–19]. Strategies that

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**Copyright:** © 2021 by the author. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). individuals can use to regulate affect might be an effective method for changing existing maladaptive eating behaviors and improving them.

The objective of the present review is to investigate the link between negative affect and maladaptive eating behavior as a regulation strategy in normal-weight individuals (with the body mass index range from 18.5 to 24.99 kg/m<sup>2</sup>). We first focus on distinctions in negative affect and in restrained eating, uncontrolled eating and emotional eating (maladaptive eating behavior). Next, we examine emotion regulation strategies and their connection with different maladaptive eating behaviors. We finish by arguing that using more adaptive strategies for emotion regulation (cognitive reappraisal) might result in downregulating integral negative affect to food and in improving eating behavior.

A search of the literature within the electronic databases PubMed<sup>®</sup>, MEDLINE<sup>®</sup> and PsycINFO was conducted. The search terms were "affect", "negative affect", "affect regulation" and "maladaptive eating behavior". In the present review, the titles and abstracts of the search results were assessed. For each paper, the type of the study (natural setting and laboratory experiments), the characteristics of the sample and the conclusion/results were defined. The key stages of literature search guidance are presented in Figure 1.

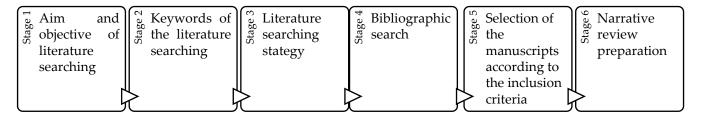


Figure 1. The key stages of literature search guidance for conducting a narrative literature review.

The author (A.B-M) identified the published studies focusing on the relationship between negative affect and maladaptive eating behaviors over a 20-month period (until 30 September 2021). Only articles published in English were considered. Population-based studies, reviews, systematic reviews and meta-analyses were included in the literature selection process. Case reports, case series, commentary letters and articles published in languages other than English were excluded.

A narrative review was proposed in an attempt to summarize the literature and to answer the research question on the relationship between negative affect and maladaptive eating behavior as a regulation strategy in normal-weight individuals, focusing especially on restrained eating, uncontrolled eating and emotional eating. The purpose of this narrative review was also to present a theoretical rationale for the relevant role of negative affect on eating behaviors, integrating research in natural and laboratory settings.

#### 2. Affect and Maladaptive Eating Behavior

The link between emotion and eating behavior has always been of interest to human behavior research. Over the last decades, it has been recognized that emotions have a strong impact on eating behavior and that eating behavior can have a powerful influence on emotions [20]. Emotions can induce changes in eating behavior [21], and eating is per se related to emotions [22]. Emotion is a complex reaction pattern being composed of subjective experience, expressive behavior (e.g., facial, bodily) and peripheral physiological responses (e.g., respiration, heart rate) [23]. Specific emotions such as anger, fear, joy and sadness affect eating responses in motivation to eat, food choice, affective reaction to food, eating speed, metabolism and digestion [24].

Furthermore, studies have found that positive and negative emotions can differ in their effects on eating. Negative emotions (such as anger, fear and sadness) may result in an increase in food intake and the consumption of unhealthy food (junk food) but in a decrease in food pleasantness in individuals who use eating as way to regulate their negative emotions [24]. Negative emotions, such as boredom, may be related to increased appetite, but sadness may be related to decreased appetite [25]. In contrast, positive emotions, such as joy or happiness, can increase food pleasantness and the intake of healthy foods [24,26–28]. Positive emotions, in general, seem to be relevant triggers for eating indulgent food amongst healthy individuals with a normal weight [28]. A metaanalysis (including 33studies with a total of 2491 participants including healthy controls and patients with an eating disorder and with obesity) on how negative and positive emotions affect food intake across laboratory settings [29] showed that, overall, negative emotions resulted in increased eating (a small effect). Other outcomes from a meta-analysis based on laboratory-based studies (which included 20 studies with a total of 3670 participants including healthy controls and individuals with pathological eating behavior [30]) found a lack of an overall effect of negative emotions on eating behavior. In addition, positive emotions had a small effect on eating behavior, and overall positive emotions lead to increased food intake.

The empirical results on emotion-induced changes in eating are contradictory. On the one hand, high-intensity emotions are believed to suppress eating (these emotions influence on the autonomic nervous system activity that triggers physiological changes, e.g., slowed gastric emptying, the release of appetite-inhibiting hormones, that may induce satiety [31]. On the other hand, high-arousal emotions (such as anger) have been found to increase food consumption [26,27] or to not decrease eating in reply to highly intense emotions (results of most laboratory studies). In relation to moderately intense emotions, it is supposed that negative and positive emotions increase food consumption among people with a more controlled eating style [32]. To sum up, empirical results reflect the same inconsistencies as the discrepancy in views on how emotions impact eating [30]. Moreover, although extensive research has been carried out on negative emotions and eating, the very fundamental question of whether negative emotions influence eating, and in whom, remains unclear. It is worth pointing out that much research has been conducted on disordered eating among patients with eating disorders or obesity, and a much smaller number of studies have been devoted to maladaptive eating behavior among normal-weight individuals without a diagnosis of an eating disorder or obesity.

#### 2.1. Negative Affect

Negative affect increases over time to the point where disordered eating occurs as a maladaptive emotion regulation strategy [33]. Some authors suggest that negative affect reduces after maladaptive eating behavior [34], whereas others point out that it does not decrease or continues to increase [35]. Taking into account the existing literature, it seems that negative affect and maladaptive eating behavior are interrelated and causally linked.

Negative affect is described as feelings of emotional distress [36]. An extensive literature has shown that negative affect leads to maladaptive eating behavior (e.g., overconsumption of high energy density foods or highly palatable foods), but it is not yet clear exactly how and why this happens. Prior work suggests that negative affect may lead to maladaptive eating because maladaptive eating reduces aversive affective states or because negative affect impairs top-down control. Thus, maladaptive eating behavior occurs, at least in part, in response to negative affective states [24,31,37].

Numerous laboratory and field studies have shown that various forms of negative affect (including stress and other negative emotional states) lead to maladaptive eating behavior, which includes both the overconsumption of unhealthy food and the underconsumption of healthy food [18,24,37]. Laboratory studies have shown that incidental and experimentally induced negative affect leads to maladaptive eating behavior with regard to both hypothetical food choices and actual eating behavior [17,38]. Cross-sectional and longitudinal field studies using self-report meal questionnaires, as well as ecological momentary assessment, have also shown that higher levels of negative affect predict maladaptive eating behavior [31,39]. Even though there is a known, robust relationship between negative affect and maladaptive eating behavior, it is not yet clear which of the two possible pathways between negative affect and maladaptive eating behavior is most important.

Negative affect can impact typical eating behavior in two ways. In the first pathway, negative affect causes an increase in tasty food craving (often unhealthy), and consumption of foods with a higher energy density (i.e., have a high calorie content, such as hamburgers or candy) [40,41]. Researchers have shown that, in at least some cases, individuals use tasty, highly rewarding high energy density food as a means of reducing negative affect [28,42]. In the second pathway, negative affect impairs top-down control over behavior, which studies have shown to be often required for choosing healthy, low energy density foods which also have vitamins, minerals and nutrients that play essential roles in a healthy diet [43,44]. While some people may find it rewarding to engage in healthy eating, the full benefits of maintaining a healthy diet come with more delay than the reward of eating tasty food [45]. Choosing to align eating (and other) behaviors with the pursuit of more delayed rewards is thought to require top-down control, and in the second pathway, negative affect disrupts the effective exercise of such control [43,46,47].

There are at least two pathways by which negative affect influences eating: by increasing the consumption of tasty, high energy density food as a means of reducing affect or by decreasing consumption of healthy, low energy-density food due to impaired top-down control [43]. In our model, these two pathways reflect the fact that negative affect might have an impact on eating either by increasing the weight given to taste or by decreasing the weight given to health in dietary decisions. As already mentioned, the taste value of a food is a marker of the immediate reward that can be used to palliate negative affect [42], whereas the health value of a food is the delayed reward that a person must represent using top-down control in order to make a healthy food choice [48]. Given that affect regulation leads to decreases in negative affect, such regulation should lead to improvements in eating behavior via both pathways, in other words, both via a decreased intake of tasty, high energy density foods and via an increased intake of healthy, low energy density foods.

It is worth adding that food choices in three eating situations, a neutral/typical meal (foods with medium nutrient density and medium energy), a healthy meal (foods with high nutrient density and low energy) and an unhealthy meal (foods with low nutrient density and high energy), provide information about a range of prototypical behaviors [49]. The actual process of making healthy choices is more difficult than making unhealthy ones in normal-weight individuals. Prior work suggests that negative affect may impair top-down control over behavior (which is needed to make healthy food choices) which may lead to underconsumption of healthy foods. Choosing tasty unhealthy foods does not require top-down control over behavior, whereas choosing untasty healthy foods does. In addition, the suppression of a thought (e.g., planning not to eat unhealthy snacks) may lead to this thought becoming more prevalent and will result in the increased consumption of unhealthy food [49].

Previous research studies have tested whether affect regulation can be used to decrease maladaptive eating [50,51]. However, in nearly all of these studies participants downregulated food-related affect (i.e., craving for food) rather than negative affect which is incidental to food (but which may be a cause of maladaptive eating behavior). Prior studies have shown that affect regulation strategies can effectively reduce craving for unhealthy foods, but all previous work has deployed these strategies to reduce craving itself rather than to downregulate the negative affect that may be a root cause of the craving [50,51]. It is worth pointing out that although many studies have examined negative affect and eating behavior, few studies have attempted to dissociate the contributions of two known pathways from negative affect to maladaptive eating behavior—the use of tasty food to cope with negative affect or the inability to choose healthy food due to impaired top-down control.

#### 2.2. Integral and Incidental Affect

Distinguishing different affective experiences could help us to understand the decisionmaking at hand, as well as the confounding findings about the influence of affect on decision making. It is important to differentiate two types of affects, integral and incidental, because they have a substantial influence on decision making and final judgment [52]. Integral (or endogenous) affect is defined as an affect stemming from consideration of the decision or judgmental target itself (i.e., food craving); a "genuine" subjective reaction to a target [53]. In other words, integral affect concerns experienced feelings about a stimulus [54]: for example, how people feel about various choice options while purchasing a food product. In making a decision, people can use their affective reactions towards options as proxies for values and use them as information in the evaluation of the options [55]. Individuals' experiences of integral affect allow them to categorize experiences on a goodbad dimension and enable them to reach a decision [56]. To sum up, integral affect is linked with the decision, decision attributes or to the decision situation. It can come about through anticipatory thinking (thinking about possible outcomes) or through activation by an actual stimulus (e.g., when a task associated with a decision is presented in a pleasant or unpleasant way) [57].

On the other hand, incidental (or exogenous) affect includes all factors that induce affect but are unrelated to the judgmental target or the decision being made [53]. In other words, feelings independent of a stimulus, such as mood states, can be misattributed to it or can have an effect on decision processes [54]. Similarly to integral affect, incidental affect can also influence judgments and decisions [58] (Figure 2). To sum up, the distinction between integral and incidental affect based on its relevance to decisions may provide the key to explaining the complex influence of affect on psychological processes.

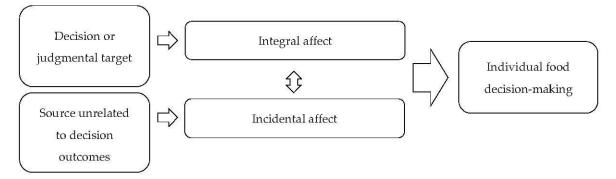


Figure 2. Integral and incidental affect and its influence on decision making.

Both mild incidental and integral affects are omnipresent in daily life [54] and interact with each other (Figure 2). Thus, a food consumption decision may be affected by two different types of affect: affect integral to the decision (food craving) and affect incidental to the choice (mood state). A previous review [52] has suggested that if integral and incidental affect is concurrently present, (1) integral affect dominates the overall response (a current incidental mood will have a significant effect on the overall judgment when integral affect is moderate or low in intensity), and (2) incidental affect has a significant influence on the integral response (when incidental affect is salient). In addition, the abovementioned review [52] has found that incidental affect congruent with the target may be beneficial to efficient decision making (it may amp up integral affect or the overall affective reaction), while incidental affect incongruent with the target may be detrimental (may attenuate the response).

Both integral and incidental affect play relevant roles in judgment and decisionmaking processes [54]. First, affect can act as information. Second, it can act as a spotlight concentrating people on different information depending on the extent of their affect and allowing them to compare the values of very different decision options or information. In this case, the two-step approach should be taken into consideration: (1) the extent (e.g., weak vs. strong affect) or type of affective feelings (e.g., anger versus fear) focuses the decision maker on new information, and (2) the new information is utilized to guide the judgment or decision. Third, affect seems to be a motivator of information processing and behavior. Lastly, affect has been associated with the extent of systematic processing in decision making [54].

#### Subtypes of Incidental Affect

Incidental affect (affect that is unrelated to the decision) influences decision making (non-normative influence). There are two sources of incidental affect: dispositional (trait) affect and situational (state) affect [53]. Dispositional affect is related to a tendency to respond in a special affective way to a diversity of events across time and situations. On the other hand, situational affect is affected by incidental moods and emotions and depends on the valence of the emotion and on specific emotion effects. Even minimal sensory cues can contribute to this type of affect and influence consecutive decision making [53]

#### 2.3. Maladaptive Eating Behavior

Eating behavior is an umbrella term that includes food choice and motives, dieting, feeding practices and eating-related pathologies such as eating disorders and obesity [59]. Eating behavior is complexly affected by psychological, physiological, nutritional, sociological and cultural factors. The modern eating patterns followed by the U.S. population are not adjusted to the Dietary Guidelines: about three-fourths of the population consume an eating pattern low in fruits, vegetables, dairy and oils, and most of the population does not follow nutritional advice and does not follow added sugars, saturated fats and sodium recommendations (U.S. Department of Health and Human Services and U.S. Department of Agriculture, 2015). Eating- and weight-related issues are highly prevalent in both the United States and Europe.

In the literature, three major maladaptive eating behaviors have been described: restrained eating, uncontrolled eating and emotional eating. It is worth pointing out that these types of maladaptive eating behaviors can be found in healthy, normal-weight individuals. Restrained eating is related to the intention to restrict food intake for the purpose of preventing weight gain or promoting weight loss [60]. Restrained eating is not equivalent to dieting, mainly because it solely illustrates the intent (not the action) of food restriction [61]. Nevertheless, restrained eating has often been linked to a total restricting dietary energy intake [62], a lower total energy intake [63] (e.g., lower meal and snack frequencies, breakfast skipping), disinhibited overeating as an effect of a loss of cognitive control [1] (restrained eating is under cognitive control rather than physiological one) and higher body mass index [60]. For decades, restraint theory indicated that restrained eating elicits counter-regulatory responses, reduces the sensitivity of the individual to satiety signals and leads to dietary disinhibition (associated with, e.g., overeating, loss of control over energy intake [64,65]. In the 1970s, laboratory-based studies revealed that individuals trying to reduce their energy intake for achieving weight control consumed more palatable foods with regard to high-calorie preloads [65]. This brought about the development of 'Restraint Theory' [1,6], according to which restrained eating is under the cognitive control of eating (e.g., eating in response to rigid dietary rules) and replaces eating in response to physiological cues. Sensitivity to internal cues for satiety is reduced and results in disinhibition and intake of large amounts of food that is not associated with hunger [35] in situations where cognitive control is weakened. Even a minor violation of rigid diet rules (e.g., by consuming high-calorie, "forbidden" foods) can lead to ignoring dietary rules (cognitive abandonment of the rule) to disinhibit the suppressed eating desires [1] and to overeating. Numerous studies have demonstrated that restrained eaters also increase food intake in response to negative emotions, possibly because these emotions deplete the cognitive resources needed for abiding by the dietary rules [29,66]. To sum up, evidence for emotional overeating is surprisingly inconsistent [66]. Cardi et al. [29] found evidence for emotional overeating in negative mood across studies among restrained eaters

and individuals with binge eating symptomatology [29], while a more comprehensive and recent meta-analysis questioned it [30]. The present meta-analysis has demonstrated that solely restrained eaters were found to be vulnerable to negative-emotion-induced eating, and negative emotions did not influence eating behavior amongst self-reported emotional eaters.

Uncontrolled eating (sometimes called external eating) is characterized by the overeating of unhealthy food in reaction to external food cues [3]. Individual differences in uncontrolled eating could be explained by two psychological processes. The first one is reduced cognitive control (processes that permit individuals to behave in a goal-directed manner, including inhibition, interference control, cognitive flexibility and working memory) [67]. The second process is automatic action tendencies towards external food cues, which are assumed to be modulated by reward networks in the brain [68]. The previous findings [69,70] have suggested that overconsumption in response to external cues may represent a general concept of uncontrolled eating characterized by low perceived self-control and high-calorie food consumption.

Emotional eating, also referred to as 'comfort eating' [71] or 'stress-induced eating' [21], indicates overconsumption in response to negative emotions [72]. Emotional eating involves a conscious or unconscious excess food consumption (including sweetened, salty or fatty foods) for reasons other than physical symptoms such as hunger. Emotion-congruent eating versus emotion-regulating eating can explain the influence of emotions on the quantity and quality of food intake. Emotion-congruent eating means that positive emotions increase and negative emotions decrease the motivation and pleasure of eating. Emotionregulating eating, also known as 'mood control eating,' explains that food intake serves to reduce unpleasant emotions (e.g., eating ice cream to relieve sadness) [73]. Several explanations regarding the psychological mechanism of emotional eating have been proposed. Psychosomatic theory underlines that overeating in response to negative emotions results from a lack of interoceptive awareness (e.g., an internal sensation of hunger), the incapacity to differentiate hunger sensations from arousal because of other aversive internal states or eating as a way to reduce negative emotions [32]. Psychological models regarding emotional eating [74,75] emphasize overconsumption in response to negative emotions as a maladaptive emotion regulation strategy. Masking theory demonstrates that overeating is an attempt to misattribute perceived stress to eating in order to divert an individual's attention from the original source of distress [74]. It is worth pointing out that while learning theories indicate that emotional overeating primarily fulfils an emotional regulatory function, cognitive theories indicate that emotional overeating results from disinhibition rather than from emotional regulation [66]. According to all these theories, before overeating occurs, individuals are unable to regulate negative affect that they experience, inducing them to use a maladptive strategy they do have access to, namely, overeating [66]. This suggests that the problem is not necessarily related to negative emotional experiences per se but rather with the absence of adaptive emotion regulation strategies available to regulate negative affect. Recent reviews have demonstrated inconsistent results as to which theory best explains emotional overeating [66].

Emotional eating may be the outcome of lower interoceptive awareness, difficulty with recognizing physiological cues of hunger or satiety and emotion regulation difficulties [76–78]. Emotional eating has also been found to be associated with overeating, excessive intake of sweet, high-fat and energy-dense foods [79], weight gain and difficulties losing weight [80], depression [81], overweight and obesity [81] and poor inhibitory control [69]. The findings propose that emotional eating might be an indicator of overconsumption generally and not specifically in the presence of negative or positive [29,69] emotions.

#### 3. Affect Regulation and Maladaptive Eating Behavior

Affect regulation is fundamentally considered as a mechanism by which individuals initiate, maintain, modulate or change the occurrence, intensity or duration of their own

emotions, moods and feelings [82] so as to pursue an affective equilibrium or homeostasis (maximize pleasant experiences and minimize unpleasant ones) [83]. According to [9], affect regulation is superordinate to coping, emotion regulation, mood regulation and traditional ego-defensive processes. One of several major forms of affect regulation is emotion regulation.

#### 3.1. Emotion Regulation Strategies

The previous section showed that negative affect is a source of many difficulties connected with eating. Therefore, downregulating negative affect could lead to improvements across a wide variety of maladaptive eating behaviors. For this, adaptive emotion regulation is advisable for successfully reducing negative affective states, strengthening or controlling positive affective states and restoring emotional balance [84].

Emotion regulation has been described as a subtype of both behavioral self-regulation and coping [85]. Emotion regulation as a construct has been described in a number of different ways. One of the best-known models [9,86] defines emotion regulation as the efforts individuals engage to impact the experience and expression of their emotions. Emotion regulation strategies are comprised of two components: antecedent- and response-focused strategies [87]. Antecedent-focused strategies are adopted before the complete activation of emotion response tendencies has taken place and have changed behavioral and peripheral physiological responding, whereas response-focused strategies appear once an emotion is already underway, thus, after the response tendencies have been generated [86]. There are two prototypical strategies that are commonly used in daily life: cognitive reappraisal and expressive suppression [86]. The first one is an antecedent-focused strategy that requires reframing or changing the way of thinking about an emotion-prompting situation in order to change the emotional effect of a situation once it has occurred. Expressive suppression, on the other hand, is a response-focused strategy that entails actively inhibiting the internal experience and external expression of emotion after emotional activation has occurred [86]. These two strategies differ in the required amount of self-regulatory resources and have different consequences. Generally, cognitive reappraisal appears to change the primary appraisals of emotional stimuli without the need for persistent self-regulatory effort over time [88]. It is associated with less experience and less expression of negative emotion, less physiological activation and more positive experience of emotion, which means that this strategy can be regarded an adaptive emotion regulation strategy. In contrast, expressive suppression entails active efforts to inhibit dominant responses, resulting in comparatively greater "resource depletion" than reappraisal [28]. It is associated with increases in physiological responding and decreases in behavioral expression, but it ends in failure to reduce the experience of emotion, which is why this strategy can be regarded as a maladaptive one [89].

It has been noted that some individuals use eating as a strategy to regulate their emotions. The means in which emotions are regulated influence eating behavior [28]. Some individuals become involved in eating as a way to downregulate negative emotions, which is likely because of using more maladaptive emotion regulation skills. Impaired emotion regulation is related to difficulties in regulating eating behavior. Deficits in emotion regulation skills result in dysregulated (overeating sweet or high-caloric foods) or overregulated eating behavior, which may result in underweight, malnutrition or excess body weight (Figure 3). The regulation strategies used to cope with negative emotions are responsible for increased eating (emotional eating). It is worth pointing out that the use of more adaptive emotion regulation strategies (e.g., cognitive reappraisal) might result in reducing maladaptive eating behavior. Cognitive reappraisal has been widely theorized to be protective against psychopathology [90], eating pathology and eatingrelated symptoms (medium to large effect size) [91]. Thus, it is plausible that cognitive reappraisal strategies, in particular those concentrating on the benefits of not eating, could potentially enhance the capacity to reduce unhealthy food intake [92]. We argue that using cognitive reappraisal might be useful in downregulating integral negative affect associated

with food by improving eating behavior (making more very healthy choices and fewer unhealthy ones, i.e., placing a higher weight on health when making dietary decisions).

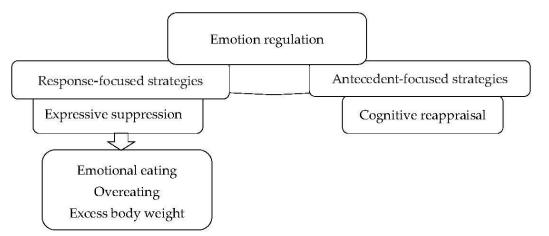


Figure 3. Emotional eating as a consequence of maladaptive emotion regulation strategy.

Emotion dysregulation has been described as a potential transdiagnostic risk factor [93] and a robust correlate of disordered eating behaviors (including a spectrum of maladaptive eating behaviors and cognitions linked to negative psychological and physiological health outcomes). Emotion regulation difficulties are related to increased binge eating [94], dietary restraint [95] and eating pathology in general [91]. Emotion regulation has mostly been recognized as a correlate of emotional eating [28,96]. Recently, Braden and colleagues [96] examined the psychological (emotion regulation and disordered eating behaviors) correlates of emotional eating across negative and positive emotional eating dimensions, determining that negative emotional eating was linked to increased emotion regulation difficulties and disordered eating behaviors. These data are consistent with research founding a positive association between negative emotional eating and emotion regulation difficulties [78].

Most studies have shown that suppression is linked to higher levels of distress and cognitive reappraisal appears to enhance subsequent behavioral self-regulation outcomes [86,88]. In addition, suppression is related to poorer behavioral self-regulation. There are few laboratory-based studies testing the impact of emotional suppression and cognitive reappraisal on eating behavior following negative mood induction. Vohs and Heatherton [97] found that when participants were requested to suppress their emotional reactions (they watched a stimulus video clip planned to induce negative affect), they reported higher levels of food intake, particularly ice cream, demonstrating that suppression is related to decreased subsequent behavioral restraint. Evers and colleagues [28] employed similar emotion-induction procedures but evaluated the impacts of habitual or trait emotion regulation styles (high or low suppression, and high or low cognitive reappraisal) of food consumption [28]. Suppression moderated the relation between sad mood and food intake, such that high suppression participants consumed considerably more than participants who do not frequently make use of suppression. In addition, the previous study demonstrated that suppression was equally efficacious as acceptance in limiting reported chocolate consumption over a week [98]; however, those in the suppression condition consumed significantly more chocolate during the follow-up laboratory session.

Previous research [28] has demonstrated that maladaptive emotion regulation strategies resulted in increased comfort food intake (sweet or salty foods) compared with adaptive strategies and with spontaneous emotion expression. The findings also revealed that (1) individuals regularly using suppression ate more when being emotional than individuals rarely using this strategy and (2) participants who suppress their negative emotions consumed more comfort foods than those who reappraise their emotions. To sum up, these mixed results confirm the thesis that the way people regulate their negative emotions modulates the amount of food intake.

Taut, Renner and Baban [99] investigated the effects of negative emotions (fear, negative affect) and emotion regulation strategies (suppression, cognitive reappraisal) on food consumption in a neutral control condition where participants chose whether and how much they desired to consume and also whether they wanted to consume at all (ad libitum food intake). The authors examined whether participants use eating as a secondary coping strategy when emotion regulation is ineffective. The majority of participants in the reappraisal group were less likely to consume both chocolate and crisps in comparison to the control and suppression groups. However, among individuals who ate, there was no difference in the amount consumed across conditions. Thus, the main discrepancy between the three emotion regulation strategies appears to be whether or not eating is utilized as a secondary regulation strategy at all rather than differences in the amount of food required for secondary regulation as indicated in previous research [28]. The main difference between suppression and reappraisal is whether or not eating is needed as a secondary coping strategy, rather than differences in the amount of food intake per person as proposed in the study by Evers et al. [28]. The findings indicate that when individuals are faced with a negative event, eating is employed as a secondary coping strategy when the adopted emotion regulation strategy is ineffective. Inversely, an adaptive emotion regulation strategy, such as reappraisal, reduces the probability of food consumption, even when emotion regulation is utilized during rather than before the unfolding of the negative event. Thus, the means people cope with negative emotions might be more appropriate for elucidating emotional eating than the distress itself [28].

Cognitive reappraisal, such as thinking of long-term health consequences of eating unhealthy food when regarding images of such foods, enhances inhibitory region activation (less inhibitory control is connected with greater weight gain), decrease reward region (hyper-responsivity of the reward region contributes to overeating) and attention region activation, as well as to prevent weight gain [100]. A recent study [101] has shown that cognitive strategies reduce unhealthy and enhance healthy food consumption (craving). Thus, changes in craving may affect the consumption of both healthy and unhealthy foods. These findings evidence that training-based interventions (specifically, regulation of craving training) affect eating behavior (increasing healthy food choices in the face of enticing unhealthy options) and can reduce unhealthy eating (reducing total caloric consumption, particularly of high-caloric or unhealthy foods) [101] and might be beneficial in helping people enhance their recruitment of inhibitory regions when faced with high-fat or high-sugar foods [100].

#### 3.2. Applying Affect Regulation to Incidental Affect in the Context of Eating

Understanding how incidental affect influences food intake is an important topic. The previous studies showed that incidental affect influences in-store shopping [102] and in-home food choice [103]. In addition, the findings showed that incidental affect (sadness and happiness) impacts food consumption within a general population [104]. A previous study [104] showed that incidental affect influences consumption levels at the individual level. Consumption levels of a hedonic product are lower for individuals in a state of happiness than for those in a state of sadness. In other words, while sad individuals presented a substantial refuse in their consumption, happy individuals appeared to be unswayed by nutritional information. Thus, it seems that happy people are already avoiding food intake, and the presence of nutritional information does not force their food intake any lower. Conversely, sad people took of liberty of trying and overcoming their negative state by eating more (in the information-absent condition) [104]. This study also showed that happy people consumed more raisins in comparison with sad people. In the lack of mood-changing cues, affective evaluation predominates, therefore, people comport according to their mood state (i.e., happy people have positive evaluations and

tend to consume more, while sad people have negative evaluations and tend to consume less) [104].

#### 4. Directions for Future Research

The present review has shown that negative affect is a source of many difficulties connected with eating. Therefore, downregulating negative affect could lead to improvements across a wide variety of maladaptive eating behaviors. For this, adaptive emotion regulation is advisable for successfully reducing negative affective states and strengthening or controlling positive affective states [85].

A rich literature has shown that people can and do use a host of different affect regulation strategies to regulate affective responses including stress responses and negative emotions and moods [85]. Maladaptive emotion regulation strategies (such as suppression of emotions) are positively associated with emotional eating [76]. While suppression appears to be maladaptive in terms of increased comfort food intake in comparison to reappraisal [28], reappraisal seems to be related to reduced food intake. Correlational studies found that negative affect did not predict eating behavior among non-clinical samples [28,99], however many limitations exist in the previous and current studies, therefore further research in this area is needed. In addition, the exact process of emotions affecting behavior is still uncharted.

Cognitive reappraisal applied to incidental affect might be more effective than some of prior efforts. Additional studies are needed in order to further investigate the effect that specific emotion regulation strategies have on maladaptive eating behavior in normalweight individuals. These results could provide worthwhile insights into the emotional mechanisms underlying maladaptive eating behavior.

Narrative review is the limitation of the present work. Future work should focus on a systematic review, currently widely considered as studies with the highest level of evidence, and follow a set of strict established guidelines, such as PRISMA or Joanna Briggs Institute guidelines.

In addition, in an ongoing global pandemic of coronavirus disease, individuals can present unfavorable changes in eating behavior [105], therefore, it would be needed to investigate changes in eating behavior and emotion regulation in normal weight individuals during the COVID-19 pandemic.

#### 5. Conclusions

The scientific literature (field and experimental studies) provides clear evidence that negative emotions and maladaptive emotion regulation strategies influence maladaptive eating behavior. It is assumed that moderate arousal or moderately intense emotions affect eating [30].

It is plausible that increased food consumption may be an attempt to downregulate negative emotion. The previous research has demonstrated that the way in which individuals cope with negative emotions (rather than experience negative emotions) may determine the influence of emotion on eating behavior [28]. The more "costly" an emotion regulation strategy is in terms of consuming self-regulatory resources, the more individuals are likely to increase food intake as a secondary regulation strategy [99]. Continuous involvement in self-regulatory acts (such as abstaining from eating or regulating one's emotion states) could lead to resource depletion and a reduction in skill to self-regulate at a later point [106].

Some individuals use eating in order to face their negative emotions and regulate them. That leads to increased or decreased eating and is linked to an increased use of maladaptive emotion regulation strategies. Therefore, it is important to teach normal-weight individuals to use more adaptive emotion regulation strategies to properly manage their emotions and affect. Maintenance of advantageous emotion regulation is necessary in those people in order to have adaptive eating behavior without over-control or loss of control over eating. The use of more adaptive strategies for emotion regulation (cognitive reappraisal) might reduce maladaptive eating behavior. Therefore, effective interventions can help to sustain eating behavior change.

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# **The Relationships among Microelement Composition of Reindeer Meat (***Rangifer tarandus***) and Adaptation: A Systematic Review and Meta-Analysis**

Sergei Andronov <sup>1,2,\*</sup>, Andrey Lobanov <sup>1</sup>, Elena Bogdanova <sup>3,\*</sup>, Andrei Popov <sup>1</sup>, Alexander Yuzhakov <sup>4</sup>, Olga Shaduyko <sup>2</sup>, Dele Raheem <sup>5</sup> and Irina Kobelkova <sup>6</sup>

- <sup>1</sup> Laboratory for Studying the Mechanisms of Physical Factors Action, Centre for Testing and Examination of Natural Healing Resources, National Medical Research Centre for Rehabilitation and Balneology, Ministry of Health of the Russian Federation, 121099 Moscow, Russia; alobanov89@gmail.com (A.L.); apopov2007@yandex.ru (A.P.)
- <sup>2</sup> Excellence Support Unit, Tomsk State University, 634050 Tomsk, Russia; dolcezzamia@mail.ru
- <sup>3</sup> Department of Economics and Management, Northern Arctic Federal University, 163002 Arkhangelsk, Russia
   <sup>4</sup> Saint Petersburg Federal Research Center, Russian Academy of Sciences, 199178 Saint Petersburg, Russia;
- alyuzhakov@yandex.ru
   Northern Institute of Environmental and Minority Law, Arctic Center, Lapland University, 96101 Rovaniemi, Finland; bamidele.raheem@ulapland.fi
- <sup>6</sup> Federal Research Centre for Nutrition and Biotechnology, 109240 Moscow, Russia; kobelkova@ion.ru
- \* Correspondence: sergius198010@mail.ru (S.A.); e.n.bogdanova@narfu.ru (E.B.)

**Abstract:** This systematic review and meta-analysis based on PRISMA statements aimed to summarise the data on the chemical composition of reindeer meat depending on the region of the *Rangifer tarandus*. We searched SCOPUS, PubMed, Embase, CrossRef, Medline, Cochrane library, eLibrary, and CyberLeninka. A total of 3310 records published between January 1980 and December 2021 were screened. We identified 34 relevant studies conducted in Russia, Norway, the USA, Canada, and Finland for the synthesis. Overall, the consumption of reindeer meat reduces arterial hypertension and atherosclerosis due to many polyunsaturated fatty acids (linoleic, linolenic, arachidonic) and vitamin C, which balances lipid fractions. Venison is an effective means of preventing obesity and adapting to cold due to the content of a complete set of essential trace elements, amino acids, and even L-carnitine. The high content of vitamin C and microelements (iron, zinc, copper) in reindeer meat is likely to increase the body's antioxidant defence against free radicals and help prevent chronic non-infectious diseases. Thus, venison is an essential component of the adaptation mechanism for the Arctic population.

**Keywords:** systematic review; reindeer meat; macro- and microelement analysis; adaptation; Arctic population; meta-analysis

#### 1. Introduction

The unique nutrition of the Arctic Indigenous Peoples is associated with their increased endurance, health, and adaptability to the harsh climate [1]. Reindeer meat, blood, and liver are the most critical elements of this traditional nutrition enriched with minerals [2,3]. Reindeer consumption is a crucial factor of successful adaptation to the cold stress, as well as a component of national culture, food, and economic security and sovereignty, affecting the well-being and health of the Indigenous population in the Arctic [4–9].

The reindeer (*Rangifer tarandus*) habitat covers territories in Eurasia and North America between 50- and 81-degrees north latitude [10] and includes continental and island territories, tundra, taiga, and mountainous areas close to them in vegetation composition and climatic conditions [11]. Reindeer live in Russia, the USA, Norway, Sweden, Finland,

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**Copyright:** © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). Denmark, Iceland, Canada, Mongolia, Great Britain, and China [10]. The largest populations of wild reindeer (*Rangifer tarandus caribou*) are in Russia (952.9 thousand; 2015) and Canada (1300 thousand; 2016) [11]. The world's largest livestock of domesticated reindeer is in Russia (1620.8 thousand reindeer in 2021) [12]. In Russia, the largest population of wild reindeer is in the Krasnoyarsky Krai, the Chukotka Autonomous Okrug, the Republic of Sakha (Yakutia), and domesticated reindeer are in the Yamal-Nenets Autonomous Okrug [13]. Such various reindeer habitats make pre-conditions for the different chemical compositions of reindeer products in different northern regions.

The macro- and microelement composition of reindeer meat is impacted by significant differences in the species and mineral composition of forages (plants and lichens), the duration of grazing seasons on winter and summer pastures, the proportion in the diet of green fodder, shrubs, lichens, mushrooms, eggs of birds, and rodents, the macro- and microelement composition of soil and water, pollution, availability of salty seawater, and the cutting of velvet antlers [14,15]. A specific feature of the northern reindeer is its seasonal migration to areas with different forage resources: Summer pastures with a predominance of herbaceous plants and shrubs and winter pastures rich in lichens [16].

The study of the macro- and microelement composition of reindeer meat started in the second half of the 20th century. In the 1970s, in Canada, O. Schaefer (1977) and K. Hoppner (1978) confirmed the high nutritional value of reindeer meat due to high protein and low fat content [17,18]. Two decades later, H.V. Kuhnlein (1992; 1996; 2000; 2002) conducted a study of micronutrient composition of reindeer products [19–22] and developed recommendations for the use of venison by patients with atherosclerosis, vitamin deficiency, diabetes mellitus, and for the prevention of heart, liver, and stomach diseases [23–25]. In the 1990s, in Alaska, the USA, the chemical composition of traditional products, including venison, was studied [26]. Currently, a national database includes the data on the complete quantitative and qualitative chemical composition of reindeer meat in Alaska [27]. In Russia, studies conducted in Yamal-Nenets Autonomous Okrug [28,29], Nenets Autonomous Okrug [30–32], Taimyr [33–35], the Republic of Yakutia [36], and on the Kola Peninsula [37–39] confirmed the nutritional and biological value of reindeer meat. Furthermore, they proved the need to include this product in a healthy diet.

*Rangifer tarandus* is highly adapted to Arctic conditions. The optimal work of enzymes that ensure adaptation to cold stress provides the accumulation of essential trace elements necessary for the practical work of enzymatic chains. The most crucial macronutrients are calcium (Ca), magnesium (Mg), phosphorus (P), potassium (K), and sodium (Na), among others, which activate enzymes, regulate the number of hormones, promote muscle and nervous activity, and therefore are essential components of the daily human diet [40–42]. Thus, the consumption of reindeer meat can increase adaptation to the Arctic conditions, reduce the risk of heart diseases, and improve metabolism [43–45].

Improving knowledge about the macro- and microelement composition of reindeer meat in different northern regions will contribute to the expansion of the use of reindeer products to prevent diseases and increase the adaptation of the Arctic population and shift workers in the circumpolar area, as well as develop effective medicinal and pharmaceutical products. Furthermore, studying the chemical composition of reindeer meat will also increase the value of exported reindeer meat, which is an important factor in promoting the economic sovereignty and well-being of the Indigenous Peoples in the Arctic.

Our systematic review and meta-analysis aim to summarise the data on the chemical composition of reindeer meat depending on the region of the *Rangifer tarandus* and analyse the effects of venison consumption on human health and adaptation in the Arctic.

#### 2. Materials and Methods

In this research, a systematic review based on the Preferred Reporting Items for Systematic Reviews and Meta-Analyses, the PRISMA statement [46,47], was conducted. The PRISMA checklist is presented in Appendix A according to the model [48].

The research questions for this systematic review were: "Does the macro- and microelement composition of reindeer meat vary in different northern regions?".

#### 2.1. Search Strategy

We searched the SCOPUS, PubMed, Embase, CrossRef, Medline, Cochrane library, eLibrary, and CyberLeninka electronic databases to identify relevant studies for the synthesis without language restrictions, using and updating them (from January 1980 to December 2020). In addition, the reference lists of all studies included and all the systematic reviews identified during the search process were checked.

The search strategy for all databases included terms of the Medical Subject Headings. Searches were made using the following keywords or their combination: "chemical composition of reindeer meat", "chemical composition of venison", combined with "sodium", "potassium", "calcium", "magnesium", "phosphorus", "iron", "zinc", "trace elements".

#### 2.2. Inclusion Criteria

Eligible studies were required to meet the following criteria: (1) Evaluate the concentration of the minerals (sodium, potassium, calcium, magnesium, phosphorus, zinc, iron) in reindeer meat; (2) the results were received in the territories located in the High North; (3) experimental descriptive or retrospective studies. We also excluded study protocols, letters to the editor, editorials, and conference abstracts with no full text available. All citations were entered into a bibliographic reference manager, and duplicate studies were excluded, automatically or manually (EndNote<sup>®</sup>, v. X7, Tomson Reuters, Philadelphia, PA, USA).

The control group included data on the macro- and microelement composition in reindeer meat obtained from our data. The content of trace elements in reindeer meat was assessed in the testing laboratory centre of the Federal Research Center for Nutrition and Biotechnology (Moscow) (certificate No. ROSS RU.0001.21IP14 dated 22 August 2014). In addition, sampling of the studied objects was carried out following the national standard GOST R 51447–99 [49]. The following standard methods were used to determine the chemical composition: (1) Identification of the content of trace elements (potassium, calcium, sodium, magnesium, phosphorus) according to R 4.1.1672-2003 [50]; (2) determination of iron and zinc under the national standard GOST No. 30178-96 [51].

Laboratory studies to identify trace elements in food were conducted in the autumnwinter season. To determine the concentration of metals, during the analysis food products were subjected to mineralisation to remove organic impurities. The determination was made using a model-Z 5300 atomic absorption spectrophotometer by atomic absorption spectrometry. The determination of the content of trace elements (calcium, magnesium, phosphorus) was implemented on a liquid chromatograph (HPLC) (model "Agilent 1100" detector DAD) in the laboratory of vitamins and minerals.

#### 2.3. Study Selection, Data Extraction and Assessment of Methodological Quality and Risk of Bias

According to the search strategy, the authors (SA, EB) screened titles and abstracts and independently assessed the full text of all potentially relevant studies for inclusion in this review. All disagreements were managed through discussion with a third author (AL). Then, following a standardised data collection form, the information was extracted from the included studies: (i) Study characteristics: Setting, study design, and countries; (ii) microelement composition of reindeer meat; and (iii) health impacts. We also evaluated the lists of references of the studied papers to identify other relevant articles to be included. Reasons for exclusion are reported in Figure 1.

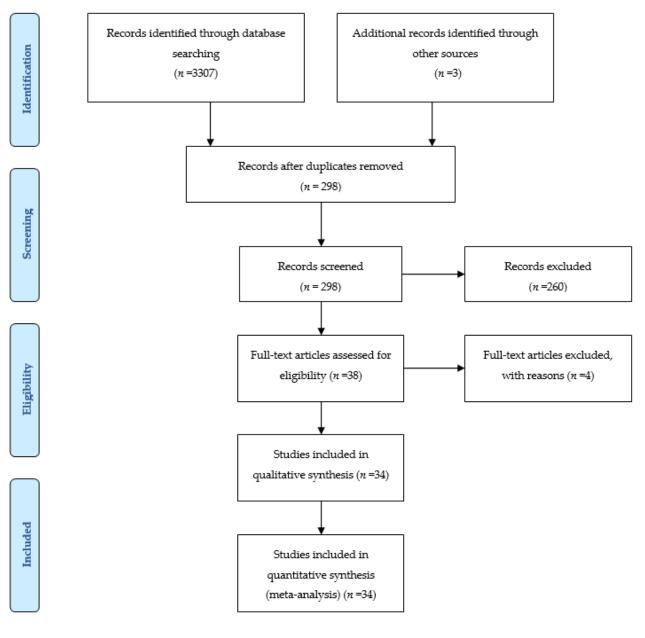


Figure 1. PRISMA flow chart of study selection.

To assess the methodological quality and risk of bias, the checklist of Esther F. Myers [52–54] was applied (Appendix B). After a detailed evaluation of the methods and results, the studies were analysed to verify the possibility of "skewed results", "confusions", and "random occurrence". Only studies with a low risk of bias were included.

#### 2.4. Data Analysis and Synthesis

We applied Cochran's Q statistics and calculated I<sup>2</sup> [55] to assess the statistical heterogeneity across studies. The interpretation of the value of I<sup>2</sup> was: 0 to 40 = low; 30 to 60 = moderate and worthy of investigation; 50 to 90 = severe and worthy of understanding; 75 to 100 = aggregate with major caution [56], and a 95% confidence interval. A *p*-value < 0.05 was considered statistically significant. The interpretation threshold for the weighted effect values was 0.8 [57]. We generated the forest plots for each analysis. A comprehensive analysis of Egger's test and Funnel Plot Visual interpretation were implemented for the assessment of the publication bias [58–60]. The standardised difference in mean values (Hedge's g) and 95% confidence intervals were calculated using a random-effects model [58–61]. The Jamovi statistical software (version 1.6, Sydney, Australia) [62] and the MAJOR module [63] were used to generate figures and run the test. Jamovi uses the Graphical User Interface (GUI) version of the R module, and MAJOR uses the R package, Metafor [64]. We used sensitivity analysis to explore the influence of each study in the pooled meta-analysis or publication bias results. This analysis was adopted in the case of substantial or considerable (50 to 100%) heterogeneity or significant publication bias (p < 0.05) [65,66].

#### 3. Results

#### 3.1. General Characteristics

A total of 3310 records published between January 1980 and December 2021 were screened. First, the abstracts of the publications were analysed. We excluded duplicated, descriptive (e.g., [67]) articles and publications that did not have information about the content of trace elements in reindeer meat or contained data about other animals (3012) (e.g., [68–81]). In total, 260 studies were excluded due to the unavailability of the full text of the publication (e.g., [82]). Therefore, 38 sources included in the further analysis were assessed by two independent reviewers.

Quantitative synthesis used 34 studies (Figure 1) published in English (n = 25) and in Russian (n = 9). In addition, fourteen studies were conducted in Russia [3,38,83–94], seven in Norway [74,95–100], six in the USA [27,101–105], four in Canada [19,21,22,106], and three in Finland [107–109]. The details of the included studies are presented in Table 1.

	Sample of	Macro- and Microelements, mg/100 g							
Region		К	Р	Na	Mg	Ca	Fe	Zn	Source
Yamal-Nenets Autonomous Okrug (control group)	10	$\begin{array}{c} 360.0 \\ \pm 18.0 \end{array}$	$\begin{array}{c} 250.0 \\ \pm 12.5 \end{array}$	$\begin{array}{c} 77.0 \\ \pm 4.5 \end{array}$	28.0 ± 1.5	$\begin{array}{c} 15.0 \\ \pm \ 0.8 \end{array}$	$5.0 \\ \pm 0.5$	2.2 ± 0.4	[own data]
Murmansk region	10	225.0 ± 11.2	226.0 ± 11.3	121.0 ± 6.1	$\begin{array}{c} 16.1 \\ \pm \ 0.8 \end{array}$	$9.6 \\ \pm 0.5$	$\begin{array}{c} 6.1 \\ \pm \ 0.3 \end{array}$	$\begin{array}{c} 3.0 \\ \pm \ 0.5 \end{array}$	[38,83,86]
Komi Republic	10	$\begin{array}{c} 333.0 \\ \pm \ 50.0 \end{array}$	*	54.16 ± 9.2	$\begin{array}{c} 31.03 \\ \pm \ 4.55 \end{array}$	7.13 ± 1.78	$\begin{array}{c} 5.55 \\ \pm \ 0.9 \end{array}$	$\begin{array}{c} 4.19 \\ \pm \ 0.7 \end{array}$	[3]
Taimyr, Krasnoyarsk Territory	30	$\begin{array}{c} 465.0 \\ \pm 10.2 \end{array}$	71.0 ± 5.0	276.0 ± 11.0	120.0 ± 10.0	$\begin{array}{c} 158.0 \\ \pm 40.0 \end{array}$	$\begin{array}{c} 18.2 \\ \pm 1.5 \end{array}$	$\begin{array}{c} 10.1 \\ \pm \ 0.8 \end{array}$	[85]
Republic of Yakutia	10	$\begin{array}{c} 316.6 \\ \pm \ 6.4 \end{array}$	$\begin{array}{c} 266.7 \\ \pm \ 6.5 \end{array}$	$\begin{array}{c} 137.2 \\ \pm \ 4.5 \end{array}$	23.7 ± 0.5	$\begin{array}{c} 14.9 \\ \pm \ 0.6 \end{array}$	15.2 ± 1.6	$\begin{array}{c} 3.0 \\ \pm \ 0.5 \end{array}$	[84,87–92]
Far East	10	$\begin{array}{c} 305.2 \\ \pm \ 15.0 \end{array}$	$\begin{array}{c} 194.4 \\ \pm \ 9.7 \end{array}$	77.4 ± 3.9	24.5 ± 1.2	$\begin{array}{c} 10.2 \\ \pm \ 0.5 \end{array}$	2.9 ± 0.15	$\begin{array}{c} 3.0 \\ \pm \ 0.5 \end{array}$	[93,94]
Finland	30	318.0 ± 15.9	230.0 ± 11.5	$95.0 \\ \pm 4.8$	26.0 ± 1.3	$\begin{array}{c} 8.1 \\ \pm \ 0.4 \end{array}$	$\begin{array}{c} 3.6 \\ \pm \ 0.2 \end{array}$	$\begin{array}{c} 3.0 \\ \pm \ 0.2 \end{array}$	[107–109]
Norway	30	$\begin{array}{c} 290.0 \\ \pm 14.5 \end{array}$	$\begin{array}{c} 189.0 \\ \pm \ 9.5 \end{array}$	$95.0 \\ \pm 4.8$	33.0 ± 2.0	7.0 ± 1.3	2.9 ± 0.7	$\begin{array}{c} 4.8 \\ \pm 1.6 \end{array}$	[74,95–100]
Canada	158	$\begin{array}{c} 451.8 \\ \pm 22.5 \end{array}$	219.5 ± 11.0	$\begin{array}{c} 49.7 \\ \pm \ 2.5 \end{array}$	33.1 ± 1.7	$5.0 \\ \pm 0.3$	$5.4 \\ \pm 0.3$	$\begin{array}{c} 3.5 \\ \pm \ 0.2 \end{array}$	[19,21,22,106]
Alaska, the USA	30	$\begin{array}{c} 320.0 \\ \pm \ 16.0 \end{array}$	230.0 ± 11.5	$52.0 \\ \pm 2.6$	$\begin{array}{c} 26.0 \\ \pm \ 1.3 \end{array}$	$5.0 \\ \pm 0.3$	$\begin{array}{c} 4.1 \\ \pm \ 0.2 \end{array}$	$\begin{array}{c} 2.1 \\ \pm \ 0.1 \end{array}$	[27,101–105]

Table 1. The data of the included studies.

\* No data.

The retrieved studies involved a total of 328 *Rangifer tarandus*, which were adult animals of both sexes with an average age of  $2.0 \pm 0.5$  years. The sample sizes ranged from 10 to 158. The mean value (mg/100 g) of macro- and microelements varied: Potassium—from  $225.0 \pm 11.2$  to  $465.0 \pm 10.2$ ; sodium—from  $49.7 \pm 2.5$  to  $276.0 \pm 11.0$ ; phosphorus—from  $71.0 \pm 5.0$  to  $266.7 \pm 6.5$ ; calcium—from  $5.0 \pm 0.3$  to  $158.0 \pm 40.0$ ; magnesium—from  $16.1 \pm 0.8$  to  $120.0 \pm 10.0$ ; iron—from  $2.9 \pm 0.15$  to  $18.2 \pm 1.5$ ; and zinc—from  $2.1 \pm 0.1$  to  $10.1 \pm 0.8$  (Table 1).

Separate meta-analyses were conducted for different macro- and microelements (magnesium, iron, zinc, calcium, potassium, sodium, and phosphorus).

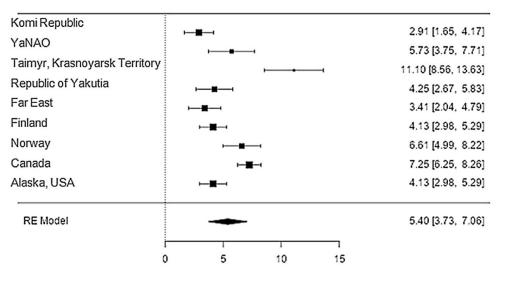
# 3.2. *Macro- and Microelement Composition in Reindeer Meat: Heterogeneity Analysis* 3.2.1. Magnesium

The iron content in reindeer meat was available in 11 studies. The standardised mean differences ranged from 2.9107 to 11.0987; most ratings were positive (100%). The estimated standardised mean difference based on a random-effects model was 5.3972 (95% CI: 3.7340–7.0604). Thus, the mean value was significantly different from zero (z = 6.3602, p < 0.0001) (Table 2, Figure 2).

**Table 2.** The content of macro- and microelements in reindeer (*Rangifer tarandus*) meat: Heterogeneity analysis.

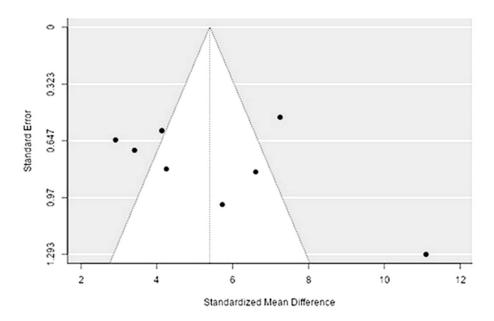
Macro- and Microelements	Random-Effects Model, k	Estimate *	se	Z	p	CI Lower	CI Upper
Magnesium	9	5.40	0.849	6.36	< 0.001	3.734	7.060
Iron	9	5.83	1.31	4.43	< 0.001	3.250	8.404
Zinc	9	0.51	0.149	3.45	< 0.001	0.22	0.804
Calcium	9	-2.12	2.45	-0.867	0.386	-6.918	2.674
Potassium	10	24.3	25.4	0.96	0.34	-25.45	73.99
Sodium	9	24.1	23.7	1.02	0.31	-22.31	70.5
Phosphorus	9	14.5	19.0	0.76	0.45	-22.7	51.7
		Heteroge	eneity Statistic	S			
Macro- and Microelements	Tau	Tau <sup>2</sup>	I <sup>2</sup>	H <sup>2</sup>	df	Q	р
Magnesium 2.419		5.8524 (SE = 3.259)	92.17%	12.776	8.000	66.719	<0.001
Iron 3.832		14.686 (SE = 7.81)	97.04%	33.77	8.000	269.34	< 0.001
Zinc 0.44		0.194 (SE = 0.0995)	97.67%	42.98	8.000	429.42	<0.001
Calcium	7.292	53.1782 (SE = 26.9478)	99.3%	142.905	8.000	488.351	<0.001
Potassium	79.87	6378.95 (SE = 3034.51)	99.44%	178.16	9.000	1970.58	<0.001
Sodium 71.00		5041.41 (SE = 2522.57)	99.94%	1779.06	8.000	8955.84	< 0.001
Phosphorus	56.8	3227.16 (SE = 1621.7)	99.54%	216.18	8.000	2146.4	< 0.001

\* Note. Tau<sup>2</sup> Estimator: Hedges.



**Figure 2.** Forest plot of the comparison of the content of magnesium in reindeer (*Rangifer tarandus*) meat by geographical regions.

The *Q*-test confirmed the heterogeneity of the sources, including the data on the content of magnesium in reindeer (*Rangifer tarandus*) meat (Q(8) = 66.72, p < 0.0001, tau<sup>2</sup> = 5.85, I<sup>2</sup> = 92.17%). The 95% interval was from 0.37 to 10.42. Publication bias was explored with a visual inspection of the funnel plot (Figure 3), where the regression test showed asymmetry in the funnel plot (p = 0.026), but not the rank correlation test (p = 0.3429) (Table 3).



**Figure 3.** Funnel plot for publication bias evaluation of magnesium content in reindeer (*Rangifer taran-dus*) meat by geographical regions.

	Test							
Macro- and — Microelements _	Fail-S	afe N	Egger's Regression					
	Value	p	Value	р				
Magnesium	1559.000	<0.001	2.221	0.026				
Iron	1284.000	<0.001	3.33	0.001				
Zinc	1689.000	<0.001	-0.099	0.921				
Calcium	226.0	<0.001	-0.14	0.89				
Potassium	735.0	<0.001	-0.14	0.89				
Sodium	735.0	<0.001	-0.14	0.89				
Phosphorus	225.0	<0.001	1.3	0.19				

**Table 3.** The statistical analysis of publication bias of the included sources with the data on macroand microelements content in reindeer (*Rangifer tarandus*) meat \*.

\* Fault-tolerant calculation of N using Rosenthal's approach.

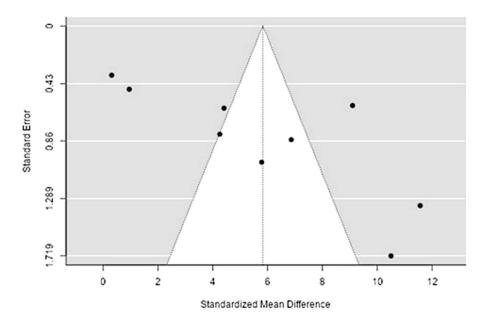
### 3.2.2. Iron

The iron content in reindeer meat was available in 11 studies. The standardised mean differences ranged from 0.32 to 11.56, and most ratings were positive (100%). The estimated standardised mean difference was 5.83 (95% CI: 3.25–8.4) based on a random-effects model. Thus, the mean value was significantly different from zero (z = 4.43, p < 0.0001) (Table 2, Figure 4).

Komi Republic	<b>⊢-H</b> I	4.26 [2.68, 5.84]
YaNAO	<b>⊢</b> ∎(	5.78 [3.79, 7.78]
Taimyr, Krasnoyarsk Territory	<b>ب</b> ر	11.56 [ 8.93, 14.19]
Republic of Yakutia	<del>بر</del>	10.50 [7.13, 13.87]
Far East		0.96 [0.03, 1.88]
Finland	<b>⊢≣</b> →	4.41 [3.21, 5.61]
Norway	H <b>H</b> I	0.32 [-0.40, 1.04]
Canada	⊨∎⊣	9.10 [7.93, 10.26]
Alaska, USA	<b>⊢∎</b> 1	6.86 [5.20, 8.53]
RE Model	-	5.83 [3.25, 8.40]
[	1 1 1	
-5	0 5 10 15	

**Figure 4.** Forest plot of the sources, including the data on the iron content in reindeer (*Rangifer taran-dus*) meat in different geographical regions.

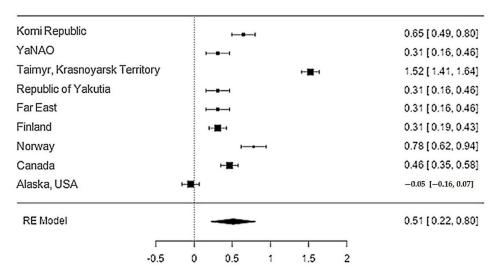
The *Q*-test confirmed the heterogeneity of the sources, including the data on the content of iron in reindeer (*Rangifer tarandus*) meat (Q(8) = 269.34, p < 0.0001, tau<sup>2</sup> = 14.69, I<sup>2</sup> = 97.04%). The 95% interval was from -2.11 to 13.77. Publication bias was explored with a visual inspection of the funnel plot (Figure 5), where the regression test showed asymmetry in the funnel plot (p = 0.0009), but not the rank correlation test (p = 0.12) (Table 3).



**Figure 5.** Funnel plot of the sources, including the data on the content of iron in reindeer (*Rangifer tarandus*) meat in different geographical regions.

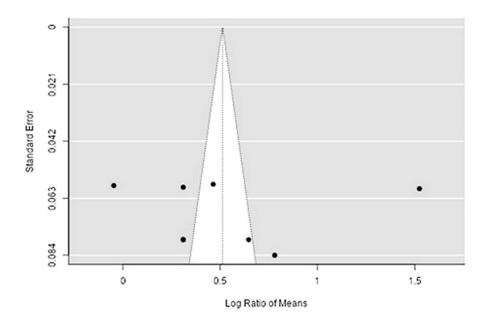
### 3.2.3. Zinc

Data on the content of zinc in reindeer meat were available in 11 studies. The standardised mean differences ranged from -0.05 to 1.52, with most ratings being positive (89%). The estimated standardised mean difference based on a random-effects model was 0.51 (95% CI: 0.22–0.80). Thus, the mean value was significantly different from zero (z = 3.45, p < 0.0006) (Table 2, Figure 6).



**Figure 6.** Forest plot of the sources, including the data on the content of zinc in reindeer (*Rangifer taran-dus*) meat in different geographical regions.

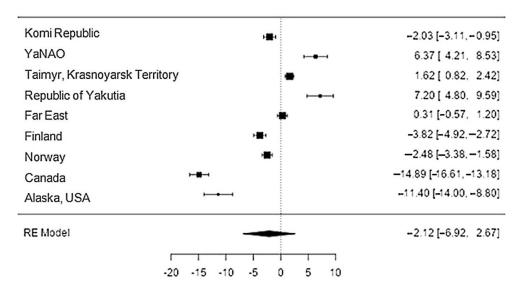
The *Q*-test confirmed the heterogeneity of the sources, including the data on the content of zinc in reindeer (*Rangifer tarandus*) meat (Q(8) = 429.42, p < 0.0001, tau<sup>2</sup> = 0.194, I<sup>2</sup> = 97.67%). The 95% interval was from -0.399 to 1.42. Publication bias was explored with a visual inspection of the funnel plot (Figure 7), where the rank correlation and regression tests were p = 0.45 and p = 0.92, respectively (Table 3).

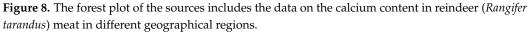


**Figure 7.** Funnel plot of the sources, including the data on the content of zinc in reindeer (*Rangifer tarandus*) meat in different geographical regions.

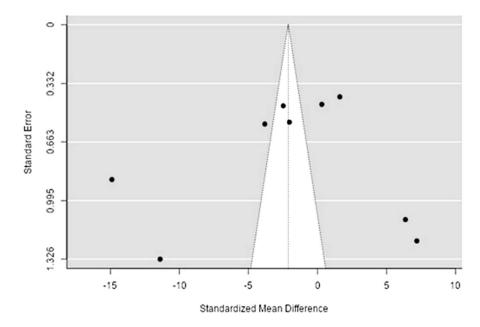
### 3.2.4. Calcium

Data on calcium content in reindeer meat were available in 11 studies. The standardised mean differences ranged from -14.9 to 7.2, with most ratings being negative (56%). The estimated standardised mean difference was -2.1 (95% CI: -6.92-2.67) based on a random-effects model. Thus, the mean value was significantly different from zero (z = -0.87, p = 0.39) (Table 2, Figure 8).





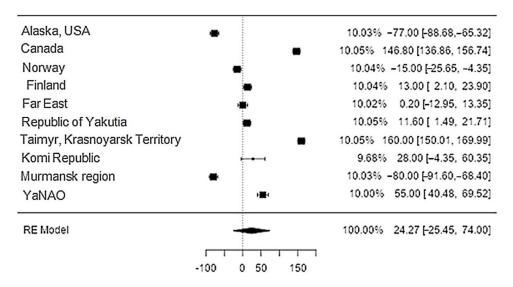
The *Q*-test confirmed the heterogeneity of the sources, including the data on the content of calcium in reindeer (*Rangifer tarandus*) meat (Q(8) = 488.35, p < 0.0001, tau<sup>2</sup> = 53.18, I<sup>2</sup> = 99.3%). The 95% interval was from -17.2 to 12.96. Publication bias was explored with a visual inspection of the funnel plot (Figure 9), where the rank correlation and regression test did not reveal any asymmetry in the funnel plot (p = 0.26 and p = 0.89, respectively) (Table 3).



**Figure 9.** The funnel plot of the sources includes the data on the calcium content in reindeer (*Rangifer tarandus*) meat in different geographical regions.

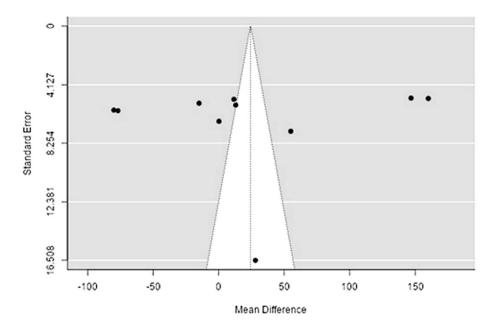
### 3.2.5. Potassium

Data on potassium content in reindeer meat were available in 11 studies. The standardised mean differences ranged from -25.45 to 73.99, with most ratings being negative (70%). The estimated standardised mean difference was 24.3 (95% CI: -25.45-73.99) based on a random-effects model. Thus, the mean value was significantly different from zero (z = 0.96, *p* = 0.34) (Table 2, Figure 10).



**Figure 10.** Forest plot of the sources, including the data on potassium content in reindeer (*Rangifer tarandus*) meat in different geographical regions.

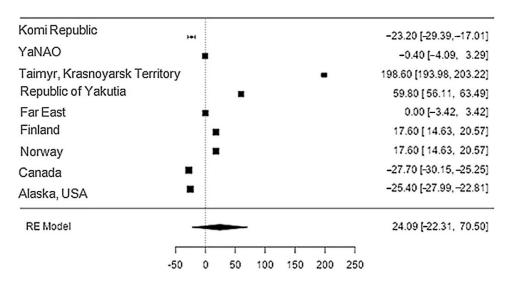
The *Q*-test confirmed the heterogeneity of the sources, including the data on the content of potassium in reindeer (*Rangifer tarandus*) meat (Q(9) = 1970.58, p < 0.0001, tau<sup>2</sup> = 6378.65, I<sup>2</sup> = 99.44%). The 95% interval was from -164.4 to 161.95. Publication bias was explored with a visual inspection of the funnel plot (Figure 11), where the rank correlation and regression tests were p = 0.48 and p = 0.88, respectively (Table 3).



**Figure 11.** Funnel plot of the sources, including the data on potassium content in reindeer (*Rangifer tarandus*) meat in different geographical regions.

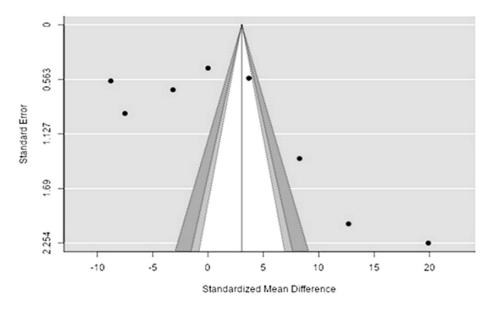
### 3.2.6. Sodium

Data on the content of sodium in reindeer meat were available in 11 studies. The standardised mean differences ranged from -27.7 to 198.6, with most ratings being negative (44%). The estimated standardised mean difference was 24.1 (95% CI: 22.31–70.5) based on a random-effects model. Thus, the mean value was significantly different from zero (z = 1.02, *p* = 0.31) (Table 2, Figure 12).



**Figure 12.** Forest plot of the sources, including the data on sodium content in reindeer (*Rangifer taran-dus*) meat in different geographical regions.

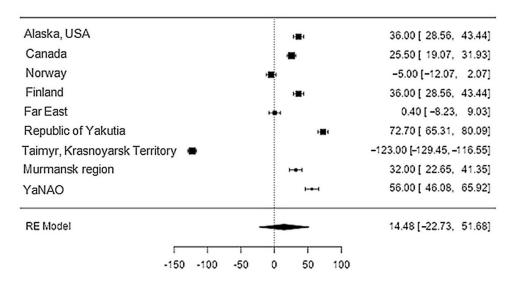
The *Q*-test confirmed the heterogeneity of the sources, including the data on the content of sodium in reindeer (*Rangifer tarandus*) meat (Q(8) = 8955.85, p < 0.0001, tau<sup>2</sup> = 5041.41, I<sup>2</sup> = 99.94%). The 95% interval was from -122.6 to 170.8. Publication bias was explored with a visual inspection of the funnel plot (Figure 13), where the rank correlation and regression tests were p = 0.14 and p = 0.46, respectively (Table 3).



**Figure 13.** Funnel plot of the sources, including the data on sodium content in reindeer (*Rangifer taran-dus*) meat in different geographical regions.

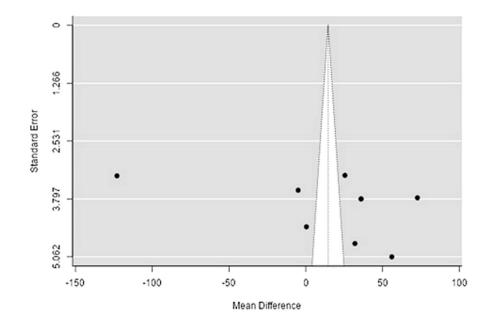
### 3.2.7. Phosphorus

The data on phosphorus content in reindeer meat was available in 11 studies. The standardised mean differences ranged from -27.7 to 198.6, with most ratings being positive (78%). The estimated standardised mean difference was 14.5 (95% CI: -22.7 to 51.7) based on a random-effects model. Thus, the mean value was significantly different from zero (z = 0.763, *p* = 0.45) (Table 2, Figure 14).



**Figure 14.** Forest plot of the sources, including the data on phosphorus content in reindeer (*Rangifer tarandus*) meat in different geographical regions.

The *Q*-test confirmed the heterogeneity of the sources, including the data on the content of phosphorus in reindeer (*Rangifer tarandus*) meat (Q(8) = 2146.4, p < 0.0001, tau<sup>2</sup> = 3227.16, I<sup>2</sup> = 99.54%). The 95% interval was from -102.9 to 131.9. Publication bias was explored with a visual inspection of the funnel plot (Figure 15), which did not present significant asymmetry: The rank correlation and regression tests were p = 0.34 and p = 0.19, respectively (Table 3).



**Figure 15.** Funnel plot of the sources, including the data on phosphorus content in reindeer (*Rangifer tarandus*) meat in different geographical regions.

### 4. Discussion

This meta-analysis has expanded the knowledge of the composition of reindeer meat in different Arctic regions. The main findings of our research showed that the highest concentration of macro- and microelements is present in reindeer meat of the following Arctic regions: Magnesium—in Taimyr, Yamal-Nenets Autonomous Okrug, Canada; iron—in Taimyr, Republic of Yakutia, Canada; zinc—in Taimyr, Komi Republic, Norway; calcium—in Yamal-Nenets Autonomous Okrug, Republic of Yakutia, Taimyr; potassium in Canada, Taimyr, Yamal-Nenets Autonomous Okrug; sodium—in Taimyr, Republic of Yakutia; phosphorus—in Republic of Yakutia, Alaska, Finland. Different proportions of macro- and microelements in reindeer meat can be a pre-condition for discussing the possible correlation between the value (nutritious and biological) and price of reindeer meat in different Arctic regions.

Different content of the macro- and microelements in the Arctic regions can be explained by ecosystems and anthropogenic (economic and industrial differentiation) factors. For example, a higher concentration of magnesium, calcium, potassium, and sodium in the Arctic regions with a harsh climate can be the outcome of a longer period of eating lichens and rags of vascular plants as a result of a long snow season. This is probably due to the higher concentration of trace elements in lichens and scrubs than green plants [110]. Higher concentrations of iron in Taimyr, the Republic of Yakutia, and Canada are probably associated with the regional features of iron accumulation in acidified soils and the high content of this trace element in the surface waters of these Arctic regions [111]. The high zinc content in Taimyr, Komi Republic, and Norway is possibly due to anthropogenic pollution caused by mining and processing polymetallic ores containing zinc [112]. However, the increased zinc content may also be of natural origin [113]. The phosphate content is probably related to the geochemical features of the soils [114]. In comparison, the soils of the Alaska and Finland regions contain more phosphates available for plants [115]. However, the primary source of the macro- and microelements in reindeer meat is their nutrition.

The supply of metals largely depends on their content in the surface layer of the soil [116]. Plants accumulate chemical compounds from the surface layer of the soil, which is typical for most of the territory of the Kola Peninsula, the Arkhangelsk Region, and the Nenets Autonomous Okrug and, to a lesser extent, with a decreasing trend in metal

concentrations in the Yamal-Nenets Autonomous Okrug and the Republic of Yakutia [117]. Zinc belongs to the elements of strong biological accumulation [118,119], so the increase in the concentration of this element in soils is strongly associated with the processes of accumulation in plants (e.g., in Western Siberia [120,121]). Consequently, zinc entry with plant litter into the soil is very intensive.

Reindeers' diet consists of lichens, mosses, and vascular plants, accumulating significant amounts of metals and metalloids [79,122,123]. Therefore, the considerable variation of reindeers' habitat causes significant differences in the reindeer's diet. For example, the macro- and microelement composition of venison is influenced by the species composition of plants and lichens and the content of trace elements in them, the duration of grazing seasons on summer pastures, the proportion of green fodder, shrubs, lichens, mushrooms, eggs of birds, and rodents, the macro- and microelement composition of soil and water, the presence of pollution, the availability of salty seawater, cutting antlers, etc. The rich diet of *Reindeer tarandus* is also explained by specific seasonal migration to areas with different forage resources. Summer pastures are rich with herbaceous plants and shrubs. In contrast, winter pastures have many lichens.

The reindeer consumes 44 shrub willows and birches, 94 species of sedges, 52 species of cereals, 24 species of legumes, and 170 species of other plants [121]. Lichens are an essential and rich part of the reindeer's diet, especially in wet and frosty seasons (mainly in winter). So, on the territories located in the Arctic tundra zone (i.e., the northern part of the Yamal-Nenets Autonomous Okrug), as the significant part of the reindeer's ratio, lichens dominate most of the year [124–126]. In venison, it results in a high concentration of iron and zinc (important elements of antioxidant systems and cytochromes of the respiratory cell chain). The concentration of many trace elements in lichens is generally higher than in bryophytes, ferns, conifers, shrubs, and grasses [110]: Lichens accumulate more Co, Ni, Mo, Au, Mg, Ca, Zn, Cd, Sn, and Pb compared with other plants in the Arctic region [127]. Due to the lack of a root system and obtaining most minerals with precipitation (snow, rains), the concentration of trace elements in lichens highly depends on the transboundary transfer of trace elements and the amount of precipitation [128]. So, in more southern and western regions of Eurasia, less magnesium and calcium are accumulated in lichens than in the eastern and northern areas due to a large amount of precipitation during the snowless period [127]. The accumulation of trace elements by lichen also depends on its type and geographical location [129], i.e., woody lichens accumulate less zinc than bushy lichens (e.g., Cetraria, Cladonia) [130].

Moss, quickly accumulating metals, is the dominant form of vegetation in Arctic tundra ecosystems [122,131]. Sea aerosol is an additional source of elements including sodium, lead, mercury, and caesium [123,132]. Some of the elements are accumulated efficiently in mosses (e.g., Cd, Co, Cr, Cu, Fe, Mn, and Zn) [122], the Zn-Cd-Cu-Mn and Mo element correlation may be explained by their dietary intake from moss tundra. Compared to other Arctic regions and Canada, the values of most trace elements in the soils of the Yamal-Nenets Autonomous Okrug is higher (except Pb, Fe, and Mn) [133]. It can impact their transition to venison and increase the nutritious value of the reindeer meat in this Arctic region.

While mosses, lichens, and shrubs mostly accumulate cationogenic elements, herbaceous plants do it with anionic ones [111]. In the northern subarctic tundras, Zn, Nb, P, Mn, and Cu are actively accumulated [134]; in the middle and southern tundras, there are Zn, P, and Mn, and in the low northern subarctic tundras close to the coastal areas, the spectrum of elements is much more comprehensive than on the uplands of the continent [111].

Sedges and grasses and cereals (e.g., arctophile, bluegrass, arctagrostis, reed grass) dominate in the reindeer's diet (over 50% in early autumn; over 40% in early autumn) during the snowless period [135], and they actively accumulate Cu, Zn, and Pb [111]. In winter, especially with a lack of lichen forage, the rags of these plants can make up even more than 60% of the reindeer's diet.

The source of zinc, silver, lead, manganese, and barium for a reindeer is vaginal fluffy (Erióphorum vaginátum), a valuable nutritious food in winter and spring [136,137]. The accumulation of these trace elements depends not only on the composition of the substrate but also on the acidity of the soil [138]. Variegated and reed horsetails included in the reindeer's diet in early spring and autumn, as well as field horsetail, marsh horsetail, marsh horsetail all year round [135], also contribute to enriching reindeer meat with manganese, silicon, and iron [111].

The high content of zinc and copper in reindeer meat can also result from consuming leaves of willows (gray willow, filiform willow, spear-shaped willow, ferruginous willow, Lapland willow, beautiful willow) and low and white birch. In early summer, the leaves of shrubs can provide up to 30% of the reindeer's diet (over 90% of them are willow leaves) [135]. Yernik and willow have the maximum accumulation of zinc [120].

Upon consuming blueberries, lingonberries, cloudberries, bearberries, crows, and rowan berries, a reindeer accumulates zinc, iron, and magnesium [134]. Likewise, mush-rooms bring zinc, selenium, lead, copper, strontium, and mercury in a reindeer's diet [139]. While grazing, a reindeer can also eat birds' eggs, lemmings and voles, rodent nests, and frozen fish, covering the deficiency of such trace elements as calcium, potassium, phosphorus, sodium and zinc [140].

The knowledge of the macro- and microelement content of reindeer meat can help develop dietary programmes to manage the health risks of Arctic residents. The concentration of valuable trace elements necessary for adaptation in the Arctic is much higher in venison than other meat types. In north-eastern Canada, Kuhnlein H.V. et al. (1996) proved that consuming traditional food (venison) results in receiving more phosphorus, iron, zinc, and magnesium compared with imported products [20]. According to Bogdan E.G. and Turshuk E.G. (2016), S.V. Andronov, and A.A. Lobanov et al. (2017), venison is rich in macro- and microelements, has high nutritional and biological value [37,141].

Some researchers recommend widely using reindeer products to increase human resistance to unfavourable environmental factors in the diet [41,141–144] because reindeer meat is especially rich in calcium, phosphorus, potassium, sodium, magnesium, iron, and zinc. The high phosphorus, magnesium, potassium, and iron content in venison provides its high efficiency for increasing adaptation to cold stress and geomagnetic activity in the Arctic [145,146]. A diet enriched with reindeer products significantly increases the antiatherogenic fraction of blood lipids, prevents overweight, atherosclerosis, and heart disease [37,144], and improves microcirculation, tissue fluid exchange, and the body's antioxidant defence against free radicals [6]. A sufficiently large amount of trace elements (iron, zinc) contained in venison can help to prevent acute infectious diseases and provide antioxidant protection of the human body from free radicals [91,102]. This explains the high efficiency of adaptation to cold stress, as well as increased prophylactic activity during hypothermia [7,8].

The important contribution of reindeer meat and its macro-nutrients towards adaptation was acknowledged in Nordic countries. According to the Nordic nutrition recommendations, reindeer meat as game meat does not present the epidemiological evidence shown with high consumption of processed or red meat increasing the risk of colorectal cancer, type-2 diabetes, obesity, and coronary heart disease [147,148].

Our study had some limitations. First, the reindeer habitat in the Arctic is huge, therefore we had to present a less-detailed analysis for some regions. Second, a number of published studies included in the analysis are characterised by heterogeneity. In our meta-analysis, we used random effects models; so, a high level of heterogeneity (>80.0%) could impact the reliability. Third, there were a number of variations in the studies that were analysed: The quality, research methods, observation period, etc. Finally, selection bias is possible because observational studies were used in this meta-analysis.

The strengths of our study are associated with the implementation of a complex approach to systematising information on the mineral composition of reindeer meat in different Arctic regions. The meta-analysis has wide geographical coverage. A comprehensive and robust search strategy was designed to avoid the loss of relevant research. Moreover, there were no studies excluded for linguistic reasons to avoid linguistic bias. In addition, routine tests and visual inspection of the funnel plot plots did not reveal any evidence of a risk of publication bias.

### 5. Conclusions

The meta-analysis revealed that the indicators of the content of trace elements in reindeer meat had a high variability depending on the geographical region. The ecosystems and anthropogenic factors strongly impacted the macro- and microelements composition of reindeer meat in different Arctic regions. In the Russian Arctic regions with the most severe climatic conditions (especially, Taimyr, Yamal-Nenets Autonomous Okrug, and the Republic of Yakutia) and Canada, venison has the highest mineral saturation, and therefore, higher nutritious and biological value due to enriched biodiversity and the rich fodder base for reindeer. This makes reindeer meat an effective means of preventing obesity and adapting to cold due to the content of a complete set of essential trace elements and amino acids. The high content of iron and zinc in reindeer meat increases the body's antioxidant defence against free radicals and helps to prevent chronic non-infectious diseases. Ultimately, future research could compare the differences in the content of macro- and microelements in venison and other types of meat in the Arctic to prove its higher biological value.

A unique macro- and microelement composition of reindeer meat also proves its economic value and will be important for nutritional policy makers in the Arctic regions. This is a good pre-condition for the negotiation of fair prices for reindeer meat exported from this region based on the balance of the nutritious/biological value and price. It contributes to increasing the profitability of reindeer herding in the Arctic regions and maintaining this significant traditional livelihood of the Indigenous Peoples.

**Author Contributions:** Conceptualisation, A.L. and I.K.; methodology, A.L.; software, S.A.; validation, E.B. and A.Y.; formal analysis, S.A. and E.B.; investigation, A.L.; resources, S.A.; data curation, A.P.; writing—original draft preparation, E.B., S.A. and A.L.; writing—review and editing, A.Y.; revising, D.R. and O.S.; visualisation, S.A.; supervision, A.L.; project administration, E.B.; funding acquisition, E.B. and O.S. All authors have read and agreed to the published version of the manuscript.

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Informed Consent Statement: Not applicable.

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Conflicts of Interest: The authors declare no conflict of interest.

# Appendix A

# Table A1. PRISMA Checklist \*.

Section/Topic	ŧ	CRECKLIST RETI	Keported on Page #
		TITLE	
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
		ABSTRACT	
Structured summary	ы	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	1
		INTRODUCTION	
Rationale	e	Describe the rationale for the review in the context of what is already known.	1–3
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	2-3
		METHODS	
Protocol and registration	ß	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	N/A
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	3-4
Information sources	4	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	3-4
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	3-4
Study selection	6	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	3-4
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	4-5
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	4–5
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	5
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	ы
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I <sup>2</sup> ) for each meta-analysis.	ы
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	5, Appendix B
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	N/A

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Table A1. Cont.

RESI         17       Give numbers of studies screened, assessed for eligibit         18       For each study, present characteristics for which d         19       Present data on risk of bias of each         20       For all outcomes considered (benefits or harms), present         21       Present results of each meta-anal         22       Present results of each meta-anal         23       Give results of additional analyses, if         24       Summarize the main findings including the strength         25       Discuss limitations at study and outcome level (e.g., ris         26       Provide a general interpretation of the		we putted unit age #
17       Give numbers of studies screened, assessed for eligibil         18       For each study, present characteristics for which d         19       Present data on risk of bias of ea         10       20       For all outcomes considered (benefits or harms), presen         21       Present results of each meta-anal         22       Present results of each meta-anal         23       Cive results of additional analyses, if         24       Summarize the main findings including the strength         25       Discus limitations at study and outcome level (e.g., ris         25       Discus limitations at study and outcome level (e.g., ris         26       Provide a general interpretation of the	RESULTS	
18For each study, present characteristics for which d19Present data on risk of bias of ea20For all outcomes considered (benefits or harms), presen conf21Present results of each meta-anal22Present results of each meta-anal23Give results of additional analyses, if DISCU24Summarize the main findings including the strength25Discuss limitations at study and outcome level (e.g., ris Provide a general interpretation of the PUNI	eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	5-6
19Present data on risk of bias of ea20For all outcomes considered (benefits or harms), presen conf21Present results of each meta-anal22Present results of each meta-anal23Cive results of additional analyses, if24Summarize the main findings including the strength25Discuss limitations at study and outcome level (e.g., ris26Provide a general interpretation of the27Provide a general interpretation of the	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	5-14
20For all outcomes considered (benefits or harms), present conf21Present results of each meta-anal22Present results of additional analyses, if23Cive results of additional analyses, if24DISCU25Discuss limitations at study and outcome level (e.g., ris26Provide a general interpretation of theFUNI	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	6-14
21 22 23 24 26 26	present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	6-14
22 23 24 25 26	ta-analysis done, including confidence intervals and measures of consistency.	6-14
23 24 25 26	Present results of any assessment of risk of bias across studies (see Item 15).	6–14
24 25 26	'ses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	N/A
24 25 26	DISCUSSION	
25 26	ength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	14–17
26 Provide a general interpretation of the FUNI	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	16–17
FUND	of the results in the context of other evidence, and implications for future research.	17
	FUNDING	
Funding 27 Describe sources of funding for the systematic rev	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	17
* According to [46].		

## Appendix B

 Table A2. Quality Criteria Checklist \*.

Quality Criteria checklists	[3]	[85]	[88]	[89]	[84]	[90]	[91]	[92]	[39]
Year	2019	2019	2010	2011	2014	2016	2017	2019	2019
Relevance questions									
1	Yes								
2	Yes								
3	Yes								
4	N/A								
Validity Questions									
1	Yes								
2	Yes								
3	Yes								
4	N/A								
5	Yes								
6	Yes								
7	Yes								
8	Yes								
9	Yes								
10	Yes								
Quality Rating (+,0,-)	+	+	+	+	+	+	+	+	+
Quality Criteria checklists	[19]	[21]	[22]	[98]	[95]	[96]	[74]	[97]	
Year	1992	2000	2002	2002	2012	2012	2012	2013	
Relevance questions									
1	Yes								
2	Yes								
3	Yes								
4	N/A								
Validity Questions									
1	Yes								
2	Yes								
3	Yes								
4	N/A								
5	Yes								
6	Yes								
7	Yes								
8	Yes								
9	Yes								
10	Yes								
<b>Quality Rating</b> (+,0,-)	+	+	+	+	+	+	+	+	

07] 95 es es es es es es es es es es	[108] 1997 Yes Yes N/A Yes Yes Yes Yes Yes Yes Yes Yes Yes Yes	[109] 2007 Yes Yes Yes N/A Yes Yes Yes Yes Yes Yes Yes Yes Yes Yes	[102] 2010 Yes Yes N/A Yes Yes Yes Yes Yes Yes Yes Yes Yes Yes	[103] 2011 Yes Yes N/A Yes Yes Yes Yes Yes Yes Yes Yes Yes Yes	[104] 2014 Yes Yes N/A Yes Yes N/A Yes Yes Yes Yes Yes Yes Yes	[105] 2020 Yes Yes N/A Yes Yes N/A Yes Yes Yes Yes Yes Yes Yes Yes	[106] 2021 Yes Yes N/A N/A N/A Yes N/A Yes Yes Yes Yes Yes N/A	
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ł	+				Yes	Yes	Yes	
		+	+					
3]				+	+	+	+	
	[100]	[27]	[87]	[38]	[101]	[99]	[93]	[94]
99	2006	2009	2009	2018	2019	2019	2020	2013
es	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
es	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
es	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
es	Yes	Yes	Yes	Yes	Yes	Yes	N/A	Yes
es	N/A	N/A	Yes	Yes	N/A	N/A	N/A	Yes
es	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
es	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
es	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
es	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
es	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
es	N/A	N/A	Yes	Yes	N/A	N/A	N/A	Yes
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Table A2. Cont.

\* According to [54].

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# Article Design and Development of an Instrument on Knowledge of Food Safety, Practices, and Risk Perception Addressed to Children and Adolescents from Low-Income Families

Sueny Andrade Batista <sup>1</sup>, Elke Stedefeldt <sup>2</sup>, Eduardo Yoshio Nakano <sup>3</sup>, Mariana de Oliveira Cortes <sup>4</sup>, Raquel Braz Assunção Botelho <sup>1</sup>, Renata Puppin Zandonadi <sup>1</sup>, António Raposo <sup>5,\*</sup>, Heesup Han <sup>6</sup> and Verônica Cortez Ginani <sup>1,\*</sup>

- <sup>1</sup> Department of Nutrition, University of Brasília, Brasília 70910-900, Brazil;
- subatistanutricionista@gmail.com (S.A.B.); raquelbotelho@unb.br (R.B.A.B.); renatapz@unb.br (R.P.Z.)
  <sup>2</sup> Departamento de Medicina Preventiva, Universidade Federal de São Paulo (UNIFESP),
  <sup>3</sup> Departamento de Medicina Preventiva, Universidade Federal de São Paulo (UNIFESP),
- São Paulo 04023-032, Brazil; elke\_nutri@hotmail.com
- <sup>3</sup> Department of Statistics, University of Brasilia, Brasilia 70910-900, Brazil; eynakano@gmail.com
- <sup>4</sup> Department of Food Science, Campus UMass Amherst, University of Massachusetts, Amherst, MA 01003, USA; mcortes@umass.edu
- CBIOS (Research Center for Biosciences and Health Technologies), Universidade Lusófona de Humanidades e Tecnologias, Campo Grande 376, 1749-024 Lisboa, Portugal
- <sup>6</sup> College of Hospitality and Tourism Management, Sejong University, Seoul 143-747, Korea; heesup@sejong.ac.kr
- \* Correspondence: antonio.raposo@ulusofona.pt (A.R.); vcginani@gmail.com (V.C.G.)

**Abstract:** In the fight against foodborne diseases, expanding access to information for different groups is needed. In this aspect, it is crucial to evaluate the target audience's particularities. This study constructed and validated an instrument containing three questionnaires to identify the level of knowledge, practices, and risk perception of food safety by low-income students between 11 and 14 years old. The following steps were used: systematic search of the databases; conducting and analyzing focus groups; questionnaires development; and questionnaires analysis. After two judges' rounds, the final version was reached with 11 knowledge items, 11 practice items, and five risk perception items. The content validation index values were higher than 0.80. The adopted methodology considered the students' understanding and perceptions, as well the appropriate language to be used. Besides, it allowed the development of questionnaires that directly and straightforwardly covers the rules set by the World Health Organization for foodborne disease control called Five Keys to Safer Food (keep clean; separate raw and cooked; cook thoroughly; keep food at safe temperatures; and use safe water and raw materials). Its use can result in a diagnosis for elaborating educational proposals and other actions against foodborne illness in the most vulnerable population.

Keywords: adolescents; children; food safety; hygiene practices; low-income; risk perception

### 1. Introduction

Even though the prevalence of outbreaks of foodborne disease (FBD) is underreported, it results in socioeconomic losses worldwide. In 2010, there were 600 million cases, with 420,000 deaths, caused mainly by agents of diarrheal disease. FBD represents a challenge for food security, human health, economic prosperity, agriculture, market access, tourism, and sustainable development.

Another important aspect is an imbalance in the disease's distribution with a higher incidence in vulnerable populations, either economically, due to comorbidities, or by the age group (children and the elderly). In addition to being more susceptible to disease complications, such as dehydration, these populations may be more exposed to disease-causing agents, mainly biological ones, viruses, parasites, and bacteria). Fungi and their

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**Copyright:** © 2021 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). associated toxins have also been characterized as a significant safety problem worldwide [1]. This fact may result from a lack of knowledge and an environment conducive to such agents' proliferation, such as inadequate conditions for handling and preserving food in the domestic environment [2].

A study carried out by Hadler et al. [3] identified this situation of greater vulnerability. Children and the elderly in poverty in the United States showed a higher incidence of Salmonellosis. The study highlighted the fact that 91.1% (n = 48.111) of the illnesses were acquired domestically. European Union data corroborate with the importance of domestic kitchens in the epidemiological scenario of FBD [4]. In Brazil, of the total number of FBD outbreaks reported between 2007 and 2017, 38.3% (n = 2922) of the cases occurred in homes, considered the places with the highest occurrence of FBD [5].

Responsibility for food safety must be taken together. Government agencies must fulfill their role in educating, informing, regulating, supervising, and ensuring fair and reliable business relationships. Besides complying with regulations and protecting food from contamination, producers from different sectors of the economy must provide consumers with clear and accurate information on handling food. Finally, consumers must recognize their role, following the relevant instructions, and adopting appropriate food hygiene measures [6,7].

Nevertheless, the high incidence of FBD in the home environment is possibly related to failures in adopting safe procedures during food handling. Individuals who handle food have a great responsibility in these cases, and they are considered the main ones responsible for foodborne outbreaks. Numerous studies have been developed to explore the food handler's involvement in controlling FBD [8–14]. These studies aimed at understanding these individuals' knowledge, practices, attitudes, behaviors, risk perception, and optimistic bias to develop diagnostic strategies that subsidize effective actions to face FBD. For this purpose, the studies use instruments developed for the target population according to the research proposal, such as specific questionnaires. They usually approach an adult audience, composed of food handlers from food services or consumers from these establishments.

Understanding such factors is essential since research related to consumers in general and food handlers demonstrated that unsafe handling practices are frequent, despite happening through an acceptable level of knowledge [8,9,15–20]. There is a need to cover other knowledge areas and age groups [8]. Children and adolescents are important targets for food safety training actions. They are at a stage where their cognitive structures reach their highest development level [21], considered crucial for developing knowledge, skills, and habits, including food safety. Therefore, with early intervention in young people's education, it is possible to influence future behaviors and habits before they are formed, benefiting the student himself, his family, and people who will be under their responsibility in the future. A solid foundation for healthy behaviors is then established [22–24].

Other aspects reinforce the importance of considering them, such as the great potential as agents of transformation [23–25], evidence of being ready to fulfill in the preparation of meals [17,22,26–29] and finally, a tendency to feel more confident in their perceptions of personal risk [24].

Despite concrete evidence about this group's important role in the area, the developed studies are more dedicated to adult consumers and their food handling practices. The fact reveals the need for more significant investments in studies and actions with this audience [28,30].

Studies directed to understand the difference in the socioeconomic status of risk factors for FBD are essential. Mainly considering the large proportion of FBD originating from the domestic environment [4,5,31,32] and the greatest vulnerability of populations in poverty situations. In this way, it will be possible to strengthen the individual role, including economically less affluent classes, as an essential link in the food supply chain [2,3].

When considering socioeconomic aspects, the studies point out significant factors that impact knowledge and the adoption of good food safety practices, such as level of education, place of residence [33], and location of the school (rural or urban), the latter when it comes to children and adolescents [34]. Other factors, such as low income and low awareness, contribute to a lesser willingness to adhere to an environment compatible with food safety in developing countries. In these countries, the difficulty of access to drinking water, safe cooling, fuel cost for cooking or adequate reheating of food, high environmental temperatures, inadequate sanitation, and low education levels discourages adopting appropriate practices [35–37].

Therefore, the connection between food safety education, school subjects, and the home environment becomes essential [38]. However, it is observed that studies with individuals in the school stage [8–14] present heterogeneity of methodologies, often adapted from other audiences, and not all of them present evidence of validation. They do not focus on economically vulnerable populations, and there may be bias not being applicable in adverse realities. Pawlowski [39] emphasizes that an instrument's development must necessarily include the target audience to formulate health programs and policies based on reality. In this case, challenges inherent to low-income individuals need to be considered to ensure that their specificities are addressed [39].

Interventions for younger consumers and investment in consumer education can reduce FBD, and savings are expected of up to ten dollars for every dollar invested [40]. In this sense, this research aims to construct and validate a structured questionnaire to assess knowledge, practices, and risk perception related to food safety in low-income students aged 11 to 14 years. The results serve as a starting point to design educational actions that aim to improve the population's food safety in which food challenges are faced daily.

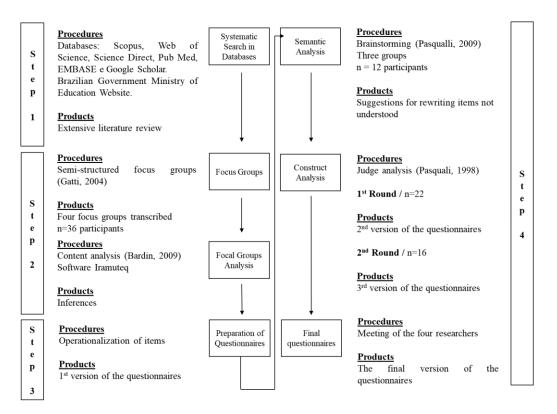
### 2. Materials and Methods

This exploratory research was conducted in the Federal District (FD)—Brazil and it was approved by the Ethics Committee of the College of Health Sciences of the University of Brasilia—CEP/FS UnB (CAAE n° 02033218.0.0000.0030). To participate in the study, three schools were selected, by convenience criteria, within a sample of the Project "Healthy eating and the school food production chain: what happens in public schools in the Federal District (FD)". The schools selected classes according to the schedule availability. The students' participation took place by presenting the terms of consent and free and informed consent.

In the form of three questionnaires, the instrument was developed for students aged 11 to 14 years. The environment of public schools was chosen to meet the criterion of low income since, according to the Programme for International Student Assessment—2015 (PISA) [41], this target audience has the lowest ESCS (Index of Economic, Social, and Cultural Status) in Brazil.

A quality instrument's development requires well-defined steps and rigorous procedures that guarantee reliable indicators [39,42]. The methodology chosen for the study recommends a process with the inclusion of specific tasks and methods carried out in a determined time sequence. These steps indicate the instrument's psychometric strength and the guarantee of the constancy of its content with the constructs.

Thus, the development of the instrument was based on the model described by Pasquali [43]. The theoretical procedures respected the following steps: (i) systematic search in the databases; (ii) conducting and analyzing focus groups; (iii) questionnaires development; and (iv) questionnaires analysis (Figure 1).



**Figure 1.** Theoretical procedures for constructing the questionnaires for assessing knowledge, practices, and risk perception related to food safety for low-income students (Federal District- Brazil).

### 2.1. Systematic Search in Databases

In addition to the database registered in Figure 1, the following documents were used to design the preliminary version of the questionnaire: Brazilian food safety resolutions based on texts from Codex Alimentarius [44]—RDC 216 and IN 16 [45,46]; *Base Nacional Comum Curricular* (BNCC)- Common Base National Curriculum [47], Protection Motivation Theory [48], Five Keys to Safer Food [49], and instruments developed by Da Cunha, Stedefeldt and De Rosso [50], De Andrade et al. [8], and Haapala and Probart [17].

### 2.2. Focus Groups (FG) Development and Analysis

Theoretical procedures, the focus of this study aims to obtain validity guaranteed by semantic and construct analyses. Thus, it was necessary to define the instrument's properties, using the focus group methodology [51]. The method offers the possibility of examining students' understanding and perceptions about food safety and verifying the appropriate language to address the target audience. It considers the view of different subjects and social contexts for less accessible information, which in many cases can only be raised through qualitative techniques [18,51,52].

Structured with an average of nine participants per group, 40 min in length, and heterogeneous concerning sex, the FGs were held in schools in the urban and rural areas of the public-school system in FD, which serves the low-income population [41]. The focal group conduction was carried out by a moderator with two transcribers' participation, following a pre-established script of 21 questions contemplating the Five Keys to Safer Food (1—keep clean; 2—separate raw and cooked; 3—cook thoroughly; 4—keep food at safe temperatures; and 5—use safe water and raw materials) [49].

The focus groups were transcribed and analyzed with the content analysis technique and by the IraMuTeQ Software (Interface de R pour les Analyses Multidimensionnelles de Textes et de Questionnaires) 0.7 alpha [53,54], aiding in the questionnaires development. The software's used followed the instructions in the manual provided by Camargo and Justo [55], and Descending Hierarchical Classification (DHC) analysis was performed.

### 2.3. Questionnaires Development

In preparing the items, the importance of assessing the knowledge, practices, and risk perception was considered. The transmission of the knowledge with low application in the practical context, associated with the low perception of risk, possibly results in unsafe practices [56]. From this premise, the dimensions assessed were defined.

Determining knowledge and food safety practices among consumers, in general, are of scientific interest, as exemplified by a study by Meysenburg et al. [18]. The study shows that unsafe handling practices are frequent, although they happen through an acceptable level of knowledge [18]. The repetition of these practices points to a significant gap that can be filled by developing educational actions capable of altering the handler's risk perception [8].

Risk perception plays a role in risk management and control in anticipating crises and supporting people in prevention strategies [57]. Another vital phenomenon included and considered in the risk perception was the optimistic bias [58]. The concept of incorporated optimistic bias is represented by the belief that individuals, who in most conditions, visualize their chances of experiencing health and safety problems as inferior to those of their peers (co-workers, friends, and family) [8,59,60].

Therefore, investigating the relationship between practice and knowledge, permeated by risk perception, will contribute to the elucidation of educational strategies aimed at an age group with little knowledge in this field [28,30].

Three possible responses were used to measure the "knowledge" construct, "yes", "no", and "I do not know". One point was assigned for each correct answer, and zero points were assigned to the wrong answer or "I do not know" [16]. Concerning the "practices" construct, a three-point frequency scale was used—"never", "sometimes", and "always".

As for the "risk perception" construct, the first version's Likert scale was based on a study developed by Swaney-Stueve et al. [61]. The study proposes a new model of pictorial scale—the emoji scale. Thus, a five-point scale was associated with emojis, ranging from extremely low to extremely high.

### 2.4. Questionnaires Analysis

### 2.4.1. Semantic Analysis

The validity procedures started with the semantic analysis of the first version of the instrument, containing three questionnaires, separately addressing knowledge, practices, and risk perception. This stage was developed with the lowest level of skill of the target population: students aged between 11 and 12 years in a FD school. The "brainstorming" technique was used, and the steps described by Pasquali [43] allowing student participation with suggestions for reformulating issues that are not understood. It was performed with this age group to ensure that the items were understandable to the group with the lowest skill, and therefore understandable for the most skilled group.

### 2.4.2. Construct Analysis (Round 1 and 2)

For construct validation, 22 specialists were available to analyze the first version of the instrument. Of these, 16 participated in the analysis of the second version. For the selection of judges, at least one of the following items was used: (i) minimum master's degree or, (ii) research development in the areas of the studied constructs or, (iii) performance in the area of food safety or, (iv) performance in the area of school meals. This validation aimed to verify the adequacy of the latent attribute(s) behavioral representation, providing information on the representativeness and clarity of each item of the questionnaires [43].

Initially, experts received the necessary information and guidance on the instrument's evaluation process, including possible participation in other rounds. All instructions for filling out the Consent Form were provided. After consent, they could access the instrument to facilitate its full view and the link that directed them to the SurveyMonkey<sup>®</sup> platform.

The items were evaluated for simplicity, clarity, relevance, credibility, variety, and evaluation of the corresponding construct. The knowledge questionnaire was also assessed for balance and the Likert scales to measure responses adequately. For the correct completion of the evaluation, the constructs' constitutive definition and the items' adequacy criteria were provided. After the judges' participation, their answers and considerations were transcribed to the Microsoft Office Excel<sup>®</sup> 2007 program.

The Content Validation Index (CVI) was used to evaluate the judges' contributions [62], which allows observing the judges' level of agreement on each item. The item's continuity was limited to a CVI more significant than or equal to 80% ( $\geq$ 0.80) [43]. Three types of CVI were applied: Item-CVI (I-CVI), Questionnaire-CVI (Q-CVI), and Scale-level CVI (S-CVI). Thus, the first refers to the values obtained from each item that makes up the questionnaires according to the criteria of simplicity, clarity, relevance, credibility, and the possibility of evaluating the corresponding construct. The second refers to the values obtained referring to the balance criteria of the knowledge questionnaire and variety criteria of the three questionnaires. Finally, the third refers to the scales used to measure the responses to each questionnaire.

### 2.4.3. Elaboration of the Final Questionnaires

The final instrument was developed based on the answers obtained in the previous step. Additionally, the four researchers' consensus for the judges' positions was also considered, with three dietitians, and one statistician, all postgraduate.

### 3. Results and Discussion

The construction process resulted in an instrument with three questionnaires that separately contemplate each dimension studied, with 11 items of knowledge, 11 items of practice, and five risk perception items (Appendix A). The steps carried out allowed the development of an instrument that contemplates the main aspects of food safety covered in the Five Keys to Safer Food [49], within a perspective specific to the reality that will be applied. When considering the reality and particular challenges inherent to the studied public, successful communication on food safety is possible within a diagnostic perspective to carry out effective interventions [63]. For Slovic (1987), those who promote and regulate health must understand how people think and respond to risk. If there is no such understanding, the formulated policies may be ineffective.

The biggest challenge during the research was to make the language simple and straightforward. The same difficulty was reported in developing the "EQ-5D-Y" instrument for quality of life-related to children and adolescents' health. The instrument was an adaptation of the "EQ-5D-3L", intended for adults, to suit the new audience. The process resulted in changes, especially in the wording (including illustrations, titles, and response options), to optimize item comprehension and improve data quality [64,65].

In a study to assess the psychometric properties of a Japanese version of the Dutch questionnaire on eating behavior for children, there was a review process by teachers. The idea was to ensure that the final version covered the students' cognitive development and avoided any incomprehensible writing [66].

In this study, specialized judges in the education area were crucial due to the difficulty of adapting the language for children and adolescents. The contributions allowed to create an appropriate wording according to the audience's stage of cognitive development.

The results of the steps required to prepare the questionnaires are detailed below.

### 3.1. Search in Databases

The studies in the literature allowed us to obtain essential information for the subsequent steps. However, some points stood out for the characterization of the instrument. When writing the items, the students' ability to think logically was considered, according to Piaget's development in which they find themselves—formal operative [20] and Pasquali's [43] criteria for understandable and objective writing. The three questionnaires that make up the instrument had an item structure similar to that developed by Da Cunha, Stedefeldt and De Rosso [50], and De Andrade et al. [8]. The decision on using the Five Keys to Safer Food [49], based on the possible lack of information on the topic by students, proved to be easy to understand and familiar to students. Additionally, these are more disseminated and more accessible information to teachers and guardians of children. The complementary search at BNCC [47] was important to verify the syllabus required for students aged 11 to 14 years from the Brazilian public education network. From the information obtained, it was possible to establish the expected level of understanding of the topic.

The risk perception questionnaire was based on concepts from the World Health Organization [67], from ISO 31000:2009 [68], and a study developed by Slovic [57]. The inclusion of the optimistic bias [8,16,58,60] brought the possibility of broadening the discussion on children's topics. Some researchers suggest that children are also susceptible to the phenomenon of optimistic bias [60,69], but the scientific literature is still scarce.

The scale for measuring the risk perception questionnaire responses was based on the WHO document [2]. The risk perception questionnaire assessed the relationship between FBD symptoms and their severity and intensity based on the content used.

### 3.2. Conducting and Analyzing Focus Groups (FG)

The four FG (n = 36) allowed the language structure's adjustment, including in the questionnaires terms and forms of communication mentioned in the discussions. The study design made it possible to include different perceptions and knowledge since social inequalities exist between urban and rural scenarios. The integration of the two realities enabled the understanding of the universe experienced by students in different contexts.

The content analysis of the discussions that emerged in the focus groups identified the language that the public understands, the knowledge of greater and lesser mastery, the habits, and different views related to food safety. This information supported the construction of the items. The units of record (UR) evoked are shown in Table 1, where it was possible to observe the most discussed Keys to Safer Food by analyzing the statements related to each recording unit [49] and the most relevant evocations (Table 2).

Themes—Keys to	Units of Record	FG1		FG2		FG3		FG4		Total by UR	
Safer Food	Units of Record	F	%	F	%	F	%	F	%	F	%
Keep it clean	Hand hygiene/ Bacterial contamination	17	26	23	36	11	17	12	21	63	39
Separate raw and cooked foods	Cross-contamination	1	100	0	0	0	0	0	0	1	0
Cook the food well	Proper cooking	0	0	1	100	0	0	0	0	1	0
Keep food at safe temperatures	Food storage	2	20	2	20	1	10	5	50	10	6
Use safe water and	Sanitation of fruits and vegetables	0	0	2	8	15	62	7	30	24	15
safe raw materials	Safe/unsafe food	15	25	13	21	22	36	10	18	60	40

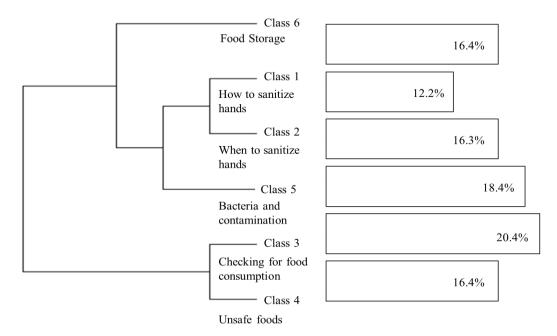
**Table 1.** Themes, units of record (UR), and relative and absolute frequencies of the evocations of the focus groups conducted with low-income students (n = 36) ages 11 and 12 (FD—Brazil).

**Table 2.** Keys to Safer Food and selected individual evocations related to the focus groups' food safety conducted with low-income students (n = 36) with school ages of 11 and 12 (FD—Brazil).

Keys to Safer Food	Evocations
Keep it clean	Hand hygiene/Bacterial contamination "If we do not wash our hands, we can infect the food, and we will eat the infected food, and the bacteria will infect us from the inside. It is always good to wash your hands if you touch the floor or something dirty." "Bacteria are tiny animals. If I touch the floor, it is full of bacteria there. Bacteria are everywhere." "You put the soap in your hand. We have to wash our hands up to the elbow. "
Separate raw and cooked foods	Cross-contamination "If you combine the moldy food with other foods, the clean one can get mold from the one that you put together."
Keep food at safe temperatures	Food Storage "You can keep the whole papaya in a warm place; when opened, you can store it in the refrigerator." "If we are going to save food to eat today or tomorrow, we will store it in the refrigerator. If we are going to save it for a week or later, it is better to save it in the freezer."
Use safe water and safe raw materials	Checking food for consumption "From the smell of fish if it is spoiled fish. Auntie, if it is food that comes in the bag, you can see the date on the label when it expires or because of the smell like beans." "There are some rotten. Others get softer sometimes." "When it expires. When it does not smell good. When the fly is climbing on top of that bad smell. Like milk. It stinks. The egg stinks when it gets rotten." "Expiration of food is when you want to eat something, and it has been in your cabinet for a long time, and you hardly eat it, and it has passed its expiration date, and if you eat it can give you a stomach ache or even kill the person." Safe/unsafe food "Unsafe food is fried, soda, fats, sweets, snacks." "Unsafe foods are those that have pesticides. If we eat food that has pesticides for several years, we will die. Get stomach pain and diarrhea." "Safe food is that there is no poison, that there is no animal." "Safe foods for me are those that do not have pesticides and those that we grow." "There are things that smell bad, but it is good to eat. Some look bad, but they are good. Some are bad, but they are good." Sanitation of fruits and vegetables "Some foods have to put on bleach, like lettuce and tomatoes. And there are some foods that we have to boil." "You have to soak the fruit, and then put a little bleach in it, then let it soak, to remove most of the microbes." "I do not eat a thing with bleach in my food." "Bleach? That is crazy, dude?"

The results obtained in the content analysis corroborate the findings from the DHC analysis of the IRaMuTeQ Software. From the DHC, the *corpus*, which is the text composed of the FG's coded transcripts, was categorized into six classes, named according to the central narrative addressed in the discussions (Figure 2).

The UR hand hygiene/bacteria contamination has the highest number of evocations (39%) in content analysis and DHC analysis (46.9%). Students demonstrated knowledge about the importance of hand hygiene and the frequency and way of performing the procedure, understand that the human body is a source of contamination for food, and associate it with bacteria.



**Figure 2.** Dendrogram of the descending hierarchical classification with the generated classes, the percentage value about the total of the analyzed corpus, and interclass relations of the focus groups conducted with 11-year-old low-income students (n = 36) (FD—Brazil).

In the BNCC [47] for students aged six to 14 years old in Brazil, these subjects are present in life and evolution. At the age of six, body hygiene habits (such as washing hands before eating) and their relationship to health maintenance should be discussed. At the age of nine, the student must have appropriate attitudes to prevent diseases associated with them, based on knowledge of transmitting some microorganisms (viruses, bacteria, and protozoa). Hand washing is a widely discussed topic, and students have more access to it. A study developed by Osei Tutu et al. [19] to assess students' knowledge and food safety practices, aged between seven and 21 years, showed that most students responded correctly about hand washing.

Then, the UR safe/unsafe food stood out (40%). Considering that the "three" and "four" classes resulting from the DHC correspond in content to this registration unit, it is noted that they stood out similarly (36.7%). Different perspectives were observed. When asked how to check when the food is fit for consumption, they responded by checking the expiration date on processed foods' packaging and the food's appearance and smell. Research with domestic food handlers has shown that some determine that food is safe because of its appearance or smell, both unreliable indicators for food safety [18].

There were differences between the participants. Many related the concept to nutritional terms for the concept of safe and unsafe food. A study developed by Gavaravarapu et al. [52] obtained similar results. Many teenagers initially confused the concept of safe food with nutritious food, listing many foods such as vegetables, cereals, milk, and meat as safe foods.

However, a large portion related the unsafe food to one that shows signs of deterioration, that is out of date and contains pesticides. The consequences of unsafe food consumption and the association between food consumption with pesticides and death were listed. Finally, in an urban school, the food was considered safe when planted and grown in the domestic environment, as there is no use of pesticides. It is noted that students have the perception that pesticides cause deleterious effects, listing symptoms such as stomach pain, diarrhea, headache, eye pain, and desire to sleep. The concern shown in the students' speeches regarding the importance of pesticidefree consumption comes in line with the significant and active role that consumers assume in the face of the movement towards achieving sustainable production and consumption. The United Nations' National Goals reinforce this movement for the Sustainable Development Goals (SDGs) [70] and the Food Guide for the Brazilian Population [71]. The Guide shows that an adequate and healthy diet derives from a socially and environmentally sustainable food system, encouraging the domestic cultivation of organic foods and exchanging between neighbors to expand access to a greater diversity of foods. Such practice leads to the production of tasty foods and protects the environment and health.

Consumers have been looking for organic foods, stimulated by health and sustainability benefits [72,73]. Consequently, children and adolescents are influenced by the environments in which they are part of. According to Bronfenbrenner's Ecological System Theory, different levels influence the construction of these individuals' lifestyles, emphasizing the importance that the environment has on them. Thus, family (parents, siblings), school (teachers, colleagues), shared activities, culture, history, customs, laws, and the economic system can influence their education and choices [74–76].

A relevant point was food consumption with signs of deterioration, common among rural school students. Thus, an instrument to investigate this habit is needed. It is noted that individuals who recognize that these foods are considered unsafe still consume such foods. PISA -2015 [41] points out that Brazilian students from rural schools had the lowest ESCS index. This lower socioeconomic level may imply less knowledge about the harms of spoiled food and the low availability of food inputs, leading to unsafe food consumption.

Furthermore, studies have pointed out that food and nutritional insecurity are more prevalent in rural areas, characterized by lower family income and low education, contributing to establishing this situation [67,77–80]. Similar data were found in a study conducted with students in China [9]. It was observed that a large majority knew that expired foods could not be consumed. Only a small number still thought that expired foods could be consumed after boiling or heating or as long as they looked good. In this case, the consumption of unsafe food, expired food, may be related to their belief that appearance is more important than the expiration date information.

The consumption of unsafe foods due to a deterioration process or expired dates causes short-term and long-term consequences. These can be chronic kidney diseases, neurological disorders, reactive arthritis, and irritable bowel syndrome. Foods containing mycotoxin are also worrisome due to their carcinogenic potential [81].

Note that the most explored keys were keeping clean and using safe water and safe raw materials in both analyses. However, topics that make up these keys, such as sanitation of the environment and utensils and drinking water, were not discussed in the focus groups. The keys: separate raw and cooked food, cook the food well, and keep food at safe temperatures obtained less space within the narratives. However, it was possible to observe cross-contamination knowledge involving fungi and food shelf life at different storage temperatures. The most debated topics are related to the direct and more usual handling of food, such as handwashing and checking food before eating. The absence of some topics and the relatively impoverished speeches of others can be attributed to the absence of more direct questions about the focus groups' subjects. Moreover, there are items related to cooking and cleaning responsibilities, which may not be part of their usual routines.

As for the sanitation of fruits and vegetables, the participants recognize the importance of adequate sanitation, but a large portion is unaware of the process that comprises three stages: washing, disinfection or sanitization, and rinsing. The disinfection or sanitization stage was the discussion point that caused the most divergence of opinions from bleach statements. This product has sodium hypochlorite as its active ingredient. This step is recommended by the Brazilian legislation [45,46], and the products must be regularized in the competent agency of the Ministry of Health, the National Health Surveillance Agency (ANVISA). Consumers have access to this information through the Food Guide for the Brazilian population [82]. Food handlers and consumers in a study developed by De Andrade et al. [8] asked whether washing vegetables and soaking them in water with vinegar is enough for this food to be safe for consumption. They obtained 57.8% and 40%, respectively. Given these data, it is inferred that the knowledge of Brazil's appropriate process may be a deficit in different audiences. Gavaravarapu et al. [52] showed that in the developed FGs, there was also recognition of the importance of cleaning fruits and vegetables and reported that raw foods should be washed carefully before consumption. However, there is no report on how hygiene should be carried out.

Finally, the concepts of gravity [67] and vulnerability [48] became evident in the evocations.

"Unsafe food can cause illness, even death, diabetes, infection, a coffin."

"The child gets more ill when he eats something bad; his intestines are more sensitive; the intestines of the adult are firmer."

### 3.3. Questionnaires Development (Questionnaires Analysis)

From the information obtained with the previous steps, the first version of the instrument was prepared. The questions were distributed among the three dimensions: 12 items of knowledge, 12 practice items, and 10 risk perception items.

Three sessions were held for the semantic analysis stage, with four students each, heterogeneously concerning sex. A construct was worked on in each session. The terms that students understood poorly were replaced by suggestions made by them or by other terms used during the explanation in this step. It was noted that very long questions hinder the correct understanding of the item. Subsequently, it was found that such questions did not meet the criteria of simplicity and clarity [43].

In the construct analysis stage, 22 judges agreed to participate, 59% have a master's degree (n = 13), 32% have a doctorate degree (n = 7), 4.5% post-doctorate degree (n = 1) and 4.5% specialization (n = 1). The judges' group comprised a pedagogue and a psychopedagogue to contribute specifically to the language's adequacy.

In the first round of the construct analysis, 28 items were approved (82.4%); they presented I-CVI values  $\geq$  of 0.80. It is noted that all items have relevance and credibility. The variety criterion was met in the three questionnaires with a mean Q-CVI = 0.94. As for the balance criterion, Q-CVI = 0.91 was obtained. The average of the I-CVI values for each item in each questionnaire is shown in Table 3. It should be noted that all items, regardless of approval, were adjusted for clarity and simplicity.

Regarding the Likert scale evaluation, the scale used in the risk perception questionnaire was approved with S-CVI = 0.82, and the scale of the practice's questionnaire presented S-CVI = 0.77, not approved.

After rewriting, removing items, and replacing the Likert scales used according to the judges' suggestions, the questionnaires was resent for further evaluation. Regarding the scale used in the "risk perception" construct, as emojis are very useful in evoking emotions and attitudes [83], they can confuse the correct measurement of responses since students can associate with feelings and check the emoji that reflects their mood. Thus, it was replaced by another five-point scale, ranging from "no chance" to "100% chance". Another five-point scale replaced the Likert scale of the "practices" construct, ranging from "never" to "always". A study by Dalmoro and Vieira [84] points out that the three-point scale is less reliable and less able to demonstrate the interviewee's opinion.

			I-C	CVI			
Items	Risk Pe	rception	Know	vledge	Practices		
	V1	V2	V1	V2	V1	V2	
1	0.93	0.99	0.84	1	0.93	0.98	
2	0.84	0.95	0.77	0.9	0.93	0.96	
3	0.86	0.98	0.79	0.88	0.88	0.86	
4	0.86	0.93	0.85	0.99	0.85	0.98	
5	0.88	0.94	0.76	0.89	0.89	0.99	
6	0.87	1	0.82	1	0.85	0.9	
7	0.91	1	0.79	1	0.92	0.98	
8	0.84	1	0.9	0.9	0.95	1	
9	0.94	-	0.86	0.93	0.88	0.96	
10	0.88	-	0.79	0.95	0.84	0.99	
11	-	-	0.84	0.93	0.78	0.96	
12	-	-	0.93	-	0.87	0.93	

**Table 3.** Average I-CVI values of the items of the versions submitted to the construct analysis of each questionnaire that make up the instrument for assessing knowledge, practices, and risk perception related to food safety for low-income students (FD-Brazil).

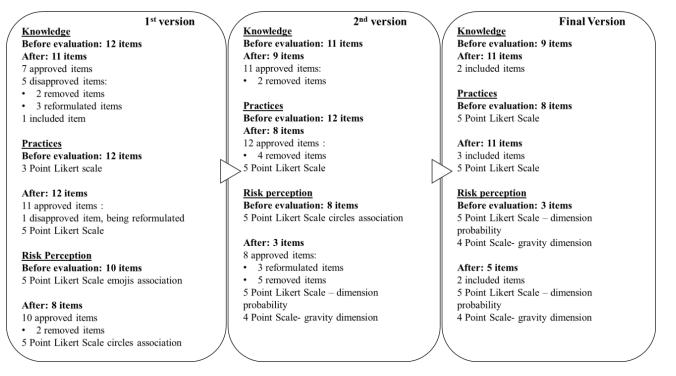
In the second assessment of the construct analysis, 16 judges agreed to participate. All items obtained I-CVI  $\geq$  0.80. The variety criterion was met in the three questionnaires with a mean Q-CVI = 0.94. As for the balance criterion, Q-CVI = 0.94 was obtained. The average I-CVI values for each item in each questionnaire are shown in Table 3. The two Likert scales obtained S-CVI = 0.94.

After completing the specialists' second evaluation, the researchers responsible for the study discussed the items resulting in the third version of the questionnaires. They analyzed the need for modifications to obtain the final version. They included items deemed pertinent and restructuring sentences to better understand and modify the Likert scale of the risk perception questionnaire.

As seen in the semantic analysis stage and according to some judges' positions, students could have difficulties understanding the concept of risk. Thus, it was decided to break down the risk perception items in their two dimensions—gravity and probability [67]. The term "chance" was used for the probability dimension because they are synonymous in the semantic context, and the epidemiological definition is not considered [85].

As the two risk dimensions were worked on separately in the risk perception questionnaire, there was a need to develop two scales to measure the responses (Appendix A). The probability dimension was measured using a five-point Likert scale associated with the ruler. The severity dimension was measured using a four-point scale associated with the figures that characterize symptoms at each severity level. Items 1.1 and 2.1 were coded from "one" to "four", with "one" being "no severity" and "four" being "high severity". The optimistic bias will be obtained by subtracting the score from item "two" (2 and 2.1) by the score from item "one" (1 and 1.1). Positive and significant differences indicate the trend of optimistic bias [14]. E.g., if in questions (1 and 1.1), the respondent obtained a score of 5 and 4 respectively, the final score was 1. In questions (2 and 2.1), he obtained scores of 4 and 2, with a final score 2. When checking the optimistic bias, it turns out that 2-1 presents a positive difference, being possible to identify the phenomenon.

The entire process of restructuring the questionnaires is shown in Figure 3.



**Figure 3.** Restructuring steps for the knowledge, practices, and risk perception assessment instrument related to food safety for low-income students (FD—Brazil).

### 4. Conclusions

The food safety scenario revealed in the discussions, and systematic database search made it possible to understand the addressed dimensions and constructs. Those were knowledge, practices, and risk perceptions, providing an instrument that considered the public's reality and perspective, especially the issue language, to be simple, straightforward, playful, and understandable. The achievement of the objective was possible through a rigorous validation process. This process resulted in a reliable and appropriate instrument consisting of three questionnaires that helped determine a starting point for the formulation of public policies aimed at the universe of low-income children and adolescents. This study's limits are represented by the fact that it does not include verifying the reliability coefficients (Conbrach's alpha and temporal stability) and validity (regression analysis and internal consistency analysis) of the items that make up the instrument. However, this is planned for future studies.

Author Contributions: Conceptualization, methodology, validation, formal analysis, investigation, data curation, writing—original draft preparation, and writing—review and editing—S.A.B.; methodology, validation, formal analysis, writing—review and editing, and supervision E.S.; validation, formal analysis—E.Y.N.; writing—original draft preparation, and writing—review and editing—M.d.O.C.; writing—original draft preparation, and writing—review and editing—R.B.A.B.; writing—review and editing, and funding acquisition—R.P.Z.; writing—review and editing, and funding acquisition—A.R.; writing—review and editing, and funding acquisition, methodology, validation, formal analysis, writing—original draft preparation, writing—review and editing, and funding acquisition—H.H.; Conceptualization, methodology, validation, formal analysis, writing—original draft preparation, writing—review and editing, supervision, project administration, and funding acquisition—V.C.G. All authors have read and agreed to the published version of the manuscript.

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**Institutional Review Board Statement:** The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by Ethics Committee of the College of Health Sciences of the University of Brasilia—CEP/FS UnB (CAAE n° 02033218.0.0000.0030).

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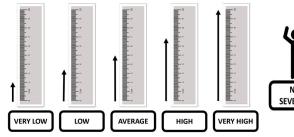
## Appendix A. Instrument Addressed for Low-Income Children and Adolescents on Food Safety Knowledge, Practices, and Risk Perception

School	Code	(filled in l	by the researcher)
Series Age:	years old Area whe	re you live: ( ) Ur	ban ( ) Rural
Gender: ( ) Female ( ) Male Date:	/ / 202	_	

#### Make an X ONLY A RECTANGLE that matches your answer.

ATTENTION: GETTING SICK = having stomach pain and/or diarrhea and/or vomiting and/or headache and/or fever because you ate contaminated food.

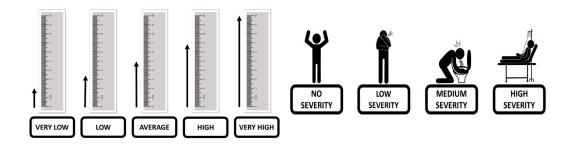
1. What is the chance that YOU will get sick	1.1 If YOU get sick from eating food served at
from eating food served at your school?	the school you study, how serious could it be?

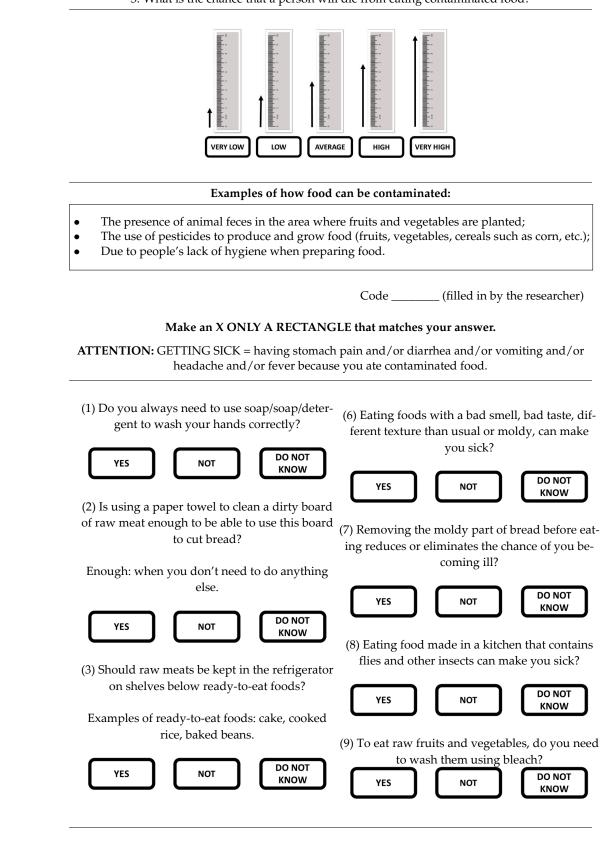


2. What is the chance that a COLLEAGUE who studies with you will get sick from having eaten the same food served at your school?

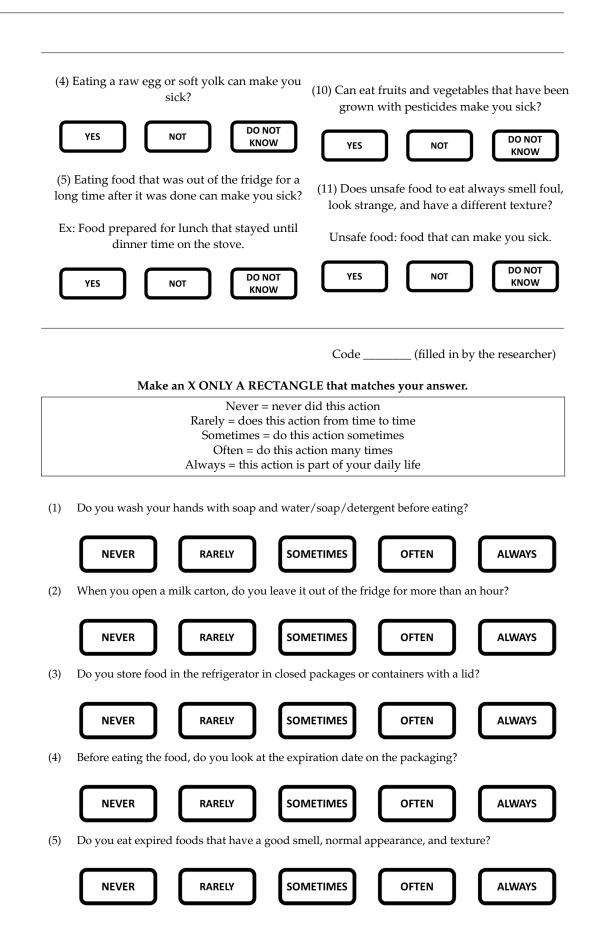


2.1 If YOUR COLLEAGUE gets sick from eating food served at the school you study, how serious could it be?

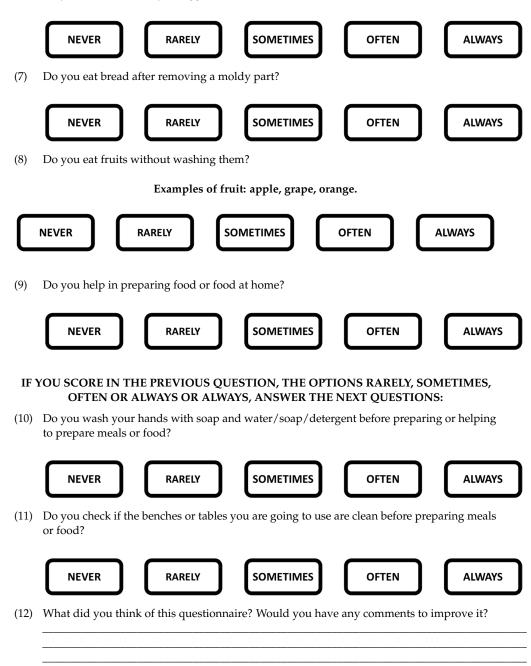




3. What is the chance that a person will die from eating contaminated food?



(6) Do you eat raw or soft yolk eggs?





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### Article The Impact of Climate Change on the Food (In)security of the Siberian Indigenous Peoples in the Arctic: Environmental and Health Risks

Elena Bogdanova <sup>1,2,\*</sup>, Sergei Andronov <sup>2,3</sup>, Andrei Soromotin <sup>4</sup>, Gennady Detter <sup>5</sup>, Oleg Sizov <sup>6</sup>, Kamrul Hossain <sup>7</sup>, Dele Raheem <sup>7</sup> and Andrey Lobanov <sup>2,3,\*</sup>

- <sup>1</sup> Department of Economics and Management, Northern Arctic Federal University, 163002 Arkhangelsk, Russia
- <sup>2</sup> Federal Autonomous Scientific Institution "Eastern State Planning Centre", 680000 Khabarovsk, Russia; sergius198010@mail.ru
- <sup>3</sup> Laboratory for Studying the Mechanisms of Physical Factors Action, Center for Testing and Examination of Natural Healing Resources, National Medical Research Center for Rehabilitation and Balneology, Ministry of Health of the Russian Federation, 121099 Moscow, Russia
- <sup>4</sup> Institute of Ecology and Natural Resources Management, Tyumen State University, 625000 Tyumen, Russia; asoromotin@mail.ru
- <sup>5</sup> Arctic Scientific Research Centre of Yamal-Nenets Autonomous Okrug, 629008 Salekhard, Russia; detter@mail.ru
- <sup>6</sup> Laboratory of Integrated Geological and Geophysical Study and Development of Oil and Gas Resources of the Continental Shelf, Oil and Gas Research Institute RAS, 119333 Moscow, Russia; kabanin@yandex.ru
- <sup>7</sup> Northern Institute of Environmental and Minority Law, Arctic Center of the Lapland University, 96101 Rovaniemi, Finland; kamrul.hossain@ulapland.fi (K.H.); bamidele.raheem@ulapland.fi (D.R.)
- \* Correspondence: e.n.bogdanova@narfu.ru (E.B.); alobanov89@gmail.com (A.L.)

**Abstract:** Climate change represents a global challenge that impacts the environment, traditional lifestyle and health of the Indigenous Peoples in the Arctic zone of Western Siberia and threatens their food security. Reindeer are an important food source for this population since reindeer herding products are used as traditional nutrition and effective preventive means and remedies for adapting to the cold and geomagnetic activity in the High North. Longer off-season periods, high summer and winter temperatures, melting ice, and forest and tundra fires have a significant impact on the trampling and degradation of reindeer pastures. These effects may lead to massive reindeer losses and changes in the traditional diet of the Indigenous Peoples in the Arctic, which result in increases in the prevalence of respiratory diseases, overweight and hypertension. This study applied a multidisciplinary approach based on ecological and medical research methods with the inclusion of socioeconomic analysis. The primary sources included data on the longitudinal dynamics of air temperature as a climate change indicator and reindeer livestock populations (1936–2018), consumption of reindeer products and physiological impacts on the Yamal Indigenous population collected during expeditions to the Arctic zone of Western Siberia in 2012–2018.

**Keywords:** climate change; food security; reindeer herding; reindeer losses; overgrazing; traditional nutrition; health risks; Arctic Indigenous Peoples; Yamal-Nenets Autonomous Okrug

#### 1. Introduction

Food security, health and well-being of the Arctic Indigenous communities depend on reindeer herding. There have been rapid social, economic, and political changes in the Russian Arctic during the last half of the twentieth century. It has impacted Indigenous traditional livelihoods, community social networks, and their lifestyle. Furthermore, the Indigenous Peoples are increasingly exposed to risks associated with climate change [1–3] which is presenting challenges for reindeer herders in the Arctic. In recent decades, Arctic and subarctic regions have experienced significant warming trends [4–7], which can have devastating environmental impacts [8]. The dynamics of the increase in the average annual

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**Copyright:** © 2021 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). temperature in the Arctic has, on average, an effect that is two times greater than that in the temperate latitudes [9]. By the end of the twenty-first century, the global mean temperature is expected to rise 1-5 °C [9]. This increase in temperature has already affected reindeer herding activities [10–13], traditional lifestyle, well-being and health of the Indigenous Peoples in Scandinavia and the Russian Arctic [1,2,14]. Adaptive capacity [15,16] and coping strategies [17] for changes in the terrestrial ecosystem caused by climate change are urgently required [14,18,19].

A growing number of studies are providing insights into how climate change is being experienced in the Arctic, by northerners, beyond the variables included in climate change models, and how Arctic people are being affected [18,20,21]. Human dimensions of climate change have been enriched with the studies of vulnerability which are increasingly considering the multiple variables that drive exposure sensitivities, and research on the interconnections between climate and non-climatic factors (industrial development of the Arctic, changing socio-ecological and economic policies, etc.) that have acted synergistically to affect individuals and communities [18,22–24]. The effect of the political context could be so large as to conceal the impact of other natural factors on reindeer populations such as climate change. However, a gradual increase of reindeer populations in the northern part of Russia in the 1960s can be associated with changes in atmospheric circulation patterns [25]. Therefore, in our research, we are mostly focused on the impact of climate change on reindeer herding practices. However, the importance of other non-climatic factors as socio-economic drivers has also been considered.

Climate change is expected to have both positive and negative impacts on reindeer herding [3,17,26,27]. Regarding the positive effects, climate change has increased the duration of the snowless season and temperatures during the growing season, which have positively impacted the productivity of plants and the growth of the reindeer's forage base [27,28]. In spring, early snowmelt and increased availability of fresh forage are favourable for lactating reindeer and the new-born calves [29–32]. These climatic changes have impacted the nomadic routes of reindeer herds and, ultimately, the traditional lifestyles of the local communities because of the decreased availability of their traditional food [33] for most of the year.

Regarding the negative impacts, warming can result in the potential re-emergence of anthrax associated with historic livestock burial sites [33–36], biological pollution, more severe insect harassment, epizootics and more frequent parasite epidemics [3,37–42]. It increases the risk of forest and tundra fires [43] (dry lichen represents an extreme fire hazard). The expansion of the taiga tick has caused the border of tick-borne encephalitis and borreliosis to move north. Longer warming periods in shallow water bodies and the thawing of cesspools previously located in the permafrost can lead to outbreaks of intestinal infections [36,44].

Warmer winters with "varying temperatures and events like rain-on-snow or thawfreeze may lead to more frequent icing of snow and basal ice" [45], which make it difficult for reindeer to access ground lichens [3,46,47]. Warm autumns can also result in the growth of mycotoxin-producing microfungi (moulds) below the snow in reindeer pastures [3,47,48]. The lengthening of off-season periods and high summer temperatures contribute to the trampling (lichen becomes brittle when it dries) and degradation of reindeer pastures [49]. This damage results in less food being available to reindeer in winter and negatively affects the adaptive abilities of their bodies and their resistance to infections (brucellosis, mycobacteriosis, and anthrax), leading to increased losses during extreme weather and decreased offspring survival rates [50]. The trampled lichen takes a long time to recover and negatively impacts reindeer husbandry for several years. The increase in the occurrence of anomalous phenomena (winter thaws and rains, late spring with ice crust, high and prolonged heat) represents a significant threat, as it can result in massive reindeer losses [51,52] because of the decreased availability of pastures and ultimately placing an effect on the subsistence-based livelihoods of the Indigenous populations [53] jeopardizing the food security of the Indigenous Peoples.

Although climate change is projected to increase fish stocks in the Barents Sea capable of offering the availability of foods [54], the decrease of reindeer livestock is more likely expected. However, reindeer meat is a rich source of protein, minerals and essential fatty acids, and its consumption is culturally acceptable in these Arctic communities [55]. The loss of this food source increases the prevalence of chronic bronchitis [56,57], overweight [58] and arterial hypertension [59,60] because consuming reindeer meat, blood and liver helps to prevent these diseases [61–64] and increases the ability of the Indigenous population to adapt to the harsh Arctic living conditions [65–68]. The maintenance of a traditional diet is closely related to the maintenance of the traditional food system [69,70], which is "tightly interwoven with the culturally, socially and environmentally embedded practices of reindeer herding. Indigenous Peoples and their traditional food systems emerged in harmony with nature and contain knowhow on the sustainable use of natural resources in ways that contribute to their health" [71].

The environmental changes in the Russian North [72], and specifically in the Yamal-Nenets Autonomous Okrug (YNAO), have resulted in the melting of ice and the extension of the sea [73,74]. The ice crust formation in the winter of 2013–2014 and the outbreak of anthrax in the summer of 2016 have provoked a discussion on Nenets' reindeer husbandry "crisis", as evidenced by the overgrowth of herds and overgrazing [75,76]. Biologists and local governments emphasized the need to significantly reduce the Yamal reindeer population "for the sake of environmental safety". However, A. Golovnev presented an alternative approach focused on a system of movement: "skilful herd-navigation and quick manoeuvring is the basis of Nenets' traditional rule "ya puna hayoda" (land after us remains). Conversely, the consequences of sluggish and stationary behaviour, including huddling around camp for a long time, is reflected in another Nenets proverb: "yadata habei" (land is turned upside down)" [77]. These are examples of traditional coping strategies for dealing with the development of adverse weather and pasture conditions that have accumulated and been exchanged within the herding communities in different Arctic countries [78]. However, the rapidly changing Arctic environment and climatic challenges have made these coping strategies ineffective, requiring the development of new methods [46,79].

Climate change and the extensive exploitation of reindeer pastures have resulted in overgrazing [80]. This has caused the Indigenous Peoples to change their nomadic routes (i.e., some reindeer herders in the Priuralsky district of the YNAO have moved to the Laborovskay tundra, which is still rich with lichen). Nomadic reindeer herders of the Tazovskaya, Tanamskaya, Gydanskaya and Yuribeyskaya tundras have experienced the reduction of winter reindeer pastures: there are significantly damaged pastures next to the slaughterhouse in the Antipayutinskaya tundra, near the trading posts ("faktoria") Yuribey and Tanama, surrounding the settlements of Antipayuta and Gyda and near fishing sites on the coast of Ob Bay (north of the settlement of Antipayuta, on the western shore of Yambuto lake, on the coast of Gydan Bay, in the area of oil and gas deposits of the Messoyakhinskaya group) [27]. Thus, climate change has had a strong influence on the Arctic ecosystems and jeopardized the Indigenous Peoples' food security and their well-being [81,82]. In this study, we focused on analysing the longitudinal trends in the climatic parameters, reindeer livestock population and consumption of traditional reindeer products and the physiological impacts on the Yamal Indigenous population. These analyses can provide relevant information about the sustainability of reindeer herding and the Indigenous communities in the Arctic zone of Western Siberia.

#### 2. Materials and Methods

## 2.1. Setting: The Yamal-Nenets Autonomous Okrug (YNAO): Geography, Population and Ethnic Structure

The YNAO, the geographic focus of our research, is an important region for the Indigenous Peoples of Russia, and it is located in the circumpolar northwest of West Siberia. It has a population of 544,008 [83] who live in an area of 769,250 square kilometres [84] with a population density of 0.71 people per square kilometre. The location of the YNAO (more than half of its territory is above the Arctic Circle) significantly influences the traditional

livelihoods in this region. It is a unique territory because almost half of the minority Indigenous population of the Russian Arctic (about 45,000 people) reside there, including the Nenets, Khanty, Selkups and Komi-Zyryans. A total of 14,600 Indigenous Peoples are nomadic, living in tundra areas [85]. The culture, health and social well-being of Indigenous Peoples are strongly linked to their traditional lifestyle and traditional livelihoods (reindeer herding, fishing, etc.), which are essential for meeting Indigenous Peoples' vital needs and helping them to survive in the severe Arctic areas.

#### 2.2. Study Design

In this paper, we present the results of a quantitative analysis of the impacts of climate change on the reindeer livestock population and consumption of traditional reindeer products and of a follow-up analysis of the increased risks of physiological health effects faced by the Siberian Indigenous Peoples in reindeer herding communities living and practicing nomadism in the remote territories of the YNAO. The objectives of our study were to determine (1) if the longitudinal dynamics of air temperature has impacted reindeer livestock populations in the YNAO in 1936–2018 and (2) if the dynamics of the reindeer livestock population has affected the consumption of reindeer products and the health of Indigenous Peoples in the Yamal Indigenous population in 2012–2018. Non-climatic factors could be also considered to explain the results.

#### 2.3. Measurement Tools, Methodology and Study Population

The study applied a multidisciplinary approach based on ecological and medical research methods with the inclusion of policy and socioeconomic analysis. The primary sources included data on the longitudinal dynamics of air temperature as a climate change indicator, reindeer livestock population, consumption of reindeer products and physiological impacts (prevalence of arterial hypertension) on the Yamal Indigenous population.

The data on the average daily, average monthly and average annual air temperatures for the period from 1936 to 2018 were obtained from the open-source platforms of the Federal Service for Hydrometeorology and Environmental Monitoring in the Russian Federation (Roshydromet) [86]. We used data collected from three meteorological stations in the following locations in the YNAO: in the city of Nadym in the Nadymsky district (65.53333: 72.51667), in the settlement of Novy Port in the Yamalsky district (67.4100: 72.5600) and the settlement of Antipayuta in the Tazovsky district (69.06667: 76.83333). The data on the number of reindeer in the YNAO for the period from 1930 to 2018 were obtained from the Department of Agroindustrial Complex of YNAO [87].

First, the correlation between air temperature and the reindeer population was studied. Spearman's rank correlation coefficient was used to determine the strength of the link between the sets of data. Due to the seasonality observed in the time series, seasonal differencing was applied to eliminate the seasonal component with the use of an autoregressive integrated moving average (ARIMA) model [88,89]. Short-term forecasting (5 years) was carried out using the Almon distributed lag method. Second, a forecasting model of the dynamics of the reindeer population was developed. Third, the correlation between the reindeer livestock population and consumption of reindeer products (reindeer meat, liver and blood) with a follow-up correlation between the consumption of these products and the prevalence of arterial hypertension (as the main reason for mortality [59]) in the Yamal Indigenous population was studied.

The data on socio-demographic characteristics, consumption of reindeer products and health status were collected during expeditions to the Arctic zone of Western Siberia in 2012–2018. The fieldwork was conducted by researchers of the YNAO Arctic Scientific Research Centre, the National Medical Research Centre for Rehabilitation and Balneology, the Northern Arctic Federal University and the Association of Reindeer Herders in YNAO (two of the researchers were Indigenous).

To study the basic patterns of consumption of traditional reindeer products, we initially conducted a cross-sectional screening with the participation of the Indigenous inhabitants of the Arctic zone of Western Siberia. The inclusion criteria for the respondents were as follows: be over 18 years of age, be of Indigenous origin, be an Indigenous language speaker, be involved in reindeer herding, live a nomadic or semi-nomadic lifestyle and have resided in the tundra or the settlements of the Arctic zone of Western Siberia for over five years. The sequence of the survey was as follows: during an expedition to the settlement between 2012 and 2018, respondents were invited to participate in the survey while undergoing a medical examination conducted by the YNAO Arctic Scientific Research Centre at health care institutions. After the aims and content of the research were explained and consent for participation was obtained, the name of each person invited to take the survey was recorded in a registry, and they were given a questionnaire. The questionnaire was developed in Russian using the methodological recommendations of the Russian Academy of Medical Science (RAMS) Nutrition Institute (1996, 2016) [90,91]. It collected information about socio-demographic factors and the consumption of reindeer products (reindeer meat, liver and blood). Data collection was performed by medical doctors who had been trained in the study procedures with the assistance of Indigenous nurses and researchers. All participants given the questionnaire were interviewed and underwent medical examinations at the beginning of the study by a general practitioner, pulmonologist and cardiologist. If a patient had been found to have severe somatic pathology, they would have been excluded from the survey, but no such cases were found. Participants filled out a confidential paper questionnaire. The consumption of reindeer products was analysed according to the following questions: "How many grams of reindeer meat did you consume over the previous 30 days?", "How many grams of reindeer liver did you consume over the previous 30 days?", "How many grams of reindeer blood did you consume over the previous 30 days?" The participants received information about the programme, both verbally and in writing, and they provided written informed consent. The consent form stated that participation was voluntary and that their confidentiality was assured. Participants' personal data and their answers were anonymised, numbered and entered into de-identified databases.

An analysis of the traditional foods (reindeer meat, liver and blood) was carried out using frequency and survey methods [63,92]. The amount of food consumed was estimated using a catalogue graphically depicting a range of portions of foods and dishes [93]. The amount of traditional products consumed over the previous 30 days was estimated in the survey. The blood pressure of the participants was measured three times according to the Korotkov method, and the presence of arterial hypertension (AH) was established in accordance with the recommendations for AH [94,95].

Statistical analyses were performed using Microsoft Excel 2016 and Statistica for Windows, v. 8.0 (StatSoft Inc., Oklahoma, OK, USA). Significant differences were defined at a *p*-value < 0.05.

#### 2.4. Ethics Approval

The study was approved by the Ethics Committee of the Arctic Scientific Research Centre of YNAO, Salekhard, Russian Federation, on 16 January 2012 (approval protocol No. 01/1-13). The research has been done in accordance with ethical concerns of working with the Indigenous Peoples in the Russian Federation (Constitution of the Russian Federation, Article 69. 14 March 2020). Communication was initiated with the Associations of the Indigenous Peoples and with representatives from national Indigenous communities of the Nadymsky, Yamalsky and Tazovsky districts in YNAO early in research planning. This resulted in an expression of interest from their representatives in having the research conducted in their communities.

#### 3. Results and Discussion

Our main findings showed that climate change (increasing average air temperature) has impacted the reindeer population mostly in the central area of the Arctic zone of Western Siberia (Nadymsky district), as we found a weak correlation between the dynam-

ics of average air temperature and reindeer livestock population in the northern areas (Yamalsky and Tazovsky districts). The weak correlation is explained with the stronger impact of non-climatic factors (i.e., the industrial development of the Arctic, changing socio-ecological and economic policies as well as tendencies of commodity production in the Indigenous reindeer herding communities). If the trend for increasing average annual temperatures continues at the same rate, the growth of the reindeer population in the YNAO will remain steady until 2025. After 2023, the dynamics of the reindeer population will slow down or become negative. Weather and extreme climatic events (the formation of an ice crust over large areas, freezing rain, heat waves) can cause catastrophic collapses in the reindeer population because many areas have insufficient reindeer pasture resources, which reduces the adaptive capabilities of the reindeer and the survival of offspring. Given the cyclical nature of these processes, the most dangerous period will be the spring of 2023–2024. However, these climatic effects and the reindeer population do not have direct impacts on food security, although the health status of the Indigenous population in the YNAO is strongly linked to the consumption of reindeer products.

The results of the correlation analysis of average air temperature and the reindeer population in three districts of the YNAO are presented in Table 1.

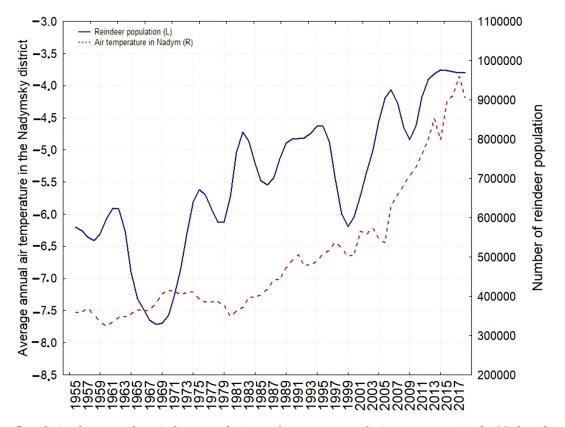
District	Spear—R	<i>p</i> -Level
Nadymsky district	0.5	0.02
Yamalsky district	0.169278	0.126048
Tazovsky district	0.4	0.0001

Table 1. Spearman rank-order correlations of average air temperature and the reindeer population.

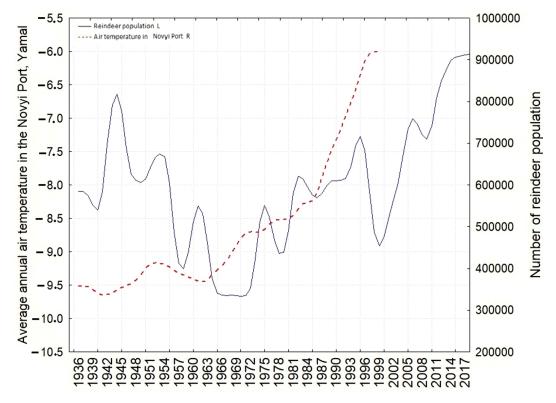
The Nadymsky district located in the central part of the YNAO has different types of landscapes, including southern shrub tundra, forest-tundra and northern taiga. In this district, we found a direct strong statistically significant correlation between the average annual air temperature and the number of domesticated reindeer (rs = 0.5; p = 0.02). As the average annual temperature increased, the size of the reindeer herd increased, which is probably associated with the increase in the reindeer forage base because of the increase in the productivity of vascular plants in the warming climate. An increase in fodder naturally increases the adaptive reserves of animals, making it possible for them to endure unfavourable periods with fewer losses, increasing the survival of offspring and increasing the number of deer (Figure 1).

For the Yamal region, which is located above the Arctic Circle on the Yamal Peninsula, no reliable correlation was found between the reindeer population and air temperature (rs = 0.169278; p = 0.126048) (Figure 2).

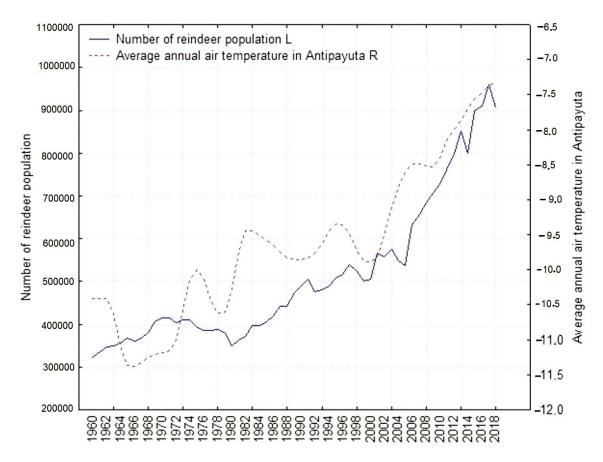
For the Tazovsky region, which is located above the Arctic Circle on the Tazovsky, Gydansky and Yavay-Sale Peninsulas, only a weak direct correlation was found between the average annual air temperature and the number of domesticated reindeer (rs = 0.4; p = 0.0001) (Figure 3).



**Figure 1.** Correlation between the reindeer population and average annual air temperature in the Nadymsky district, 1960–2018.



**Figure 2.** Correlation between the reindeer population and average annual air temperature in the Yamalsky district, 1936–2018.



**Figure 3.** Correlation between the reindeer population and average annual air temperature in the Tazovsky district, 1960–2018.

Understanding of vulnerability of reindeer husbandry requires one assess at least three separate aspects: the external impacts on the social-ecological systems, the ability of these systems to cope and adapt to these impacts, and the extent to which environmental or societal conditions hinder herders in adapting to change [96]. Climate change is one of the important external impacts on reindeer herding. The differences in the impact of climate change on the reindeer population in the Nadymsky, Yamalsky and Tazovsky districts are partly connected with the thermal balance in the northern and central areas of the YNAO. Klokov K.B. et al. [97] stated that the areas for wintering with the best thermal balance are located in the northern (tundra) parts of the Nadymsky, Purovsky and Shuryshkarsky districts of the YNAO. In July–August, the optimal grazing conditions (without going beyond the thermoneutral zone) are in the northern part of the Yamal Peninsula. On the Gydansky and Tazovsky Peninsulas, the thermal balance sometimes goes beyond the thermoneutral zone.

The positive impact of climate change on the reindeer population can be explained by the increase in the snowless period and, consequently, the increased productivity of vascular plants in the warming climate. Finnish reindeer herders have also noted this favourable effect of climate change on increased forage: a rainier but warmer and longer growing season may increase the growth of vegetation and availability of high-quality forage, such as mushrooms, for reindeer [3,17]. Arctic areas are sensitive to such changes in forage conditions. In the Arctic zone of Western Siberia, reindeer consume more than 600 species of lichens, grasses, shrubs, trees and mushrooms [98], while in Chukotka and Alaska, there are over 1000 species of vascular plants [99]. Thus, reindeer can survive without lichen if there are many wet plants under the snow, such as horsetail and evergreen grasses, which contain many vitamins, proteins and microelements. Climate change in Siberia has had environmental effects, including inducing changes in species composition [100–103],

the abundance of animals and plants [104–106] and annual biological cycles [2,107]. It has a strong influence on the grazing cover of reindeer pastures, especially in the central parts of the YNAO, where thinner snow cover, milder weather and shorter periods of low temperatures are favourable for reindeer because of the higher availability of forage. For example, the tendency for the increased growth of deciduous shrubs observed in recent decades is apparently closely related to the higher and more intense summer temperatures in Western Siberia [108]. S. Rasmus et al. also noted the positive effects of the advanced development of vegetation and the consequent higher availability of fresh forage plants for milk-producing dams and their calves, as the calves would be fit by the time the calf marking period starts in the summer [3,107]. However, in the more northern parts of Siberia and Fennoscandia, "foraging conditions have been deteriorated due to hard snow and icy layers formed on the soil and snow cover resulting in declined availability of ground lichens for reindeer. Hard snow and rime accumulated on the branches of trees have also decreased the availability of arboreal lichens" [3]. Walker et al. [109] demonstrated this phenomenon of a faster increase in the aboveground biomass of circumpolar arctic tundra vegetation in more southern Arctic areas: southern tundra subzones exhibited approximately 20–26% biomass increases, whereas northern tundra subzones had increases of 2–7%. However, these processes are much slower in the YNAO. Kovalevskaya N.M. et al. [49] mentioned that the results of their analysis of satellite data suggest that over the past three decades and more, there has been a relatively small (compared to that in other Arctic regions) increase in the productivity of Yamal vegetation and a slight increase in near-surface temperatures. While the degradation of reindeer pastures is increasing (Figure 4) that results in destroying reindeer health (Figure 5) and changing the reindeer diet.



Figure 4. Degradation of reindeer pastures in Yamal.

Warming has an unclear effect on Arctic reindeer herding and can also result in higher reindeer losses because of increased risks of entomoses [110] and helminthic diseases [42]. Besides, reindeer are better adapted to hypothermia than to overheating. In hot weather, the physiological activity of metabolic processes in reindeer decreases, and they are not able to accumulate a sufficient supply of nutrients for successful wintering [97]. Therefore, reindeer losses are a likely outcome of climate warming in the Arctic zone of Western Siberia.



Figure 5. Teeth of a three-year-old deer worn out on the mineral base in the tundra.

The rather weak impact of climate change on the reindeer population in the northern areas of the YNAO (Yamalsky and Tazovsky districts) can be explained by the more significant contribution of non-climatic factors. This region is known as the place with the most intensive industrial development in the Arctic region of Russia. Industrial development is associated with the degradation of reindeer pastures due to the disruptive effect of developing logistic and industrial infrastructure. Anthropogenic activities [2] in recent years and technogenic emissions of combustion products from fuel and energy enterprises into the atmosphere [111–114], as well as metallurgical production and mining, which produce excessive amounts of some macro-and microelements, all of which affect the physiological parameters of reindeer. In the areas near industrial centres, atmospheric pollution affects lichen first, causing its disappearance from the vegetation cover of pastures [51]. Nevertheless, YNAO is the only Arctic region in Russia where the number of domesticated reindeer has constantly increased during the last 50 years even in the conditions of intensive gas and oil extraction, political shifts and during the crisis of the 1990s [115]. It can be a result of the positive impact of industrial development and extending urban areas which provided stable markets for reindeer products. Oil and gas companies gave a stable tax base which was reinvested also in the rural economy including reindeer enterprises [25]. A high degree of nomadism among the Indigenous Peoples and dominance of individual reindeer husbandries (60% of reindeer belong to individual reindeer herders [71]) in YNAO became the preconditions for stronger socio-ecological and economic resilience of reindeer herding livelihood to shifting political context in 1960–2018. While in other Russian Arctic regions, there were dramatic declines in reindeer numbers associated with the years of institutional reforms wherein ownership rights changed; the reorganization of kolkhozes (enterprises with collective ownership) into sovkhozes (enterprises with state ownership). Later from 1991 to 2003, a decrease in the reindeer population was caused by the withdrawal of state subsidies. Finally, up to 2003, the reindeer population in reindeer enterprises decreased to approximately 35% of their previous populations [25] in all Arctic reindeer herding regions (except YNAO) of Russia.

Nowadays, one of the key factors impacting the reindeer population in the northern areas of the YNAO (Yamalsky and Tazovsky districts) is the increase in velvet antlers production. Mainly nomadic reindeer husbandries of Tazovskaya, Messoyakhinskaya, Antipayutinskaya, Tanamskaya tundras are integrated into the commodity production of meat and velvet antlers. This is due to the relatively good logistics, the presence of reindeer slaughtering houses and the possibility to sell them velvet antlers. In this group, over the past four years, the sale of velvet antlers has gradually become the main source of income for reindeer herders, and the delivery of meat is an auxiliary one. Subsistence reindeer herding, working mainly to provide the family with food and clothing, is widespread in the northern part of the Gydansky Peninsula, on the coast of the Yuratskaya Bay and other parts of the region, logistically remote from settlements, slaughter facilities and large deposits. This is explained by the high cost of gasoline and the cost of exporting reindeer products and delivering goods to the tundra. The further from the logistics centres, the less profitable commodity production becomes due to the high costs [27]. However, the risk of climate change should also not be ignored in the northern areas of the YNAO, as its influence in the neighbouring territories of the Nenets Autonomous Okrug [82] is rather evident based on changes in the vegetation cover of the tundra and reduced availability of lichen for reindeer under the ice and snow.

In general, climate change can have catastrophic effects on forage for reindeer herding in different Arctic areas. Rees et al. [116] argued that climate change impacts are likely to harm the livelihoods of those who practice reindeer husbandry in Norway and Sweden but have a neutral impact in Finland. The main factors that dictate these impacts are the changes in vegetation distribution caused by the changing climate, especially winter temperature and winds. Nevertheless, Rees et al. [116] suggested that the effect of these changes is expected to be relatively small and well within the range of previous experiences of reindeer herders dealing with climate variability. However, in Western Siberia, climate changes (autumn, winter and summer warming) may become a serious challenge for the preservation of nomadic reindeer herding [117].

A forecast model for the number of reindeer correlated to the average annual air temperatures was developed. We analysed the dynamics of average annual temperatures and the number of reindeer using the ARIMA variable method. It was found that the number of reindeer livestock undergoes seasonal fluctuations every 5 and 10 years and exhibits an overall upward trend. The analysis of the number of domesticated reindeer in the YNAO using autocorrelation and partial autocorrelation with the ARIMA model revealed the presence of a decreasing trend and lag in steps 1 and 7 and the presence of seasonality of 10. Based on the data obtained, a forecast for the number of reindeer in five years was developed. According to this forecast, under the optimistic scenario with the continued increasing rates for average annual temperatures, the number of reindeer livestock will increase; under the pessimistic scenario, it will stabilize at the 2016 values (Figure 6).

The ARIMA forecasting model showed that, with the continued increasing rates of average annual temperatures, the growth of the reindeer population in the YNAO will continue until 2025. After 2023, the dynamics of reindeer population growth will slow down or become negative. This model describes only long-term trends. Weather and climatic extremes (the formation of an ice crust over large areas, freezing rain, heat waves) can have catastrophic effects on the reindeer population because, in many areas, reindeer pasture resources are insufficient, which reduces the adaptive capabilities of the reindeer and the survival of offspring. Given the cyclical nature of these processes, the most dangerous period was predicted to be the spring of 2023–2024. However, Klokov K.B. et al. [118] were not as optimistic regarding climate change increasing reindeer livestock populations. With each increase in the average monthly air temperature of 2 °C, the boundaries of the thermoneutral zone can move north by about 100 km, which will lead to worse conditions for keeping reindeer, especially in warm years. As a result, the southern part of the Yamal Peninsula may become a zone of "risky reindeer herding", and the zone of comfortable grazing will be reduced.

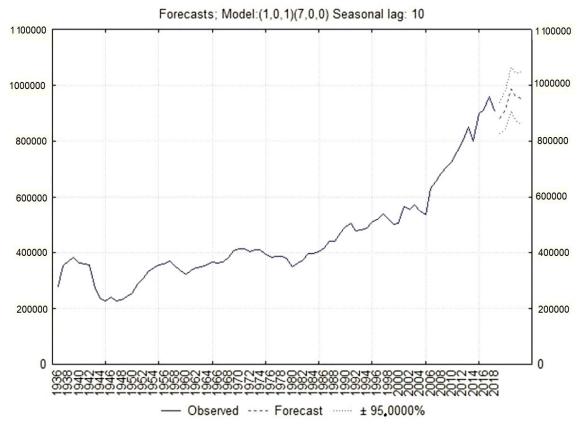


Figure 6. Forecast model for the reindeer population in the Yamal-Nenets Autonomous Okrug (YNAO), 1936–2023.

The dynamics of the reindeer livestock population will likely have an impact on the food security of Arctic Indigenous Peoples because of the increased availability of reindeer products. We assessed the correlation between the reindeer population and consumption of the most important [65] reindeer products (reindeer meat, liver and blood). A total of 1280 Indigenous inhabitants of the Arctic zone of Western Siberia participated in the study (Table 2). The age class distributions of the participants are presented in Table 3. The average age of all participants was  $45.7 \pm 14.3$  years; 396 (30.9%) of the participants were men, and 884 (69.1%) were women. From year to year, there were no differences between the proportions of males/females and various age classes sampled, as the sample remained constant throughout the study.

**Table 2.** Data on the respondents recruited for the survey, Yamal-Nenets Autonomous Okrug,2012–2018.

Year	Total Indigenous Population Included in the Study ( <i>n</i> = 1280)	Age, Years (45.7 $\pm$ 14.3)
2012	151	$41.0\pm12.5$
2013	277	$42.0\pm12.7$
2014	144	$43.0\pm13.6$
2015	150	$45.1 \pm 13.7$
2016	236	$45.0\pm14.0$
2017	136	$46.5\pm13.2$
2018	186	$47.0\pm12.8$
Total	1280	-

Year	Forecast	Lower—950,000%	Upper—950,000%	Std. Err.
2018 2019	909,626 906,607	702,724.2 683,151.4	1,116,528 1,130,062	102,958.1 111,195.3
2020	1,076,528	837,681.8	1,315,374	118,854.0
2021	1,082,704	829,418.1	1,335,991	126,039.7
2022	1,088,395	821,464.7	1,355,326	132,829.3
2023	1,087,580	807,684.6	1,367,475	139,280.7

Table 3. Forecast data for the reindeer population in the YNAO, 1936–2023.

The results of the correlation analysis were inconsistent: an increase in the reindeer livestock population was associated with the decreased consumption of reindeer products (Table 4; Figures 7–9). This does not represent a logical outcome of increases in the number of reindeer in herds. However, this phenomenon is frequently discussed in socioeconomic studies and considered to be a clear outcome of transition processes involving traditional lifestyles [27] and the traditional Indigenous economy—for example, the transfer from a subsistence economy in Western Siberia to commodity production. During the last 10 years, rising trends in the export of traditional reindeer products have decreased local Indigenous Peoples' access to venison and had a negative impact on their health [71]. The export potential of the non-edible parts of reindeer (i.e., velvet antlers, reindeer skins, camuses) should be promoted to support the food security of the Indigenous Peoples, while government policies should focus on improving the access of the Indigenous communities to the edible and medicinal portions of the carcass.

**Table 4.** Spearman rank-order correlations for the reindeer population and consumption of reindeer products.

<b>Reindeer Products</b>	Spear—R	<i>p</i> -Level
Reindeer meat	-0.8	0.01
Reindeer liver	-0.8	0.01
Reindeer blood	-0.8	0.00214

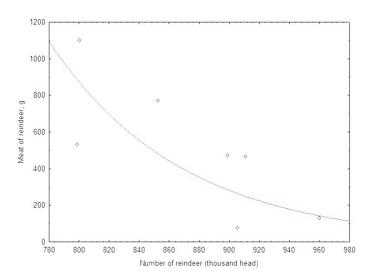


Figure 7. Correlation between the reindeer livestock population and consumption of reindeer meat.

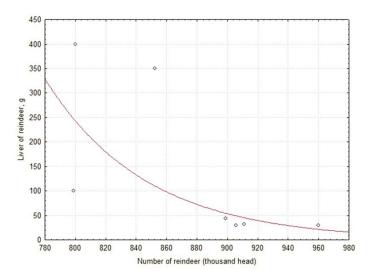


Figure 8. Correlation between the reindeer livestock population and consumption of reindeer liver.

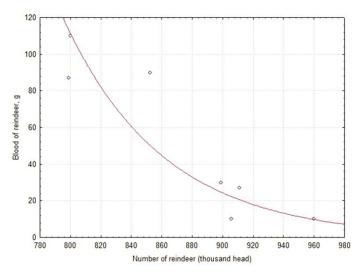
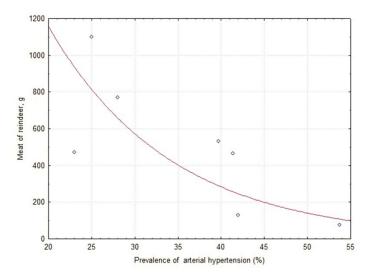


Figure 9. Correlation between the reindeer livestock population and consumption of reindeer blood.

Traditional nutrition is an important part of the Indigenous traditional culture and serves as a remedy to cold stress and increases their adaptation to the severe Arctic climatic conditions. Therefore, as expected, "the Spearman rank-order correlation revealed a strong negative association between the prevalence of arterial hypertension and the consumption of reindeer products" (Table 5; Figures 10–12).

**Table 5.** Spearman rank-order correlations for the consumption of reindeer products and risk of arterial hypertension.

<b>Reindeer Products</b>	Spear—R	<i>p</i> -Level
Reindeer meat Reindeer liver	$-0.8 \\ -0.8$	0.01 0.01
Reindeer blood	-0.77	0.01



**Figure 10.** Correlation between the consumption of reindeer meat and the prevalence of arterial hypertension.

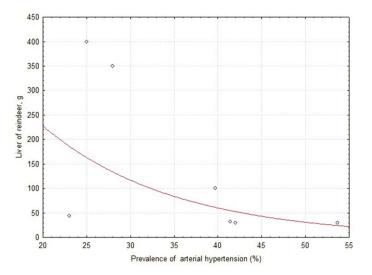
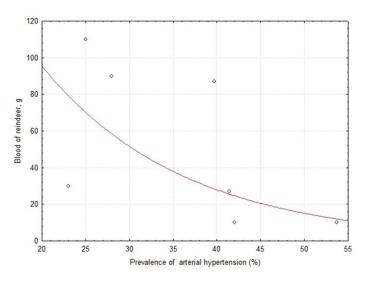


Figure 11. Correlation between the consumption of reindeer liver and prevalence of arterial hypertension.



**Figure 12.** Correlation between the consumption of reindeer blood and the prevalence of arterial hypertension.

The results of our previous study showed that there was a dramatic decrease of almost 50% in the consumption of reindeer products by the Indigenous and non-Indigenous Peoples in the YNAO, and only one-third of the studied population still ate venison once or twice daily [65]. This shift threatens their health because a diet rich in venison significantly increases antiatherogenic blood lipid fractions, contributes to the maintenance of normal body weight, and improves microcirculation, tissue fluid exchange and antioxidant protection against free radicals; these effects may explain the high prophylactic activity of venison [62] and its strong positive effects on adapting to cold stress [66] and geomagnetic activity in the Arctic [67]. Venison has been shown to effectively reduce hypertension [59] and the risk of chronic nonobstructive bronchitis [56]. These characteristics make reindeer products an important part of the local population's diet. Maintaining a traditional diet is also an important part of the Indigenous culture that strongly contributes to promoting the Indigenous Peoples' health (Figure 13). Saving these nutritious practices has a positive effect on keeping reindeer herding culturally and environmentally embedded [119].



Figure 13. Traditional Yamal Nenets ritual of drinking fresh blood of a slaughtered reindeer in the tundra.

Dissemination of knowledge on the importance of reindeer products for the Indigenous Peoples health as well as strengthening social policy to support reindeer herding husbandries in YNAO could encourage reindeer herders to keep on following nomadic lifestyle and, correspondingly, a traditional diet with a prevalence of reindeer meat and other-by reindeer products.

The main strength of our study was using unique empirical quantitative research data collected from the reindeer herders during expeditions that took place over seven years (2012–2018) and data from the longitudinal monitoring of reindeer livestock in the period 1936–2018 provided by researchers and local authorities. Most similar studies examined fragmented populations and time frames, with unclear results. However, our study had several limitations. The methodological quantitative approach was focused on using a limited number of variables for representing climate and diet changes since we were intended to analyse the impact of climatic factors on the food security of the Indigenous Peoples. While non-climatic drivers were also considered to explain the weak correlation of the factors. The studied population was recruited while undergoing a medical examination at health care institutions—municipal hospitals and feldsher-midwife medical stations in remote settlements. Participation was voluntary and did not include all

representatives of the reindeer herding communities of the studied territories, which may limit the generalizability of the findings. It would also be of value to examine food security in reindeer herding and fishing communities and the impact of traditional reindeer and fishing products and plants on the health and wellbeing of the local communities.

#### 4. Conclusions

Studying climate change and its impacts on reindeer herding while considering the input of anthropogenic and technogenic factors can provide new insights into the temporal and spatial warming variabilities in the Arctic zone of Western Siberia. In our study, the different data sets on the dynamics of annual air temperatures and reindeer livestock populations in three districts of the YNAO enabled us to not only study the ongoing climate changes in general but also examine their specific impacts on the Indigenous Peoples' food security. We conclude that as the average annual temperature increases, the size of the reindeer herds will grow, which is probably associated with the increase in the forage base for the reindeer because of the increase in the average annual temperature was correlated with an increase in the reindeer population and, in the long term, did not depend on the socio-economic model of the organization of reindeer husbandry, despite the fact that from 1936 to 2019, there were significant political and economic changes. This trend for the impact of climate change was more evident in the central districts of the YNAO.

Climate change is occurring and will continue to occur, faster in higher latitudes than in other regions. Climate change's consequences for livelihoods dependent on reindeer herding should be analysed in the context of the impacts of associated challenges and opportunities, such as exploration of the Arctic's bioresources and involvement of the Indigenous Peoples in bioproduction. Adaptation to climate change could be used as an opportunity to improve the living conditions and food security of the Indigenous Peoples and to sustain their livelihoods in the context of all related issues. Thus, climate change becomes an opportunity for supporting Arctic life and livelihoods. Any policies or regulatory measures should be developed, implemented, monitored and enforced with the full and fair participation of the Indigenous Peoples.

Climate change is already occurring and is unlikely to be curtailed soon, meaning that the effects must be addressed. The impacts of climate change on the Indigenous Peoples can be reduced by working collaboratively to ensure that indigenous interests are respected and that indigenous needs are met without precluding the involvement of others in the region and without being overwhelmed by climate change's detrimental impacts.

The results presented in this work will hopefully encourage dialogue among local practitioners, researchers and policymakers. Our study focused on reindeer husbandry, but the approach is applicable to other traditional Indigenous nature-based livelihoods (e.g., fishing, hunting, and gathering) facing the need to adapt because of the changing climate.

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Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The study did not report any data.

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Article



# **Extraction of Essential Oil from River Tea Tree (***Melaleuca bracteata* **F. Muell.): Antioxidant and Antimicrobial Properties**

Mursleen Yasin <sup>1,\*</sup>, Adnan Younis <sup>1</sup>, Fahad Ramzan <sup>1</sup>, Talha Javed <sup>2,3</sup>, Rubab Shabbir <sup>2</sup>, Hamza Armghan Noushahi <sup>4</sup>, Milan Skalicky <sup>5</sup>, Peter Ondrisik <sup>6</sup>, Marian Brestic <sup>6</sup>, Sabry Hassan <sup>7</sup> and Ayman EL Sabagh <sup>8,\*</sup>

- <sup>1</sup> Institute of Horticultural Sciences, University of Agriculture Faisalabad, Faisalabad 38040, Pakistan; adnanyounis@uaf.edu.pk (A.Y.); fahidflorist@yahoo.com (F.R.)
- <sup>2</sup> College of Agriculture, Fujian Agriculture and Forestry University, Fuzhou 350002, China; mtahaj@fafu.edu.cn (T.J.); rubabshabbir28@gmail.com (R.S.)
- <sup>3</sup> Department of Agronomy, University of Agriculture Faisalabad, Faisalabad 38040, Pakistan
- <sup>4</sup> College of Plant Science and Technology, Huazhong Agricultural University, Wuhan 430070, China; hamzanaushahi143@gmail.com
- <sup>5</sup> Department of Botany and Plant Physiology, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences Prague, 16 500 Prague, Czech Republic; skalicky@af.czu.cz
- <sup>6</sup> Department of Plant Physiology, Slovak University of Agriculture, 949 01 Nitra, Slovakia; peter.ondrisik@uniag.sk (P.O.); marian.brestic@uniag.sk (M.B.)
- <sup>7</sup> Department of Biology, College of Science, Taif University, P.O. Box 11099, Taif 21944, Saudi Arabia; sabryh@lycos.com
- <sup>8</sup> Department of Agronomy, Faculty of Agriculture, University of Kafrelsheikh, Kafrelsheikh 33516, Egypt
- \* Correspondence: mursleenyasin@gmail.com (M.Y.); ayman.elsabagh@agr.kfs.edu.eg (A.E.S.)

**Abstract:** Tea tree oil (TTO) from the genus *Melaleuca* L. has antimicrobial, antibacterial, antifungal, and antioxidant properties and is used by the cosmetic, pharmaceutical, and horticultural industries. In Pakistan, *Melaleuca bracteata* can be exploited for essential oil purposes, as this species is well adapted to Pakistan's agroclimatic conditions. The objective of the present study was to evaluate the yield of *M. bracteata* essential oil together with its antioxidant and antimicrobial properties under local prevailing conditions of the subtropics. Essential oil was extracted through the hydrodistillation method. Using this method, six batches of 8 kg samples (fresh leaves and branches) underwent a distillation process for 4–5 h. The average yield obtained was about 0.2%. The GCMS was used to identify the components of extracted essential oil. Eugenol methyl ether is the major component in extracted essential oil, i.e., 96% of the total. A high content of flavonoids and phenolics and a Fe-reducing power ability of *M. bracteata* were observed. The oil was also found effective against *B. subtilis, B. cereus,* White rot, and *A. flavus.* Hence, it is concluded that there is a possibility to use TTO for its biocidal properties, and it must also be inspected and then commercialized in Pakistan by the agriculture and cosmetic industries.

**Keywords:** *Melaleuca bracteata;* essential oil; hydrodistillation; eugenol methyl ether; antioxidant; antimicrobial

#### 1. Introduction

Natural products from plants are under immense consideration in the organic world. One such utilization is of essential oils that are complex volatile compounds extracted from leaves, twigs, flowers, seeds, branches, buds, fruits, and roots. These secondary compounds are then stored in secretory cells, glandular trichomes, canals, cavities, and epidermal cells [1]. Use of essential oils (EOs) is expeditiously increased because of their antibacterial, antiviral, antifungal, insecticidal [1], anti-inflammatory [2], and antimicrobial traits [3]. EOs also have antidepressant and anxiolytic activities [4]. These characters are possessed by certain chemical compounds, mainly phenolics, alkaloids, flavonoids, glycosides, and flavonoids [5].

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**Copyright:** © 2021 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). The Myrtaceae family would rank among the most important families of plants which are enriched with essential oils. Genus Melaleuca is one of the prominent members of the family Myrtaceae that is also well known for its essential oils. *Melaleuca* is indigenous to Australia yet can adjust into several agroclimatic conditions. The volatile oil product of this genus is familiar to the world with the name of tea tree oil (TTO). The species that are under commercial use for essential oil production are *Melaleuca alternifolia*, *M. cajuputi*, *M. bracteata*, and *M. quinquenervia* [6]. Plant extract including oil was used by Aboriginals and was also utilized by soldiers as a general antimicrobial and insect repellent in World War II [7]. Today, TTO is gaining popularity in different industries and being used in aromatherapy, herbal and allopathic medicines, cosmetics, and as natural biocide, fungicide, and preservative. The oil is slightly yellow colored to transparent with a strong camphor-like odor and menthol-like cooling effect [8,9]. The composition of TTO has almost 100 different chemicals, predominantly monoterpenes (terpinen-4-ol, terpinolene, p-cymene,  $\alpha$ -pinene,  $\gamma$ -terpinene, 1,8-cineole), sesquiterpenes, and their respective alcohols (monoterpene, alcohol-terpineol) [10].

In Pakistan, the *Melaleuca* genus has been cultivated in the Punjab region for a long time, but its properties and products are yet not investigated here. *Melaleuca bracteata* is one of the species that is well adapted to the agroclimatic conditions of Pakistan [11]. Tea tree oil contains antioxidant, cytotoxic, antifungal, insecticidal, and medicinal properties [12–14]. Reports have shown methyl eugenol and phenylpropanoids to be the prime components of tea tree oil from *M. bracteata*. Moreover, it can be inferred from literature that TTO can be used effectively by the horticulture sector as a chemical-free fungicide, bactericide, and for enhancement of the shelf life and vase life of fruits, vegetables, and flowers. Hence, it is necessary to test the effectiveness and appropriate use of this essential oil so it can be extracted and employed by the local industry more cost-effectively. Therefore, this study aims to investigate the local adapted species of tea tree oil for more exploration and assessment of this essential oil.

#### 2. Materials and Methods

#### 2.1. Plant Material

Fresh plant material including leaves and twigs were collected from the University of Agriculture Faisalabad and Qadir Baksh Nursery Farms Faisalabad, Pakistan (31.43° N, 73.07° E). The sample for extraction was prepared by clipping leaves and young twigs into smaller pieces.

#### 2.2. Extraction Method

Essential oil was extracted from Melaleuca via the hydrodistillation technique at the Department of Chemistry, University of Agriculture Faisalabad, Pakistan (31.43° N, 73.07° E). The unit consisted of a distillation flask/tank, condenser, and thermometer. About 8–10 kg of plant material was added in flask and submerged into 12 L of water. Then, the set up was airtightly closed, and the process was started at 250 °C temperature. The procedure continued for 4–5 h. Oil was collected in a separating funnel. The %age yield of the extracted oil (v/w) was calculated by the following formula [15], and then the oil was stored at a cool dry place [16].

Essential Oil Yield % = Volume of extracted oil/Fresh weight of plant material (g)  $\times$  100. (1)

#### 2.3. Analysis of Tea Tree oil by GCMS

The extracted oil samples were subjected to compound analysis via the gas chromatography–mass spectrometer technique. The detector of Agilent 5975C was used, and it was provisioned with 7890A GC and column of HP-5MS with 30 m length, 250 micrometer width, and 0.25 micrometer internal diameter with a temperature of 450°C. The oven program was kept on, and the equilibrium time for the oven was 0 min. The program was run for 36 min, and the temperature of the heater was 240 °C. The inert gas

used as a carrier was Helium with a flow rate of 0.7 mL/min. Mass spectrometry was performed in EMV mode at relative voltage of 59eV and scan range from 50 to 550amu. The mass spectrometer was equipped with a gold standard quadrupole analyzer at 150 °C (max 200 °C). The temperature of MS source was 240 °C (max 250 °C). The Agilent MSD Productivity ChemStation6 was the software which identified the compounds receiving signals at the same time from both detectors [15].

#### 2.4. Antioxidant Analysis

#### 2.4.1. DPPH Radical Scavenging Activity

Radical scavenging activity is the ability of an antioxidant compound to protect cells from free radicals produced during normal cell metabolism. To check this character of tea tree oil, DPPH (2,2-diphenyl-1-picrylhydrazyl) scavenging activity was carried out. For this purpose, 1.0 mL at 0.3 M of 1,1-diphenyl-2-picrylhydrazyl was added to the 2.5 mL solution of the sample and gallic acid standard. Standards of gallic acid were prepared at 20, 40, 60, 80, and 100 ppm. Then, all the test tubes were incubated at room temperature for 20 min and kept in the dark. After that, absorbance was recorded at 518 nm by spectrophotometer (T60 U Spectrophotometer, PG Instruments Ltd., Leicestershire, UK). DPPH 100 ppm was used as control treatment, and absorbance was recorded as Abs control. A blank experiment of 80% ethanol was run as well, and absorbance was calculated as Abs blank. The DPPH scavenging activity was determined from the following expression [17].

DPPH scavenging activity (%) =  $100 - \{[(Abs sample-Abs blank) \times 100]/Abs control]\}$ 

#### 2.4.2. Total Flavonoid Contents

Flavonoid compounds are antioxidants present in plants and all fruits and vegetables. To evaluate this in tea tree oil, 1.0 mL of samples and catechin standards at 20, 40, 60, 80, and 100 ppm were added in 4.0 mL of distilled water. The solution was prepared in a volumetric flask of 10 mL capacity. After this, 5% NaNO<sub>2</sub> weighing 0.3 mL was mixed in the above prepared solution. 1 M NaOH (2 mL) was added after 1 min, and later, after 5 m, 0.3 mL of 10% AlCl<sub>3</sub> was also mixed. After 60 s, 2 mL of 1 M NaOH was added to make the total volume up to 10 mL by deionized distilled water. We mixed it thoroughly, and absorbance was observed at 510 nm using reagent blank. Catechin equivalent in milligram per gram dry plant material was used to present the total flavonoids in TTO [18].

#### 2.4.3. Total Phenolic Compounds

To estimate the phenolic compounds of tea tree oil, gallic acid standards at 20, 40, 60, 80, and 100 ppm were used. To 1.0 mL volume of both samples and standards, 5.0 mL of Folin-Ciocalteu (Fisher chemicals) and 4 mL of Na<sub>2</sub>CO<sub>3</sub> (7% w/v) were added with continuous shaking. Then, all the solutions were kept in dark for 30 min, and later on, absorbance was detected at 765 nm with the help of a spectrophotometer (T60 U Spectrophotometer, PG Instruments Ltd., Leicestershire, UK). A blank sample used was a reagent solution. A gallic acid equivalent (GAE) was used to express the amount of total phenolics based on milligram per gram of plant dry weight [19].

#### 2.4.4. Reducing Power Ability (RPA) of the Plant

It is determined by adding 1.0 mL gallic acid standard solutions and sample to 0.2 molar solution of phosphate buffer solution (2.3 mL) at 6.6 pH. Then, 2.5 mL of 1% potassium ferricyanide ( $K_3$ [Fe(CN)<sub>6</sub>]) was added to the solution. Incubation of the mixture was done at 37 °C for 20 m. Then, 10% trichloroacetic acid (2.5 mL) was mixed and then centrifuged at 1000 rpm for 10 m. The supernatant with a 2.5 mL volume was mixed with distilled water and 0.5 mL of 0.1% FeCl<sub>3</sub>. After 10 m, absorbance was determined at 700 nm using a spectrophotometer (T60 U Spectrophotometer, PG Instruments Ltd., Leicestershire, UK) [20].

#### 2.5. Antimicrobial Analysis

The inhibitory zone (IZ) assay of tea tree oil was tested on microbes using the disc diffusion method as proposed by Al-Abd et al. [21]. Firstly, an agar plate was prepared by spreading the microbe, then paper disks of antimicrobials were added. This method is mostly used to determine the best antibiotic against a new or drug-resistant pathogen. The zone of inhibitions was measured with the help of a transparent scale in millimeters [22]. The bacterial strains tested by this procedure were *Bacillus subtilis* and *Bacillus cereus*. The fungal strains evaluated against tea tree oil were White rot and *Aspergillus flavus*.

#### 2.6. Statistical Analysis

The lab experiments were conducted through a completely randomized design (CRD). Data were analyzed using *Statistix 8.1* software (https://www.statistix.com/ (accessed on: 9 February 2020)), and mean analysis was conducted through Tukey's honestly significant difference test on a 0.05 level of probability [23].

#### 3. Results and Discussion

#### 3.1. Essential Oil Yield

Yield of essential oil via the hydrodistillation method was about 0.2%, as shown in Table 1. The volume of extracted oil could vary based on the agroclimatic and geographical conditions of the area. A study by Siddique et al. [24] reported the %age yield of *Melaleuca bracteata* to range at  $0.14 \pm 0.01\%$  in Pakistan. The percentage yield of extracted oil of each hydrodistillation batch is shown in Table 1.

Table 1. % age Yield of tea tree oil from *M. bracteata* leaves.

Batch No.	% Age Yield
1	$0.25\pm0.017$
2	$0.2\pm0.017$
3	$0.15\pm0.015$

#### 3.2. GCMS Analysis

The GCMS analysis of tea tree oil from *M. bracteata* showed the 7 peaks as shown in Figure 1. Table 2 indicates that methyl eugenol is the supreme compound of tea tree oil extracted from *Melaleuca bracteata* species. It represents 96% of the total, and the other six contents were found as just trace elements. The other elements in order of percentage are p-Mentha-2,8-diene-1-ol, Methyl cinnamate, Germacrene D, Terpinolene, Linalool, and alpha-phellandrene. Previous literature has also proclaimed methyl eugenol as the major component of *M. bracteata* oil, with 86.5% of the total volume [24,25]. The latter also indicated methyl cinnamate as the second highest compound, representing 11% of the total. However, another team of scientists documented the percentage of methyl eugenol to be greater than 95% in the tea tree oil of *bracteata*, which is quite related to the present finding [26].

**Table 2.** GC–MS analysis of tea tree oil from Melaleuca bracteata.

Peak No.	Identified Compounds	Retention Time (min)	% of Total
1	alpha-phellendrene	8.568	0.49%
2	p-Mentha-2,8-diene-1-ol	9.278	0.92%
3	Terpinolene	11.071	0.64%
4	Linalool	11.595	0.50%
5	Methyl cinnamate	19.359	0.77%
6	Methyl eugenol	19.897	96.02%
7	Germacrene D	20.663	0.67%

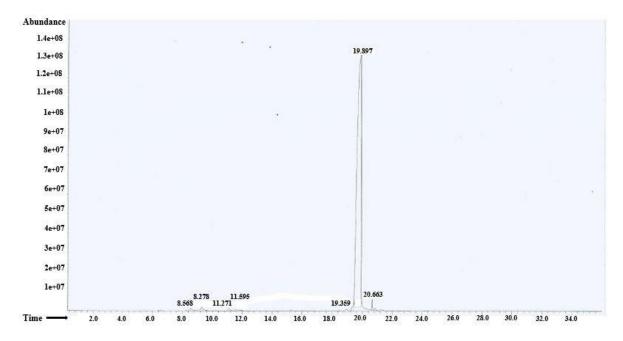


Figure 1. Chromatogram of TTO by GCMS analysis.

Methyl eugenol (ME) belongs to phenylpropanoids, and its occurrence is found in 450 species of plants. In nature, ME is known for plant chemical defense as an antifungal, antibacterial, antinematode and has toxic activities against insect pathogens [27]. The oil from Echinophora sibthorpiana (Apiaceae) also has methyl eugenol. Kivanc [28] found that its oil ( $\sim 0.1\%$ ) and ME at 0.05-0.1 % have negative effects on the growth of fungi and bacteria. Later, Kivanc and Akgul [29] identified that eugenol methyl ether at 1000 ppm also detained mycelium and spore formation of Aspergillus and Penicillium strains. It has also been found to inhibit fungal infestation in peanuts by Sudhakar et al. [30]. ME has been shown to act as a floral synomone in the coevolution of orchid species in the genus Bulbophyllum with fruit flies. It was recognized as the best insect repellent and most effective in knockdown and killing effects among the major constituents of EOs [31]. The importance of methyl eugenol can also be inferred from the findings of Rossi et al. [32] that volatile oil from carrot was successfully evaluated to inhibit the growth of Campylobacter jejuni, a major gastroenteritis-causing bacterium across the globe. Methyl eugenol, a component of carrot oil, was also found effective separately at a minimum inhibitory concentration of 250 µg/mL.

#### 3.3. Biochemical Assays

The oil extracted from *Melaleuca* species exhibited competent antioxidant properties. The result is supported by Table 3, which shows the antioxidant values of *M. bracteata* oil along with the mean and standard error values.

 Table 3. Average antioxidant values of TTO from Melaleuca bracteata.

Replication No.	TFC Value (mm/g)	TPC Value (mm/g)	RPA Value (mm/g)	DPPH %Age
R1	955.67	108.945	300.79	89.13
R2	942.16	108.945	314.88	86.95
R3	955.67	112.145	307.81	84.45
Mean	951.16	110.01	307.83	86.84
S.D	7.80	1.84	7.04	4.22
S.E	4.50	2.60	4.06	2.43

TFC: total flavonoid contents, TPC: total phenolic compounds, RPA: reducing power ability, DPPH: 2,2-diphenyl-1-picrylhydrazyl.

#### 3.3.1. DPPH Scavenging Activity

In Table 3, the average scavenging activity of tea tree oil is displayed. DPPH values for *M. bracteata* oil ranged at  $86.848 \pm 2.43 \text{ mg/mL}$  from the three replicates.

#### 3.3.2. Total Flavonoid Contents

From Table 3, it is inferred that TTO has a relatively higher amount of flavonoids, i.e.,  $951 \pm 4.50 \text{ mg/mL}$ . Flavonoids are produced in plants as a response to oxidative pressure and photodamage via secondary metabolic processes [33,34].

#### 3.3.3. Total Phenolic Compounds of Tea Tree Oil

The TPC of tea tree oil were exploited with respect to the gallic acid standard. The slope expression obtained from the standard curve was used to calculate the concentration of phenolics as a gallic acid equivalent (GAE) in mg/g of plant dry weight basis. Table 3 represents a higher concentration of phenolics, i.e., 110 mg/g of the dry weight of *Melaleuca bracteata* plant.

#### 3.3.4. Reducing Power Ability

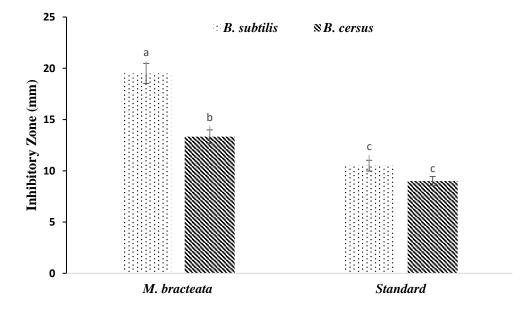
Antioxidants reduce Fe<sup>3+</sup> to Fe<sup>2</sup>, i.e., ferricyanide complex to the ferrous form. This transformation is observed at 700 nm [35]. The reducing ability is amplified with increased absorbance [36]. RPA values were evaluated as equivalent to the standard. About 308 mg/g of the dry weight of the plant was found in *Melaleuca bracteata* oil. A study by Hou et al. [37] provided evidence regarding the antioxidant potential of *Melaleuca bracteata*. When extraction was done with conventional methods, the total phenolic compounds found were 88.6  $\pm$  1.3 mg, and the total flavonoids were 19.4  $\pm$  0.2 mg. The DPPH scavenging activity was high at 86  $\pm$  0.3%. After conditions were optimized, all the values of antioxidants were heightened and found to be at their peak values of 98.7  $\pm$  1.2 mg, 21.6  $\pm$  0.3 mg, and 94.7  $\pm$  0.8% for TPC, TFC, and DPPH, respectively. Siddique et al. [24] also reported that scavenging activity in *M. bracteata* ranged between 35.3 and 89.2  $\pm$  0.4%. This potential increased with the increase of concentration of oil from 20 to 100 µg/mL.

Tea tree oil is an effective antioxidant agent as its commercial species, i.e., *Melaleuca alternifolia*, has also been observed to be enriched with this potential by several reports. One such is the study of Noumi et al. [38] in which DPPH scavenging activity was recorded at 12.5  $\mu$ g/mL expressed as IC50 values (the concentration of antioxidant at which 50% of the reaction was inhibited). The power of TTO as an antioxidant was also found to be better than that of common synthetic antioxidant butylated hydroxytoluene (BHT) at 30 mM. Previous researchers proposed TTO as a good alternative to maintain the oxidative stability of the food matrix. Additionally, when compared with other natural oxidants such as vitamin C, vitamin E, and quercetin, *Melaleuca alternifolia* oil expressed strong free radical scavenging properties and inhibited lipid peroxidation [39]. This is because of the presence of phenols in this oil, which reduce the aerobic oxidation of organic matter [40]. Hence, it is concluded that the present detection of antioxidants in tea tree oil from *Melaleuca bracteata* is in accordance with previous reports of several scientific studies, and this species could also be explored as an antioxidative essential oil along with its patent plant species (*Melaleuca alternifolia*).

#### 3.4. Antimicrobial Properties

*Bacillus subtilis* and *Bacillus cereus* were tested against tea tree oil from *Melaleuca bracteata*. The zone of inhibition was measured in millimeters for all treatments. It is the measure of a clear region with no microbial growth around the paper disc on the agar surface. The clear area represents the resistance or the effective inhibition of microbial growth by antimicrobial agent. The larger the region is, the greater the antimicrobial effect of the agent. the effect of tea tree oil on the growth of *Bacillus subtilis* and *Bacillus cereus* is elaborated on in Figure 2. The zone of inhibition obtained by the application of *M. bracteata* 

oil was 19.5 and 13 mm  $\pm$  0.43. The oil was efficient in controlling bacterial growth better than standard treatment.



**Figure 2.** Zone of inhibition calculated from *M. bracteata* oil sample and standard against *Bacillus* species.

*Melaleuca* species possess good antibacterial effects against *Bacillus* strains. Previously, in a finding by Goswami et al. [41], the zone of inhibition for *B. subtilis* by *M. bracteata* oil was measured to be 6–7 mm, which showed a moderate antifungal effect of essential oil on the bacterial strain. The growth of *B. subtilis* subsp. *Spizizenii* was also inhibited by this oil species at 250 µg/mL up to 44 mm zone of inhibition [24]. EO from this species was also found to be effective against *Staphylococcus aureus* strains, *S. epidermidis, Klebsiella pneumonia*, and *Streptococcus mutans* when tested by Goswami et al. (2017) [41]. The MIC of *M. alternifolia* oil counted for *Bacillus cereus* was 0.3% (v/v) by Griffin et al. [42]. Previous literature reported that TTO has a bactericidal activity against *Staphylococcus* species, *Lactobacillus, Actinomyces viscosus*, and *E. coli* [43–46]. The antimicrobial traits of *M. alternifolia* have been employed to its terpinen-4-ol compound [47,48]. Moreover, terpinen-4-ol and methyl eugenol from both the species have been recognized to have efficient antibacterial, antifungal, and antinematode properties [14,27].

#### 3.5. Antifungal Properties

The potential of fungicidal effects of tea tree oil was evaluated by measuring its zone of inhibition for *Aspergillus flavus* and *Phanerochaete chrysosporium* (White rot). From Figure 3, it is inferred that the growth of White rot fungi and *A. flavus* was restricted up to 11.67 mm and 10.5 mm by the application of oil from *M. bracteata* species, respectively.

Shin [49] has documented the antifungal aspects of commercial TTO from *M. alternifolia* against *Aspergillus flavus*. The concentration required for the effective control on fungus ranged between 0.3 and 0.7 (% volume by volume). The oil has been found to be effective against other *Aspergillus* species such as *A. niger* with a minimum inhibitory concentration of 0.016 to 0.4 (%volume/volume). The effective antifungal agent is terpinen-4-ol in this oil. Moreover, the zone of inhibition formed by methyl eugenol from the oil of another *Melaleuca* species was  $24.3 \pm 0.3$  mm for pure oil and  $46 \pm 0.6$  mm at 100 µg/mL of oil against *Fusarium oxysporum* [50]. Homeyer et al. [51] observed that within 15 min of exposure, TTO reduced the log of *Fusarium oxysporum* up to -4 as compared to control treatment.

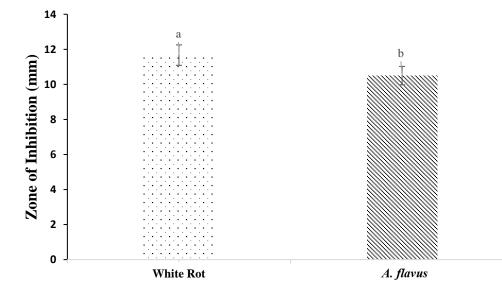


Figure 3. Zone of inhibition calculated from *M. bracteata* oil against White Rot and Aspergillus flavus.

## 4. Conclusions

The present study aimed at the extraction of essential oil from *Melaleuca bracteata* to explore its antioxidant and antimicrobial properties. It has been found from the results that the yield percentage for essential oil is about 2% through the hydrodistillation method, and it is enriched with eugenol methyl ether. A high antioxidant activity is observed when measured on scales for total flavonoids, total phenolics, reducing power ability, and DPPH. The laboratory application of TTO on microbes reduces their activity. Thus, tea tree oil from *Melaleuca bracteata* species has significant potential as an antioxidant and antimicrobial to be used as a natural microbicide in the food and agriculture industries. The *Bracteata* species is well adapted to Pakistan and specifically the Punjab region's climate. Thus, it must be exploited by the local industry to produce raw and refined oil products to commercialize its use in horticulture, pharmaceuticals, and cosmetics.

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# A Comprehensive Appraisal of the Wild Food Plants and Food System of Tribal Cultures in the Hindu Kush Mountain Range; a Way Forward for Balancing Human Nutrition and Food Security<sup>†</sup>

Abdullah Abdullah <sup>1</sup>, Shujaul Mulk Khan <sup>1,2,\*</sup>, Andrea Pieroni <sup>3,4</sup>, Aminul Haq <sup>5,6</sup>, Zahoor Ul Haq <sup>1,7</sup>, Zeeshan Ahmad <sup>1</sup>, Shazia Sakhi <sup>8</sup>, Abeer Hashem <sup>9,10</sup>, Al-Bandari Fahad Al-Arjani <sup>9</sup>, Abdulaziz A. Alqarawi <sup>11</sup> and Elsayed Fathi Abd\_Allah <sup>11</sup>

- <sup>1</sup> Department of Plant Sciences, Quaid-i-Azam University, Islamabad 45320, Pakistan; abdullahkhan@bs.qau.edu.pk (A.A.); zahoornaturalist@gmail.com (Z.U.H.); zeeshanahmad78@vmail.com (Z.A.)
- <sup>2</sup> Pakistan Academy of Sciences, Islamabad 45320, Pakistan
- <sup>3</sup> University of Gastronomic Sciences Pollenzo, Piazza V. Emanuele II, I-12042 Bra/Pollenzo, Italy; a.pieroni@unisg.it
- <sup>4</sup> Department of Medical Analysis, Tishk International University, Erbil 44001, Kurdistan, Iraq
- <sup>5</sup> Department of Botany, University of Peshawar, Peshawar 25130, Pakistan; aminulhaq\_bot@yahoo.com
- Department of Botany, GPGC Khar Bajaur 18650, Pakistan
- <sup>7</sup> Department of Botany, SBBU Sharingal Warai campus Dir, Sheringal 18200, Pakistan
- <sup>8</sup> Center for Plant Sciences and Biodiversity, University of Swat, Mingora 19130, Pakistan; shaziasakhi@hotmail.com
- <sup>9</sup> Botany and Microbiology Department, College of Science, King Saud University, Riyadh 11451, Saudi Arabia; habeer@ksu.edu.sa (A.H.); aalarjani@ksu.edu.sa (A.-B.F.A.-A.)
- <sup>10</sup> Mycology and Plant Disease Survey Department, Plant Pathology Research Institute, ARC, Giza 12511, Egypt
- <sup>11</sup> Plant Production Department, College of Food and Agricultural Sciences, King Saud University,
- Riyadh 11451, Saudi Arabia; alqarawi@ksu.edu.sa (A.A.A.); eabdallah@ksu.edu.sa (E.F.A.) \* Correspondence: smkhan@qau.edu.pk
- † This article is dedicated to the memory of Habib Ahmad (TI), Emeritus of Hazara University, Pakistan & Fellow of the Pakistan Academy of Sciences who passed away on 7 April 2021; Habib was an extraordinary scholar and great human being and he has represented an irreplaceable academic guide for generations of young botanists, plant ecologists, and agricultural scientists across the globe.

Abstract: The tribal belt of the Hindu Kush mountains is famous for its unique culture, ethnography, wild food plants, food systems, and traditional knowledge. People in this region gather wild plants and plant parts using them directly or in traditional cuisine, or sell them in local markets. However, there is a huge lack of documentation of the food system, particularly that related to wild food plants (WFP). In the current study, we focus on the uses and contributions of WFPs in the traditional tribal food system of the Hindu Kush valleys along the Pakistan-Afghanistan border. Ethnobotanical data were gathered through questionnaire surveys of 84 informants, including 69 men and 15 women, belonging to 21 different villages of the chosen area. In tribal societies men and women rarely mix and thus very few women took part in the surveys. We documented 63 WFP species belonging to 34 botanical families, of which 27 were used as vegetables, 24 as fruits, six in different kinds of chutneys (starters), and six as fresh food species. Fruits were the most used part (41%), followed by leaves (24%), aerial parts (24%), seeds (7%), stems (3%), and young inflorescences (1%). The reported uses of Carthamus oxyacantha, Pinus roxburghii seeds, and Marsilea quadrifolia leaves are novel for the gastronomy of Pakistan. The results reveal that WFPs provide a significant contribution to local food systems and play a role in addressing human nutritional needs, which are usually not met through farming practices. The tribal peoples of the Hindu Kush use WFPs for their nutritional value, but also as a cultural practice—an inseparable component of the tribal community's lifestyle. This important traditional knowledge about the gathering and consumption of WFPs, however, is eroding at an alarming rate among younger generations due to the introduction of fast-food, modernization, and globalization. Therefore, appropriate strategies are imperative not only to safeguard traditional

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**Copyright:** © 2021 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). plants and food knowledge and practices, as well as the cultural heritage attached to them, but also to foster food security and thus public healthcare via local wild foods in the region.

**Keywords:** gastronomy; livelihood; public healthcare; traditional knowledge; wild food plants; valleys of the Hindu Kush mountain range

# 1. Introduction

Wild food plants (WFP) have played a crucial role in food systems since the beginning of human civilization [1]. Wild food plants refer to the non-cultivated plant species that are gathered or harvested by local communities from their surrounding ecosystems for utilization in their food systems [2]. Ecosystems in the tribal belt are diverse, being composed of plains, forests, and pastures, providing habitat to various kinds of wild fruits and vegetables [3]. Not only are these WFPs important from a nutritional point of view, but they have important medicinal value [4-7]; various WFPs have been evaluated for their pharmacological properties in many other parts of the world [5,8,9]. Recently, a number of WFPs have been identified as "functional foods" due to their nutritional value. They provide a healthy diet and can contribute to the prevention of some illnesses [10,11]. According to researchers, most WFPs are a rich source of bioactive compounds, such as essential fatty acids, vitamins, and complex sugars [11–13], and can contribute significantly to the alleviation of malnutrition [3]. Their importance in income generation, poverty reduction, nutritional balance, food security, and agricultural diversification has been emphasized in several studies [14,15]. One of the major contributions of WFPs is their use in food security by providing alternative food sources and food diversity in traditional communities [16]. Wild food plants play a crucial role in human survival during times of famine [17] and food shortages; recent examples include their use during the COVID-19 pandemic crisis, and the geopolitical and tribal unrest around the world.

Very few studies have been conducted to document food plants in Pakistan and the area under consideration in this article has never been studied before. Such comparisons are imperative as the Hindu Kush receives less summer precipitation than the Himalayas which gives rise to different kinds of WFPs.

Pakistan is a lower-middle-income country and the 6th most populous in the world [18]. It experiences all four seasons and has a wide diversity of natural resources, especially plants [19], and it still ranks as the 11th most food insecure country in the world [20]. About 60% of the people in the country are food insecure [18]. Food insecurity increases in the tribal belt of the country due to conflicts, distance from cities, and the prevailing dry climate. The Pakistani–Afghan border in the Hindu Kush mountain range has always been the center of tension due to its geopolitical position. Man-made catastrophes, the dramatic increase in the human population, limited access to food, and local livelihood strategies are the other major reasons for food insecurity and poverty in the tribal belt [21]. Wild food plants are an important resource for poor local communities and can be of tremendous help in alleviating hunger and malnutrition if properly managed and utilized. To date, little research has been conducted on the wild vegetable and fruit species used in the food systems of Pakistan in general and Pakistani–Afghan border regions in the Hindu Kush mountain range in particular [16,19,22–28]. The book by Abbasi et al. [29] on the ethnobotanical and nutraceutical aspects of the wild edible vegetables of the Lesser Himalayas is one of the major contributions in this regard.

#### Tribal Culture

The people of the region practice and enjoy a centuries-old culture known as Pashtunwali or Pukhtunwali. Pashtunwali is a very common cultural heritage of the tribal communities of Pathans living along the Pakistani–Afghan border in the Hindu Kush mountain range. It is a way of life or legal social code of conduct that governs their lives. According to [30] it is a 2000–3000 years old tradition that remains the central pillar of tribal societies. In this era of modernization, Pashtunwali is perceived as culturally idiosyncratic, significantly influencing perceptions, behaviors, and thoughts in everyday life [31,32]. Code of honor (Nang), hospitality (Melmastia), and revenge (Badal) are the three core pillars of Pashtunwali [33]. The code of honor, or self-respect and dignity, is a key component and characteristic of Pashtun society. Melmastia is a sum of conventions and an immediate reward from local societies regarding local cultural values, while Badal is like a debt which ends with revenge. These three cultural customs are the sum of tribal communal expectations from members of their society, as well as from outsiders [34]. These customs are related to WFPs in that Pathans offer food to each other and safeguard their natural resources. Although the tribal societies living along the Pak-Afghan border in the Hindu Kush mountain range are well known for their unique traditional food system, it has not been explored by researchers to document the valuable traditional knowledge about WFPs. The Bajaur area is the most populous, climatically varied, and ethnographically rich in the tribal belt of the Hindu Kush mountain range and it exemplifies the culture and traditional knowledge of the entire Pak-Afghan border and thus it was selected for the current study. It is a region composed of relatively dry valleys in the Hindu Kush mountain range. As for the food system in the tribal belt, especially in the area under investigation, it is worth mentioning that people are financially poor and most of the time they use WFPs. They eat at home most of the time, and the practice of hoteling and eating out in restaurants or other food points is uncommon. All of this strengthens the ties between people and wild food resources, including plants. Climate is one of the main drivers affecting all facets of life including wild food and food systems, with considerable variation in the different administrative units (tehsil) of the study area, i.e., Barang in the south, Nawagai and Chamrkand in the southwest, and Mamund, Salarzai, and Utmankhel tehsils in the north and northeast. Local communities collect wild vegetables and fruits from side valleys, mountains, and cultivated fields. They know the best and most suitable gathering seasons of wild vegetables using traditional ecological knowledge (TEK). The proper time and method of collecting various WFPs is crucial in terms of nutritional value. Women usually cook them in traditional tribal styles. The demand for wild vegetables and fruits increases in different seasons when cultivated fruits and vegetables are not available. Bearing in mind the importance of WFPs in the food system of tribal communities, the current study was designed to (1) document the diversity of WFPs gathered and consumed by tribal communities; (2) assess traditional knowledge about WFPs, their role and contribution in nutrition and the traditional tribal food system; and (3) compare the Hindu Kush valleys with other areas in regard to wild food plants.

# 2. Methodology

#### 2.1. Study Area

The study region is a tribal area that remained part of the Federally Administered Tribal Areas (FATA) up to 2018. The tribal areas of Pakistan form a long narrow belt along the historical Durand Line (Pakistani–Afghan border). Federally Administered Tribal Areas consisted of six frontier regions (FR), i.e., Kohat, Bannu, Tank, Dera Ismail Khan, Peshawar, and Lakki Marwat, and seven agencies, namely Bajaur, Mohmand, Khyber, Orakzai, Kurram, North Waziristan, and South Waziristan. According to certain writers [35], in the mid-19th century the British faced difficulties and challenges in tax collection and effective control over the tribal belt and, thus, devised a legal and administrative system known as the Criminal Tribes Act of 1871. The law was "designed for the registration, surveillance, and control of certain tribes" [36] that was later enacted under the frontier crimes regulation (FCR). This remained in effect in the tribal belt until 2018. In that year, the government of Pakistan merged the whole tribal belt of FATA into the mainstream province of Khyber Pakhtunkhwa in the hope of promoting harmony and development following the drafting of the constitution of Pakistan. The area selected for the current study is a mountainous territory, with an area of 1296 km<sup>2</sup>, situated in northwestern Pakistan, located at 34–51° N

latitude and 71–31° E longitude [37]. It shares a 52-km-long border with Afghanistan west of the Panjkora River, possessing three important passes, i.e., Nawa Pass, Ghakhi Pass, and Letaisar [38]. These three passes play a significant role in trade and the sharing of traditional knowledge between both countries. The research area is mainly inhabited by the Utmankhel and Tarkanri tribes, which speak different dialects of Pashto. The Utmankhel tribe resides in the southeast, bordering the districts of Malakand, while the Tarkanri tribe lives in the northwest on the Pakistani-Afghan border of the Hindu Kush mountains. The Utmankhel tribe is divided into the sub-tribes of Alizai, Shamozai, Aseel, Gharshamozai, and Mandal [39]. The Tarkanri tribe comprises the sub-tribes Mamund, Salarzai, Safi, Khalji, and other sub-ethnic groups who live in the former state of Khar [40]. According to Sher 2019, [40], the Utmankhels are descendants of the Kirlan tribe of Waziristan who migrated to the valleys of Arang and Barang in Bajaur during the 12th century. On the other hand, the Tarkanri people are descendants of Afghani Pashtuns. Before the events of 11 September 2001, people of both areas used to visit the two countries easily for various purposes. In short, Afghan culture has had a great impact on the Tarkanri people. Most of the people of the district live in rural parts of the area (Figure 1).

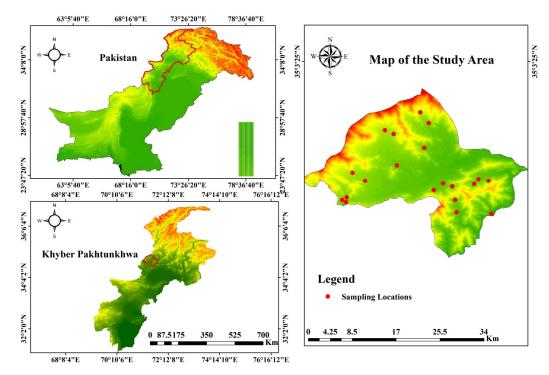


Figure 1. Map of the study area, showing the 21 studied villages.

The district has many natural resources, such as different kinds of stones, including marble, and other minerals. Local communities are involved in the extraction of marble, limestone, manganese, chromites, and talc, among other mineral resources. Geographically, the study area lies at the end of the Hindu Kush mountain range which creates considerable disparity and uncertainty in the pattern of monsoon rains from year to year. The climate of the district varies from extremely hot to bitter cold while traveling from the southeast to the northwest. The weather remains pleasant for most of the summer, but the temperature drops below freezing during winter. The area receives about 800 mm of annual rainfall. Variation in the climatic conditions of the area leads to a considerable diversity of plants and, thus, WFPs [38]. The people are mostly farmers, and a few others are teachers, drivers, cobblers, and businessmen. A considerable number of local people work in other parts of the country or abroad and depend on remittances for their livelihood. The local people have been collecting WFPs for nutritional and ethnomedicinal purposes for countless generations [41,42].

# 2.2. Data Collection

In the current study, 21 different villages were selected for interviews and gathering data on WFPs using a random table method where 3 villages from each of the tehsils (administrative units) of the study area were selected for questionnaire data collection (Table 1). Two visits to each village were arranged for this purpose between March and November 2016. Interviews were conducted following the code of ethics of both the International Society of Ethnobiology and the American Anthropological Association [42]. Knowledgeable informants were selected through the snowball sampling method [43–45]. A total of 84 informants were interviewed (four from each village) including 69 men and 15 women. The age of the informants ranged from 17 to 85 years, while education level ranged between illiterate to university degree (Table 2). The interviews were conducted in the Pashto language. Participants were asked about the local names, parts used, collection times, recipes and modes of utilization, uses in other dishes, and the sale or marketing of the WFPs species they gathered in the past and still gather today [45]. The first author comes from the study area and has family ties with a few of the villages studied, which helped considerably in gathering data about the uses of WFPs and selecting the informants. Each interviewee was verbally informed that the data gathered would be published and shared globally, which could help them by garnering international interest in their traditional knowledge and use of food plants, as well as the plants they associate with.

S No	Tehsil Name	Population	Tribes	Studied Villages	Latitude N °,′,″	Longitude °,′,″	Altitude (Meters)
				Arang Bagh	34 42 49. 42 N	71 39 13.01 E	1005
1	Utmankhel	107,356	Shamozai	Taoheed Abad	34 43 21 .73 N	71 41 38.56 E	1156
				Ghazi Baba	34 43 39.08 N	71 39 57. 31 E	941
				Kamal Dara	34 38 02.82 N	71 36 17. 07 E	906
2	Barang	76,558	Aseel	Nazar Mina	34 40 07. 65 N	71 36 02 .20 E	1013
				Targhao	34 37 47 .60 N	71 42 07. 79 E	688
			Alizai,	Batai	34 42 23. 92 N	71 35 32.97 E	994
3	Khar	247,510	Mamund and	Shinkey	34 42 56.00 N	71 34 00. 74 E	931
			Salarzai	Sara Mena	34 41 46.70 N	71 32 26.70 E	963
				Dara	34 48 55. 51 N	71 30 50.10 E	964
4	Salarzai	268,517	Salarzai and Mashwani	Gabar Cheena	34 54 52. 93 N	71 30 10. 15 E	1276
			Washwan	Tofann Shah	34 53 05. 17 N	71 31 36.50 E	993
				Karkani	34 44 45.15 N	71 18 46.86 E	1161
5	Chamarkand	2868	Sahibzadgan, Molyan	Kotkai	34 43 21.57 N	71 20 53.18 E	1060
			10101 y ult	Lar Chamarkand	34 41 12. 54 N	71 12 45. 37 E	1057
				Nawagai	34 40 07.88 N	71 17 02. 82 E	959
6	Nawagai	79,002	Safee and Miangan	Sro Gato Miagan	34 40 32. 89 N	71 17 48.55 E	1004
			mungun	Khanano Kalay	34 39 45. 39 N	71 17 39.80 E	1032
			Mamund and	Barkhalozo	34 51 14. 67 N	71 25 41.36 E	1185
7	Mamund	311,873	Wara	Umarey Mamund	34 45 58.62 N	71 26 13.95 E	955
			Mamund	Mena	34 51 52.66 N	71 24 13.47 E	959

Table 1. The geographical position of the 21 studied villages in the seven tehsils of the study area.

Lite	racy/Education Level of the Inform	ants
Literacy level	No. of informants	Percentage
Illiterate	39	46.43
Primary	21	25.00
Middle	13	15.48
Secondary	7	8.33
University	4	4.76
	Age Ranges of the Interviewees	
Age group	No. of informants	Percentage
17–27	7	8.33
28–37	9	10.71
38–47	16	19.05
48–57	15	17.86
58–67	13	15.48
68–77	15	17.86
78–85	9	10.71

Table 2. Ages and literacy levels of the informants in the study area.

The interviewees were asked about the settlement they live in, as well as their age, place of birth, and occupation. They were also asked the following questions: (1) What wild vegetable species do you collect? (2) What wild fruit species do you collect and use? (3) What species do you use in sauces, salads, and chutneys (4) What WFPs do you use as raw foods (5) What wild herbal drinks do you consume? (6) What wild edible seeds do you collect and use? (7) Do you collect WFPs for marketing purposes? (8) How frequently do you use the plant in a season? (9) What are the names of WFPs in your language? (10) Which parts do you use? (11) What recipes you prepare? (12) What is the best season for collecting? (13) What traditional dishes are made with these WFPs? (14) Did you use any WFPs in the past which you did not mention previously? Thematic analysis was carried out on the basis of our interviews and observations. All the data based on these questions were arranged in tabulated form. After interviewing four people in each village, a short field excursion with the interviewees was arranged to collect all available WFPs.

Collected plant specimens were identified with the help of the Flora of Pakistan [46] and the Wild Plants of the Swat Valley [47], and then plants names were cross-checked against the Plant List database [48]. Plants uses were also compared with previous studies [16,19,22–29,47].

Secondary data were also gathered by collecting and reading local as well as scientific literature about the local names, cuisines, and tribal food systems for finalizing the article in hand.

#### 2.3. Data Analysis

Data on WFPs, i.e., botanical name, local name, plant family, used parts, growth habit, mode of utilization, marketing, use reports, relative frequency of citation, and previous literature that reported their use, were gathered via a questionnaire and literature survey.

#### 2.3.1. Relative Frequency of Citation (RFC)

The data on wild food plants, collected during the questionnaire survey, were also analyzed qualitatively via the relative frequency of citation (RFC) to highlight the local significance of each WFP species in the region following [5,49]:

$$RFC = \frac{FC}{N} (0 < RFC < 1)$$
(1)

where FC denotes the number of informants mentioning a specific WFP species, while N is the total number of informants interviewed during the survey.

# 2.3.2. Classification of WFPs Based on Mode of Consumption

All WFPs were grouped into different food categories on the basis of consumption mode following Thakur et al 2017 [3]. Those plant species cooked as food were categorized as vegetables, for example, *Caralluma tuberculata, Malva neglecta,* and *Digera muricata*. Species that were consumed without cooking were classified in the category of fruits dry or fresh, i.e., *Ficus carica, Morus alba,* and *Celtis caucasica*. Another category consisted of plants used, together with salt and spices, to make sauces, salads, and chutneys, for example, *Mentha longifolia, Mentha royleana,* and *Zanthoxylum armatum. Salvia moorcraftiana, Saccharum bengalensis,* and *Silene conoidea* were considered as raw food species, or those plants, other than fruits, whose parts were consumed raw without cooking.

#### 3. Results

#### 3.1. Tribal Food System and WFPs

Tribal people have a unique and simple food system primarily based on WFPs, dairy products, and cereal crops they cultivate in their fields. Almost every family in the study area raises domesticated animals (sheep, goat, cow, or buffalo) to meet their dairy requirements [50]. They customarily collect WFPs while grazing their animals. They usually have a piece of land where they cultivate local vegetables and crops. They prepare and consume various kinds of cultural foods from these three abovementioned sources. Some of the well-known simple foods of this tribal region include Jawaro Neeny (roasted corn seeds), Ghanamo Neeny (wheat grains are roasted and then a little brown sugar is added to form small balls), Dhal (ground wheat grains are boiled with brown sugar and butter oil), Gongrhi (boiled wheat grains), Shedano rotay (wheat flour is mixed with cow oil and Carthamus oxyacantha seeds to make a bread that is baked in a tandoor), and Kakori (wheat flour is mixed with peanuts, resin, walnuts, coconut, and sugar to make a special type of bread). Kakori is prepared mostly when an infant start walking for the first time. Ghunzakhi (wheat flour mixed with vegetable oil, sugar, and *Cuminum cyminm* seeds) is a special type of sweet prepared and gifted mostly by women when they move from their mother's house to their in-law's house. Elderly individuals cook lentils and pulses in a unique traditional style: when the dish is prepared a cup or more of butter oil is added. This dish is offered at special cultural gatherings and ceremonies, such as Ashar. Ashar is a centuries-old tradition of the Pathans in which members of a local community gather to help each other in their work without consideration of compensation, monetary or otherwise. Unfortunately, today, this important cultural tradition is on the verge of extinction due to urbanization, modernization, and globalization. These traditional foods are considered an important feature and significantly contribute to the tribal food system. Tribal people of the study area living in valleys and the foothills collect WFPs to fulfill their food requirements. They are wholly or partially dependent on WFPs resources.

#### 3.2. WFPs and Their Taxonomic Categorization

Overall, 63 folk taxa belonging to 34 botanical families were recorded. A high proportion of these WFPs belong to the Amaranthaceae and Leguminosae families (five species each), followed by Rhamnaceae, Rosaceae, Lamiaceae, and Polygonaceae (four species each), and then Brassicaceae and Moraceae (three species each). The most dominant WFP categories included vegetables (27 species), fruits (24 species), chutneys and sauces (7 species), and raw food species (six species). Field observations showed that the vegetable category was mainly composed of weeds locally called *Gayyah* (unwanted plant species occurring in or along crop fields). The traditional communities gathered these species for their own food uses, as well as that of their animals.

# 3.3. Traditional Knowledge Related to Gathering WFPs

Wild food plants were reported to be gathered from diverse localities, including agricultural fields, foothills, and forests. The diversity and availability of plants vary with seasonal changes. Local people possess traditional knowledge regarding the seasonal availability and time of collection of WFPs [51]. Spring (March–May) and Summer (June–October) are the peak seasons for WFPs collection in the study region. Most WFPs are not available from November to February due to the cold temperatures [24]. Leaves and pot herbs grow well in April and August, while the best time for fruits ranges from June to October. Different vegetable species remain available for 2 to 9 months, whereas certain fruit species, such as *Sideroxylon mascatense*, *Punica granatum*, *Vitis jacquamontiana*, *Morrus*, as well as *Ficus* and *Zizphus* species, are only available for a few months.

#### 3.4. Shepherds and Their Role in Gathering WFPs

Shepherd communities, in search of grasses and fodder for their cattle, migrate from one region to another in a cyclic manner. They graze their sheep, goats, and cows on hills and in the mountains and collect WFPs for their own dietary needs. They are totally or partially dependent on WFPs and homemade dairy products.

One elderly participant (81-year-old man) belonging to the Utmankhel - Alizai tribe mentioned his view about the role of WFPs in the food system of shepherds and grass collectors in these words: "In our time, when we used to visit the mountains for cattle grazing or grass and fuelwood collection, and we felt hungry, we ate mint (*Mentha longifolia*) and heart wing sorrel (*Rumex hastatus*) leaves with green chilies, onions, tomatoes, and prickly ash (*Zanthoxylum armatum*) seeds with wheat or barley bread. We ground these on big rocks with the help of small round stones along streams or springs. We used the creeping wood sorrel (*Oxalis corniculate*) as an alternative to *Rumex hastatus* leaves sometimes. Some people used to eat brown sugar with wheat or barley bread". Today, this practice of eating WFPs has decreased due to the modernization, globalization, and urbanization of communities.

#### 3.5. WFPs and Their Uses in Traditional Cuisine

Our findings show that fruits were the most commonly used parts (41%) of WFPs, followed by leaves (24%), aerial parts (24%), seeds (4%), stems (3%), and young inflorescences (2%), (Figure 2). The use of various parts varied from species to species and area to area within the region. Leaves and aerial parts were mostly used as vegetables, in sauces, or consumed raw, while fruits were mostly eaten raw. In terms of growth habit, the recorded WFPs were categorized as 57% herb, 22% tree, 19% shrub, and 2% climber species.

#### 3.5.1. Vegetable Species

During the questionnaire survey, local people were asked (1) what wild vegetable species they collect? In response to our question, interviewees mentioned 27 species used as vegetables in the studied villages. According to the use reports, the most cited and used vegetable species were *Nasturtium officinale* (47), *Oxalis corniculata* (44), *Trifolium repense* (44), *Solanum villosum* (37), *Digera muricata* (31), *Portulaca quadrifida* (29), *Malva neglecta* (28), *Vicia sativa* (27), *Cichorium intybus* (21), and *Caralluma tuberculata* (19). *Nasturtium officinale*, locally called *Termera*, was the most used vegetable species in the region and considered a digestive. The species was collected by local communities beside water bodies such as streams, springs, ponds, and lakes from March to November. It was favored for its unique taste and availability. *Oxalis corniculata* was the most readily available species, which grows in the shade of other plants or humid places from March to December. Its widespread availability, long growing season, and special taste made it one of the most consumed vegetable species. *Trifolium repense* is a semi-domesticated species that grows alongside

water bodies. It was widely consumed as a vegetable throughout the region. For example, Chokanr, or *Trifolium repense* leaves with rice, was a very popular traditional dish across the whole tribal belt. *Solanum villosum, Solanum americanum, Descurainia sophia*, and *Sisymbrium irio* leaves and young shoots were boiled in concentrated milk and offered to patients as well as older people as a digestive.

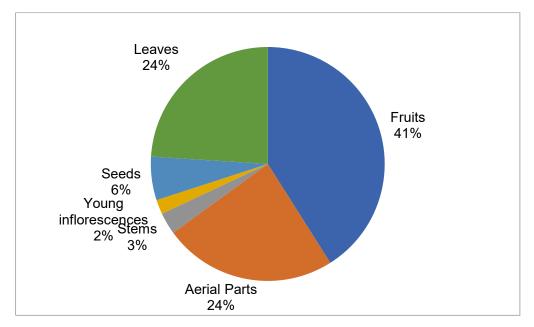


Figure 2. Contribution of the WFP's parts used in the tribal food system.

# 3.5.2. Fruit Species

In total, 24 species were collected from the wild and consumed raw by local inhabitants. According to the use reports, the most important wild fruit species were *Ficus carica* with 64 use reports, followed by *Berberis lyceum* (58), *Myrtus communis* (53), *Olea ferruginea* (48), and *Sideroxylon mascatense* (48). *Ficus carica* was a very common species in the study region and local people collected its fruits in the morning to avoid the hot weather, as it bears fruit during the hottest months of June and July. *Berberis lyceum* fruits were collected and mostly consumed raw, although some people also used the fruit to make a refreshing juice. *Sideroxylon mascatense*, which occurs in the low elevation hills, was another important fruit species of the study area. People collected ripe *Sideroxylon* fruits in large bottles or pots to maintain their shape and avoid bruising the fruit.

#### 3.5.3. Sauces and Chutneys

In response to our questionnaire, informants reported only six species used in making chutney. The custom of herbal sauce or chutney making was not very common throughout the region but included the use of *Mentha longifolia* (40), *Mentha royleana* (34), *Cuminum cyminum* (21), *Zanthoxylum armatum* (37), *Allium ampeloprasum* (14), and *Thymus linearis* (7). Interestingly, all these species are aromatic and were used in fresh and dried forms as well. The local people shade-dried these species in their respective seasons for utilization throughout the year.

#### 3.5.4. Species Used Raw

This category was also not common in the region. Fresh raw food species were collected from the wild and just masticated or chewed for their flavor or [52] to quench thirst or suppress appetite. Interviews confirmed only six plants, including the young shoots of *Saccharum benghalensis* (17), the fruits of *Silene conoidea* (22), the young shoots of *Salvia moorcraftiana* (9), and the aerial parts of *Indigofera* species (3), were consumed in the

region. Additionally, the seeds of *Carthamus oxyacantha* and *Pinus roxburghii* were used as food.

# 3.5.5. WFPs Used in Herbal Teas, Drinks, and Decoctions

*Myrtus communis, Mentha royleana,* and *Mentha longifolia* were used in herbal teas and decoctions. *Myrtus communis* leaves were boiled with green tea and *Mentha royleana* or *Mentha longifolia* were used to prepare herbal drinks. These drinks were used to treat vomiting, diarrhea, heartburn, and other stomach-related problems. The local people kept it in dry form in their houses for off-season uses. *Berberis lyceum, Punica granatum,* and *Morus nigra* were used in fresh drinks. The juices of *Berberis lyceum* and *Punica granatum* were considered important drinks in the area. Indigenous people used these plants for cooling the body and quenching thirst. *Berberis* fruits have a very short period of availability and may be available in the market for only 20–30 days. On the other hand, *Punica granatum* fruits were stored by local communities. They stored it in grain bins and used it for a few months. *Morus nigra* is very common and produces large amounts of fruits, but its juice was not common in the study area. Very few informants reported its use as an herbal drink.

## 3.6. Summary of WFP Cuisine

Various recipes for cooking WFPs were recorded during the interviews with local informants. The traditional communities used WFPs in different ways, and the knowledge of these plants and their mode of consumption were passed down from their ancestors. There were different methods used for the preparation of wild foods depending on the nature of the plant. Wild vegetables, i.e., Amaranthus vridis, Amaranthus crispus, Chenopodium album, Rumex dentatus, Rumex histatus, Malva neglecta, Digera muricata, Nasturtium officinale, Lathyrus aphaca, Vicia sativa, Medicago polymaorpha, Medicago denticulata, Portulaca quadrifida, Cichorium intybus, Polygonum aviculare, Polygonum plebium, Solanum villosum, Solanum americanum, and Marsilea were cut into small pieces and boiled in water. After boiling these vegetables, onions were fried in oil, and then tomatoes and garlic were added [22]. After the addition of garlic, a special kind of sauce was made in the traditional tribal style, which is locally called Dagh or Tarka. Some people also added green chilies depending on preference and taste. Subsequently, the boiled vegetables were added and heated until the poured oil comes out on top of the dish. Some vegetables, such as Solanum villosum, Solanum americanum, Descurainia Sophia, and Sisymbrium irio, were boiled in concentrated milk [25]. Bitter vegetables like Caralluma tuberculata were cut into small pieces, boiled in water 2-3 times, and then fried in oil with onion, tomatoes, chilies, and condiments. Some people also cooked *Caralluma* with minced meat (Keema/Qeema).

# 3.7. Storage and Uses of WFPs in the Off-Seasons

The storage and drying of WFPs is an important practice that provides stability to the food system throughout the year [24]. In the study area, a number of plants, such as the aerial parts of *Mentha longifolia*, *Mentha royleana*, and *Punica granatum* fruits, were stored by local communities in dried form for consumption during the off-season. Our findings showed that in ancient times, local people used to store fruit of pomegranates in grain bins (which were made of mud), and then they used them during times of need. *Morus alba, Sideroxylon mascatense*, and *Prunus armeniaca* (Kakhta) fruits were dried and used in the winter season. In April, *Allium carollianum* leaves were collected by traditional communities to prepare local spices or masala in their homes. They shade-dried its leaves and then used the dried form with *Curcuma longa* (Korkaman), *Cuminum cyminum*, and *Punica granatum* seeds. The use of *Punica granatum* seeds in spices was a custom of the traditional communities. *Myrtus communis* leaves and fruits were stored in homes and used in lassi and herbal tea for their enticing aroma and appetizing flavor.

#### 3.8. Relative Frequency of Citation

In the present study, RFC values ranged from 0.01 to 0.8. The highest RFC was recorded for Ficus carica (0.8), followed by Myrtus communis (0.7), Berberis lyceum (0.7), Nasturtium officinale (0.6), and Olea ferruginea (0.6) (Table 3). The high RFC values of certain wild fruit species demonstrate their abundant use and extensive knowledge among the tribal communities. Myrtus communis and Berberis lyceum have high RFC values but both species are facing conservation threats in the area. This continuous practice of gathering WFPs has caused an alarming decrease in the populations of some species. On the other hand, due to the cultural and religious importance of certain species, such as Ficus carica and Olea ferruginea, some plants are highly valued and thus distributed widely in the region. Therefore, on the basis of our field observations and interviews, we think that the extensive use of WFPs depends not only on the abundance of a species, but also on local preferences and its high nutraceutical value. However, traditionally people have used such food plants irrespective of their knowledge of the plant's impact on health. Rather than thinking about their health benefits, it has been more customary to use wild plants because of long-established traditions, but such uses are of tremendous benefit to these tribal people.

# 3.9. Selling and Marketing of WFPs

During the interviews, we asked the participants whether they collected or still collect WFPs for marketing purposes. In response, the interviewees reported 10 plant species that are available in the local markets of Bajaur, i.e., Khar, Pashat, Inayt Kalay, Nawagai, Memola, Chenagai, and Tawheed Abad. These 10 marketable species included four wild fruits, five vegetables, and one species used as a flavoring agent. The fruit species included *Sideroxylon mascatense, Berberis lyceum, Ziziphus sativa*, and *Diospyrus lotus. Mentha longifolia, Mentha royleana, Caralluma tuberculata, Spinacia quadrifida*, and *Malva neglecta* were the wild vegetables species, while *Zanthoxylum armatum* was used as a flavoring agent in various traditional dishes. All these species were available fresh in the market except *Sideroxylon mascatense*, whose fruits were available in the market in dried form and during the off-season as well (Table 3).

S. No	Botanical Name of WFPs	Local Name of WFPs	Family	HB	Part Used	Recipes and Mode of Utilization in Cuisines	Contribution to Tribal Food System (Use in Other Cuisines)	Coll. Time	Marketing	UR	RFC	Previously Reported in Pakistan for Food Usages
	Allium car- olinianum DC	Ogai	Amaryllidaceae	н	Leaves	Leaves are used as a salad or fried in oil with tomato and red chilies, and mixed with different ingredients in variety of cousins	i. Chicken Handi, ii. Traditional bread iii. Flavoring agent	Feb-Jun	+	14	0.17	
р	Amaranthus crassipes Schltdl.	Churlai	Amaranthaceae	Н	Leaves	Leaves are boiled in water and then fried in oil with tomato, onion, and red chilies	i. Indian squash ( <i>Praecitrullus</i> <i>fistulosus</i> )	Apr-Dec	ı	×	0.10	
ო 112	Amaranthus viridis L.	Ganar	Amaranthaceae	Н	Leaves	Leaves fried in oil with tomato, onion, and red chilies	i. Pulses ii. Indian squash	Apr-Dec	+	23	0.27	[22,23,25,26,28]
4	Asparagus officinalis L.		Asparagaceae	S	Aerial parts	Leaves boiled in water and then fried in oil with tomato, onion, and red chilies	ı	]unnul	+	13	0.15	
Ŋ	<i>Berberis</i> lyceum Royle	Koaray	Berberidaceae	S	Fruit	Used fresh	i. Juices	Mar-Nov	+	58	0.69	[1,25,27]
9	Caralluma tuberculata N.E.Br.	Pamankay	Asclepediaceae	Н	Stems	Leaves fried in oil with tomato, onion, and red chilies	i. Qeema ii. Meat	Jun-Nov	ı	19	0.23	[22-24,28]
~	Carthamus oxyacantha M.Bieb	Kareza	Asteraceae	Н	Seeds	Seeds are cooked in bread and then the bread is topped with butter oil	i. Caramelized candy	Jul-Sep	ı	13	0.15	
8	Celtis caucasica Willd	Tagh	Cannabaceae	Г	Fruit	Taken as fruit	ı	Mar-Jun	ı	27	0.32	[24,26,27]

	Previously Reported in Pakistan for Food Usages	[19,22,24,25,28]	[23,25,28,29]		[28]	[25]	[26,27]	[16,26,27]	[16,27]
	RFC i	0.13 [	0.25	0.23	0.10	0.37	0.40	0.76	0.26
	UR	11	21	19	8	31	34	64	22
	Marketing	ı	ı	·	ı	ı	+	+	
	Coll. Time	Mar-Jun	Apr-Jul	Apr-May	Mar-Jun	Jun-Oct	Oct-Jan	Jun-Aug	May-Sep
	Contribution to Tribal Food System (Use in Other Cuisines)	i. Pulses ii. Yogurt	i. Pulses ii. Rice	i. Meat ii. Pulses iii. Rice iii. Custard iv. Spices, etc.	i. Milk ii. Pulses	i. Pulses ii. Potatoes iii. Rice			i. Jams ii. Local ice cream iii. Custard iv. Sweets
Table 3. Cont.	Recipes and Mode of Utilization in Cuisines	Leaves boiled in water and then fried in oil with tomato, onion, and red chilies	Leaves boiled in water and then fried in oil with tomato, onion, and red chilies	Seeds used for aroma and taste in various traditional foods	Leaves boiled in water and then fried in oil with tomato, onion, and red chilies	Boiled leaves are fried in oil with tomato, onion, and red chilies	Taken as fruit	Taken as fruit	Taken as Fruit
	Part Used	Leaves	Leaves	Seeds	Leaves	Leaves	Fruit	Fruit	Fruit
	HB	Н	Н	Н	Н	Н	Т	Т	Н
	Family	Amaranthaceae	Asteraceae	Apiaceae	Brassicaceae	Amaranthaceae	Ebenaceae	Moraceae	Saxifragaceae
	Local Name of WFPs	Sarmay	Kashni	Zeera	Jenjar	Sur gulay	Tor amlook	Inzar	Da zamkay toot
	Botanical Name of WFPs	Chenopodium album L.	Cichorium intybus L.	Cuminum cyminumL.	<i>Descurainia</i> sophia (L.) Webb ex Prantl	Digera muricata (L.) Mart.	Diospyros lotus L.	Ficus carica L.	<i>Fragaria</i> <i>nubicola</i> (Lindl. ex Hook.f.) Lacaita
	S. No	6	10	113	12	13	14	15	16

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							Table 3. Cont.						
	S. No	Botanical Name of WFPs	Local Name of WFPs	Family	HB	Part Used	Recipes and Mode of Utilization in Cuisines	Contribution to Tribal Food System (Use in Other Cuisines)	Coll. Time	Marketing	UR	RFC	Previously Reported in Pakistan for Food Usages
	17	Indigofera spp.	Gedarghog	Leguminosae/ Fabaceae	S	Aerial parts	Flowers and leaves are eaten raw	ı	Mar-May	ı	б	0.04	
	18	Lathyrus aphaca L.	Korkaman	Leguminosae/ Fabaceae	Н	Aerial parts	Boiled leaves are fried in oil with tomato, onion, and red chilies	i. Pulses ii. Rice	Mar-Jun	I	17	0.20	[23,25]
114	19	Malva neglecta Wallr.	Panderak	Malvaceae	Н	Leaves	Boiled leaves are fried in oil with tomato, onion, and red chilies	Mixed with i. Rice ii. Potatoes iii. Pulses iv. Yogurt v. Beef	Mar-Nov	+	28	0.33	[22,23]
	20	Marsilea quadrifolia L.	Chopatra	Marsilleaceae	Н	Leaves	Boiled leaves are fried in oil with tomato, onion, and red chilies	i. Rice ii. Potatoes iii. Pulses	May–Jul	1	3	0.04	
	21	Medicago polymorpha L.	Speshtaray	Leguminoseae	Н	Aerial parts	Boiled leaves are fried in oil with tomato, onion, and red chilies	i. Rice ii. Potatoes iii. Pulses iv. Yogurt v. Beef	Mar–Jun		17	0.20	[22,23,25]
	22	Mentha longifolia L.	Podina	Lamiaceae	Н	Aerial parts	Leaves and young shoots are eaten as a salad	i. Flavoring agent ii. Rice iii. Custard iv. Sweets, etc.	Mar-Nov	+	40	0.48	[19,22,23,28]
	23	<i>Mentha</i> royleana Wall. ex Benth	Enalay	Lamiaceae	Н	Aerial parts	Leaves and young shoots are eaten as a salad	i. Flavoring agent ii. Rice iii. Custard iv. Sweets, etc.	Mar-Nov	Υ	34	0.40	[22,23]
	24	Morus nigra L.	Tor toot	Moraceae	H	Fruit	Taken as fruit	i. Juices ii. Jams iii. Syrup	Apr-Jul	ı	23	0.27	[16,26,27]

	Previously Reported in Pakistan for Food Usages	[16,26,27]	[16,26,27]	[53]	[22,54]	[22,23,25]	[16,24,26,27]	[16,27]	[22,23,25,27,28]
	RFC	0.33	0.06	0.63	0.13	0.56	0.57	0.06	0.52
	UR	28	ß	53	11	47	48	Ŋ	44
	Marketing	ı	ı	+	ı	ı	,		
	Coll. Time	Apr-Jul	Jul-Sep	Sep-Nov	Sep-Oct	Mar-Nov	Aug-Oct	Sep-Oct	Mar-Dec
	Contribution to Tribal Food System (Use in Other Cuisines)	i. Juices ii. Jams	1	i. Flavoring ii. Rice iii. Curd iv. Milk v. Custard vi. Sweets, etc.	ı	i. Potatoes ii. Pulses	ı	ı	i. Rice ii. Potatoes iii. Pulses
Table 3. Cont.	Recipes and Mode of Utilization in Cuisines	Taken as fruit	Taken as fruit	Taken as fruit	Taken as fruit	Boiled leaves are fried in butter oil with tomato, onion, and red chilies	Taken as fruit	Taken as fruit	Boiled leaves are fried in oil with tomato, onion, and red chilies
	Part Used	Fruit	Fruit	Fruit	Fruit	Leaves	Fruit	Fruit	Aerial parts
	НВ	F	S	S	N	Н	Н	S	Н
	Family	Moraceae	Primulaceae	Shrub	Arecaceae	Brassicaceae	Oleaceae		Oxilidaceae
	Local Name of WFPs	Spin toot	Manrogayah	Myrtaceae	Mianzarai	Tarmera	Khona	Tafnra	Trewakay
	Botanical Name of WFPs	<i>Morus alba</i> L.	Myrsine africana L.	Myrtus communis L.	Nannorrhops ritchieana (Griff.) Aitch	Nasturtium officinale R. Br	Olea ferruginea Wall. ex Aitch	<i>Opuntia</i> dillenii (Ker Gawl.) Haw.	Oxalis corniculata L.
	S. No	25	26	27	80 77 15	29	30	31	32

	Previously Reported in Pakistan for Food Usages			[27]	[25,28]	[22]	[23–25]	[24,26,27]	[22]	[16,22,24]
	RFC i	0.06	0.10	0.25	0.10	0.07	0.35	0.32	0.10	0.05
	UR	Ŋ	8	21	8	6	29	27	8	4
	Marketing	I	I	ı	ı	ı	+	ı	ı	ı
	Coll. Time	Mar-May	Dec-Feb	May–Jun	Mar-Nov	Mar-Nov	Jun-Sep	Sep-Oct	Dec-Feb	Aug-Oct
	Contribution to Tribal Food System (Use in Other Cuisines)	i. Rice ii. Potatoes iii. Pulses	ı	i. Jams	i. Rice ii. Potatoes iii. Pulses	i. Rice ii. Potatoes iii. Pulses	i. Beans ii. Rice iii. Potatoes iv. Pulses	i. Juices ii. Spices iii. Rice	i. Fried and mixed with corn grains (rarely used)	,
Table 3. Cont.	Recipes and Mode of Utilization in Cuisines	Boiled leaves are fried in oil with tomato, onion, and red chilies	Seeds are eaten raw	Taken as fruit	Boiled leaves are fried in oil with tomato, onion, and red chilies	Boiled leaves are fried in oil with tomato, onion, and red chilies	Boiled leaves are fried in oil with tomato, onion, and red chilies	Taken as fruit	Taken as fruit	Taken as fruit
	Part Used	Leaves	Seeds	Fruit	Aerial parts	Aerial parts	Aerial parts	Fruit	Fruit	Fruit
	HB	Н	F	F	Н	Η	Н	F	F	s
	Family	Papaveraceae	Pinaceae	Rosaceae	Polygonaceae	Polygonaceae	Portulacaceae	Punicaceae	Fagaceae	Rosaceae
	Local Name of WFPs	Reday	Nakhtar	Zardalo	Bandakay	Bandakay	Warkharay	Anangoray	Serai	Khwrach
	Botanical Name of WFPs	Papaver rhoeas L.	Pinus roxburghii Sarg.	Prunus armeniaca L.	Polygonum aviculare L.	Polygonum plebejum	Portulaca quadrifida L.	Punica granatum L.	Quercus baloot Griff	Rosa moschata Herrm
	S. No	33	34	35	96 116	37	38	39	40	41

	sly 2d n for ges	_			,25]				
	Previously Reported in Pakistan for Food Usages	[19,27]	[16,24]	[23,25]	[19,22,23,25]			[24]	[24,54]
	RFC	0.27	0.10	0.18	0.13	0.20	0.44	0.11	0.51
	UR	23	8	15	11	17	37	6	43
	Marketing	I	I	ı	I			+	ı
	Coll. Time	Jul-Sep	Jul-Sep	Mar-Jun	Mar-Nov	Mar-Apr	May–Jun	Mar-Apr	]un–Jul
	Contribution to Tribal Food System (Use in Other Cuisines)	i. Juices	i. Juices	i. Rice ii. Potatoes iii. Pulses	i. Rice ii. Potatoes iii. Pulses		·	ı	,
Table 3. Cont.	Recipes and Mode of Utilization in Cuisines	Taken as fruit	Taken as fruit	Boiled leaves are fried in oil with tomato, onion, and red chilies	Boiled leaves are fried in oil with tomato, onion, and red chilies	Young inflorescences eaten raw before blooming	Taken as fruit	Young stems eaten raw	Taken as fruit
	Part Used	Fruit	Fruit	Leaves	Leaves	Young inflores- cences	Fruit	Stems	Fruit
	HB	S	S	Н	Н	Н	S	Н	F
	Family	Rosaceae	Rosaceae	Polygonaceae	Polygonaceae	Poaceae	Rhamnaceae	Lamiaceae	Sapotaceae
	Local Name of WFPs	Karwara	Karwara	Tarookay	Shalkhay	Sharghashay	Momanra	Kharghwag	Gorgora
	Botanical Name of WFPs	Rubus fruticosus G.N.Jones	Rubus ulmifolius Schott	Rumex hastatus D. Don	Rumex dentatus L.	Saccharum benghalen- sis Retz	Sageretia thea (Osbeck) M.C. Johnst	Salvia moor- croftiana Wall. ex Benth.	Sideroxylon mascatense (A.DC.) T.D.Penn.
	S. No	42	43	44	42 117	46	47	48	49

	Previously Reported in Pakistan for Food Usages	[19,22,25]	[22,28]	[23,24,27]	[22]	[25]	[24]	
	RFC	0.26	0.21	0.08	0.44	0.15	0.27	0.15
	UR	22	18		37	13	23	13
	Marketing	ı	ı	ı	ı	ı	ı	ı
	Coll. Time	Mar-May	Mar-Jun	Mar-Sep	Mar-Sep	Mar-May	May-Oct	Jun-Sep
	Contribution to Tribal Food System (Use in Other Cuisines)	ı	i. Rice ii. Potatoes iii. Pulses iv. Milk	i. Rice ii. Potatoes iii. Pulses	i. Rice ii. Potatoes iii. Pulses	i. Rice ii. Potatoes iii. Pulses	i Meat ii Pulses iii Rice iii Custard iv. Spices, etc.	i. Potatoes
Table 3. Cont.	Recipes and Mode of Utilization in Cuisines	Taken as fruit	Taken as fruit	Leaves boiled in water and then fried in oil with tomato, onion, and red chilies	Leaves boiled in water and then fried in oil with tomato, onion, and red chilies	Leaves boiled in water and then fried in oil with tomato, onion, and red chilies	Aerial parts used in spices	Leaves boiled in water and then fried in oil with tomato, onion, and red chilies
	Part Used	Fruit	Leaves	Aerial parts	Aerial parts	Aerial parts	Aerial parts	Leaves
	HB	Η	Н	Н	Н	Н	Н	Н
	Family	Caryophylaceae	Brassicaceae	Solanaceae	Solanaceae	Caryophyllaceae	Lamiaceae	Amaranthaceae
	Local Name of WFPs	Mangotay	Khob kalan	Kachmacho	Kachmacho	Khorenakay		Ghana
	Botanical Name of WFPs	Silene conoidea L.	Sisymbrium irio L.	Solanum ameri- canum Mill	Solanum villosum Mill.	Stellaria media (L.) Vill.	Thymus linearis Benth.	Trianthema portulacas- trum L.
	S. No	50	51	25	ር <u>ና</u> 18	54	55	56

	Previously Coll. Time Marketing UR RFC in Pakistan for Food Usages	May-Oct - 44 0.52 [22]	Mar-Jun - 27 0.32 [19,28]	Jul-Aug + 13 0.15 [16,27]	Mar-Jun - 37 0.44 [16,26,27]	Aug–Nov + 19 0.23 [16,24,26,27]	Aug-Sep - 12 0.14 [16,24,27]	Beera Rhamnaceae T Fruit Taken as fruit - Oct-Nov - 17 0.20 [24,26,27,54]
	Contribution to Tribal Food System (Use in Other Cuisines)	i. Rice ii. Potatoes iii. Pulses iv. Yogurt	i. Meat ii. Spinach iii. Rice iv. Indian squash	i. Juices	i. Curd ii Pulses iii Rice iii Custard iv. Spices etc.	·	·	,
Table 3. Cont.	Recipes and Mode of Utilization in Cuisines	Leaves boiled in water and then fried in oil with tomato, onion, and red chilies	Legume is eaten raw or fried in oil with tomato, onion, and red chilies	Taken as fruit	Seeds are powdered and then mixed with curd, mint leaves, tomato, and red chilies to make chutney	Taken as fruit	Taken as fruit	Taken as fruit
	Part Used	Aerial parts	Fruit	Fruits	Seeds	Fruit	Fruit	Fruit
	HB	Н	Н	L/S	F	S	Τ	H
	Family	Leguminosae	Leguminoseae	Vitaceae	Rutaceae	Rhamnaceae	Rhamnaceae	Rhamnaceae
	Local Name of WFPs	Shaftal	Arwarai	Gedarkwar	Dambara	Enalai	Markhanrai	Beera
	Botanical Name of WFPs	Trifolium repens L.	Vicia sativa L.	Vitis jacque- montii R. Parker	Zanthoxylum armatum DC.	Ziziphus oxyphylla Edgew.	Ziziphus jujuba Mill	Ziziphus nummula- ria (Burm.f.) Wight and Arn
	S. No	57	58	59	60	61	62	63
				119				

HB = Habit, H = Herb, S = Shrub, T = Tree, Coll. Time = Collection Time, UR = Use reports, RFC = Relative Frequency of Citation, + = Marketable, - = Nonmarketable.

# 3.10. Storage and Uses of WFPs in the Off-Season

A few of these plant species are available in the market and contribute significantly to the local economy.

Inhabitants of rural areas collect and sell WFPs in the local markets for income generation. Abbasi et al. [25] reported that *Dryopteris ramosa*, *Bauhinia variegata*, *Chenopodium album*, *Portulaca quardifida*, *Nasturtium officinale*, *Malva parviflora*, and Solanum nigrum are sold in the markets of Rawalpindi and Abbottabad, while we did not report the market value for any of these species. According to the findings of Abbas et al. [22], wild vegetable species available in the local markets of Kurram district include *Caralluma tuberculata*, *Mentha spicata*, *Lepidium draba*, *Rumex dentatus*, *Portulaca oleracea*, *Malva neglecta*, *Trifolium repens*, *Stellaria media*, and *Nasturtium officinale*. In the current study, we documented *Caralluma tuberculate* (Figure 3), *Malva neglecta*, and *Mentha* species, which is similar between the two study areas. *Rumex dentatus*, *Malva neglecta*, *Trifolium repens*, *Stellaria media*, and *Nasturtium officinale* were very common species and used by tribal communities as vegetables, but we did not observe them in the markets.



**Figure 3.** (a) Fruit of *Morus nigra*, (b) Fruit of *Morus alba*, (c) a young man collecting fruit of Mazri Palm, (d) *Caralluma tuberculata*, (e) a young man collecting different wild vegetables, and (f) Seeds of *Carthamus oxyacantha*.

#### 4. Discussion

# 4.1. The Reported WFPs Compared to Previous Studies

We studied the contribution of WFPs to the food system and balancing the nutrition of tribal communities living along the Pakistan-Afghanistan border in the Hindu Kush mountain range. Vegetables were the dominant use category of WFPs across the study region (with 27 different species). Similar studies have also been carried out by other authors on wild vegetables and their nutritional value. For example, Abbas et al. [22] reported 53 wild vegetable species from the Kurram district. Likewise, Abbasi et al. [25] documented 45 wild vegetables from the Lesser Himalayas of Pakistan. In comparison to our study area, the district of Kurram and area of the Lesser Himalayas have a rich diversity of wild vegetable species due to greater rainfall and thus suitability for the growth of WFPs. Aziz et al. [19] reported 21 vegetables used in the remote Yasin and Ishkoman valleys of Gilgit Baltistan, while Ahmad et al., 2019 [23], reported 25 wild vegetable species from Northwest Pakistan. The areas of the Pakistani–Afghan border in the Hindu Kush mountains are characterized by dry and harsh climatic conditions which are quite similar to the climatic conditions of the Yasin and Ishkoman valleys and Northwest Pakistan. Therefore, those areas are home to a small number of wild vegetable species. Another study [28] reported 59 wild vegetables from the district of Harnai Baluchistan. The traditional communities of Harnai district use various species that were not mentioned in our study area, such as *Boerhavia procumbence*, *Erodium cicutarium*, and *Fumaria indica*. This disparity in the use of wild vegetable species may be due to the unavailability of food resources or ethnic differences. On this basis, we believe that the use of wild vegetables varies from area to area due to their availability, the climatic conditions, and ethnography.

In this study, fruits were the second most dominant category with 24 species among the recorded WFPs. On the other hand, Abbasi et al. [16] recorded 35 wild fruit species used by the tribal communities of the Lesser Himalayas, and Khan et al., 2015 [27] recorded 47 species of wild fruits from the Swat Valley. The Lesser Himalayas and Swat Valley exhibit a greater diversity of plants and receive more precipitation during the monsoon season compared to the study region. The area of the Hindu Kush including Bajaur, receives very little precipitation during the summer monsoon. These climatic factors distinguish this area from Swat Valley and the Lesser Himalayas in terms of fruit flora. Ahmad and Pieroni [24] studied 31 wild fruit species from the Thakhte-Sulaiman Hills, Northwest Pakistan. The Thakhte-Sulaiman hills are characterized by a dry and harsh climate with mostly bushy and thorny fruit plants such as Berberis calliobotrys, Ziziphus species Cotoneaster microphyllus, Cotoneaster minutus, and Cotoneaster pruinosus. On the other hand Perez-Negron and Casas 2007 [54] documented 11 wild fruit species from the district of Dera Ismail Khan. Dera Ismail Khan is also characterized by very harsh climatic conditions and is considered a hub for *Phoenix dactylifera* (Dhaki variety) and Nannorrhops ritchieana, especially in the Abdul Khel and Rahmani Khel areas. The Hindu Kush valleys near the Pakistani–Afghan border, including Bajaur, have similar plant taxa such as Sideroxylon mascatense, Nannorrhops ritchieana, and Ziziphus species. In the recent past, ethnobiologists were unwilling to acknowledge the use of Quercus fruits as human food [55], but their consumption is very common in various parts of the world, including Turkey [56,57] and the Iberian Peninsula [55]. Mendez-Baceta et al. [52,55] mentioned that in Gorbeialdea Quercus acorns are considered food for livestock or eaten during times of food scarcity. In the current study, we reported that Quercus acorns were not used as a staple food; however, roasting them is still a hobby in the tribal belt and people come from plain areas to the mountains for acorn collection. Therefore, we suggest that the distribution, diversity, and consumption of wild fruit species vary from region to region depending on their availability, the climatic conditions, nutritional importance, and cultural knowledge.

We also reported various plant species that were used as flavoring agents in sauces and chutneys, i.e., *Mentha* species. Abbas et al. [22] documented that in the Kurram district *Mentha* species were used in salads. Aziz et al. [19] reported that, in the Ishkoman and Yaseen valleys of Gilgit Baltistan, *Thymus linearis* was used in making herbal tea, while in the current study we reported that it was used as a spice. Thus, we believe that variation in the use of WFPs in chutney or sauce making varies from one area to another, perhaps due to ethnography or traditional knowledge. The practice of utilizing raw foods species was also not very common as only six species were reported to be used in raw form. All these plant species were not described as raw food species in other studies previously conducted on the WFPs of Pakistan. *Silene conoidea* was described by Aziz et al., 2020, Abbas et al., 2020, Abbassi et al., 2013b-[19,22,25] as a cooked vegetable species, while in the current study region it was used as a raw food species rather than as a cooked vegetable.

The use of plants in herbal drinks is an important cultural practice among tribal communities. Aziz et al. [19] mentioned that *Carum carvi* seeds, *Elaeagnus angustifolia* bark, and *Thymus linearis* aerial parts were used as seasonings or herbal drinks. In the current research, we reported that the aerial parts of *Thymus linearis* were used as an herbal spice, but not as an herbal drink. The other two species, *Elaeagnus angustifolia* and *Carum carvi*,

were not reported in the study area. There is great variation in the climatic conditions and topography of Gilgit Baltistan and areas of the Hindu Kush mountains. Gilgit Baltistan occurs in the Karakoram mountain range, while areas of Pakistani–Afghan border in Bajaur are part of the Hindu Kush mountain range, which suggests that variation in topographic and climatic factors generates variations in floral diversity and traditional uses of plants among local societies.

The tribal belt of Pakistan has a unique food system. Wild food plants are still used in different ways to improve their taste, nutritional value, and health benefits. The local inhabitants prepared many traditional dishes by mixing WFPs with other wild or cultivated vegetables and other foods, i.e., rice, meat, chicken handi, qeema, pulses, Indian squash, potatoes, traditional bread, milk, yogurt, custards, etc. Various other products were also prepared with WFPs, such as juices, syrups, and jams. *Carthamus oxycanthus* seeds were mixed with brown sugar and heated until caramelized, which is locally called *Kaboray* (caramel candy).

Guarrera and Savo [12] discussed this tradition in a review article titled "Wild food plants used in traditional vegetable mixtures in Italy". While investigating the wild vegetables of the Lesser Himalayas, Abbasi et al. [25] also mentioned that some vegetable species are cooked in a mixture with other vegetables or in concentrated milk. Abbas et al. [22] documented in their study of the wild vegetables of Kurram district that "all the seasonal wild vegetables are combined intermittently and a particular dish of 7 to 10 species is cooked during festivals (Nowruz) in the Shia community". Therefore, we suggest that the consumption of WFPs, especially that of wild vegetables, mixed with other foods is a long-standing tradition of human societies to improve their taste, nutritional value, and health benefits.

#### 4.2. Management and Conservation of WFPs

The territory has a great diversity of wild fruit species, including *Ficus carica*, *Ficus palmata*, *Morus alba*, and *Morus nigra*, which occur in abundance across the region. Many of these plants are not currently harvested by humans, but there is potential to use them to meet important dietary requirements. These plants can be conserved in their natural habitats and some of them domesticated as well. Very few people dry and store these fruits for consumption in other seasons of the year when these fruits are not available. There is no plan for the management and use of these species. In the present scenario of COVID-19 pandemic-driven situations, people rely on plant-based recipes to manage health care-related issues [57] and such research findings may be of vast attention for people in the rural areas.

# 5. Conclusions

The current research represents the first attempt to study the contribution of WFPs to the food system of the tribal belt of Pakistan at the Pak–Afghan border in the Hindu Kush mountain range. The local inhabitants are economically poor and most of the time utilize local food resources at home. The results showed that WFPs are still collected and consumed in the region. In addition to their nutritional value, 10 species have market value and contribute to the economy of local communities. The uses of *Carthamus oxyacantha, Pinus roxburghii* seeds, and *Marsillea* leaves are novel for the gastronomy of Pakistan after comparison with other regions of the country. *Myrtus communis, Berberis lyceum, Prunus armeniaca, Nannorrhops ritchieana, Zanthoxylum armatum,* and *Vitis jacquemontii* are the species facing conservation issues due to overexploitation, soil erosion, and climatic changes.

Knowledge related to the consumption of WFPs is decreasing in the younger generations of tribal societies. Therefore, more studies are needed to conserve this important cultural gastronomic heritage of the study region where modernization, urbanization, and industrialization are at their peak. We believe that this study will play a crucial role in the conservation and fostering of the traditional knowledge of WFPs cooking and consumption. **Author Contributions:** A.A. carried out the fieldwork, collected data and prepared the first draft of the manuscript. S.M.K. supervised the project, helped in data analyses, provided the logistic support, read all the revisions and correspond the paper. A.P. contributed to the study design and to draft the MS. A.H. (Amunul Haq) helped during Field Work. Z.U.H. helped in specimens processing. Z.A. contributed in data analyses and preparation of GIS map. S.S. helped in literature review and preparation of paper draft. A.H. (Abeer Hashem), A.-B.F.A.-A., A.A.A. and E.F.A. helped in revising and improving the manuscript and provided partial financial support for the research activity and manuscript processing fee. All authors work parallel to produce the research article in hand shape. All authors have read and agreed to the published version of the manuscript.

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# Article Analysis of Nutritional Quality Attributes and Their Inter-Relationship in Maize Inbred Lines for Sustainable Livelihood

Sapna Langyan <sup>1,2,\*</sup>, Zahoor A. Dar <sup>3</sup>, D. P. Chaudhary <sup>1</sup>, J. C. Shekhar <sup>1</sup>, Susila Herlambang <sup>4</sup>, Hesham El Enshasy <sup>5,6</sup>, R. Z. Sayyed <sup>7,\*</sup> and S. Rakshit <sup>1</sup>

- <sup>1</sup> ICAR-Indian Institute of Maize Research, PAU Campus, Ludhiana, Punjab 141004, India; chaudharydp@gmail.com (D.P.C.); jcsdmr@yahoo.co.in (J.C.S.); pdmaize@gmail.com (S.R.)
- <sup>2</sup> ICAR-National Bureau of Plant Genetic Resources, Pusa Campus, New Delhi 110012, India
- <sup>3</sup> Dryland Agriculture Research Station, SKUAST, Kashmir 190001, India; zahoorpbg@gmail.com
- <sup>4</sup> Department of Soil Science, Faculty of Agriculture, Pembangunan Nasional Veteran University, Yogyakarta 55293, Indonesia; susilaherlambang@upnyk.ac.id
- <sup>5</sup> Institute of Bioproduct Development (IBD), Universiti Teknologi Malaysia (UTM), Skudai, Johor Bahru 81310, Malaysia; henshasy@ibd.utm.my
- <sup>6</sup> City of Scientific Research and Technology Applications, New Burg Al Arab, Alexandria 21934, Egypt
- <sup>7</sup> Department of Microbiology, PSGVP Mandal's Arts, Science, Commerce College, Shahada 425409, India
  - Correspondence: sapna@icar.gov.in (S.L.); sayyedrz@gmail.com (R.Z.S.)

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**Copyright:** © 2021 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). Abstract: The present investigation was planned to understand the variability and inter-relationship among various nutritional quality attributes of maize kernels to identify potential donors of the respective traits for future hybridization programs. Sixty-three maize inbred lines were processed for the estimation of protein, starch, fat, sugar, 100-kernel weight, specific gravity, and moisture level of the grain. The results reveal that a wide variability among protein, starch, 100-kernel weight, specific gravity, and fat was seen, with special emphasis on the protein concentration that varied from 8.83 to 15.54%, starch (67.43–75.31%), and 100-kernel weight (9.14–36.11 gm). Factor analysis revealed that the protein concentration, starch, and 100-kernel weight, the three major components, comprise 68.58% of the kernel variability. Protein exhibited a significant negative correlation with starch and 100-kernel weight, indicating that an increase in the protein concentration will down-regulate the starch and 100-kernel weight. The inbred lines are proposed as donors for the development of high cultivars for their respective traits, viz., high protein (DMR WNC NY 403 and DMR WNC NY 404), high starch concentration (DMR WNC NY 2163, DMR WNC NY 2219, DMR WNC NY 2234, DMR WNC NY 2408, DMR WNC NY 2437, and DMR WNC NY 2466), high 100-kernel wt. (DMR WNC NY 2113, DMR WNC NY 2213, DMR WNC NY 2233, DMR WNC NY 2234, DMR WNC NY 2414, DMR WNC NY 2435, DMR WNC NY 2465, and DMR WNC NY 2474), sugar (DMR WNC NY 2417), and specific gravity (DMR WNC NY 2418). Genetic distance analysis revealed that DMR WNC NY 397 and DMR WNC NY 404 are the farthest apart inbred lines, having major differences in their protein, fat, starch, and sugar contents, followed by DMR WNC NY 2436 and DMR WNC NY 2394, DMR WNC NY 2212 and DMR WNC NY 2430, DMR WNC NY 396 and DMR WNC NY 2415, DMR WNC NY 404 and DMR WNC NY 2144, and DMR WNC NY403 and DMR WNC NY 2115. Moreover, this study proposes that these possible combinations of lines (in a breeding program) would result in genetic variability with simultaneous high values for the respective characteristics.

Keywords: clustering; correlation; fat; protein; starch; sugar; specific gravity

# 1. Introduction

Food insecurity and hunger affect more than 900 million people worldwide each year. People from poor and underdeveloped and some developing nations are at greater risk. About 5 million hungry people in the world die each year from nutrient deficiency

causes [1,2]. Women and children are more susceptible to nutrient deficiency due to reproduction and growth demands, respectively [3]. Maize (*Zea mays* L.) is one of the world's important cereal grain crops after rice and wheat. The United States, the European Union, China, Brazil, Mexico, and India are the world's leading producers of maize [4]. Its popularity as a crop is largely due to its diverse functionality as a food source for both humans and animals. Maize has been the major source of the world's protein and calories [5,6]; hence, it is the dietary staple food crop for more than 300 million people [7].

India, since independence, has achieved a manifold increase in food grain production owing to the Green Revolution of the late 1960s. Indian agriculture research, until lately, insisted on increasing productivity per se, rather than quality. India's Human Development Index rank of 130 in the year 2020 reflects a major deficiency in the quality of life of people. Various initiatives of the government have largely remained unsuccessful in bringing down the menace of malnutrition. The latest National Family and Health Survey (NHFS2018-19) data released by the government are indicative of the limited progress made in improving the nutritional status and quality of health services for infants and children, adolescent girls, and women [8]. This is because an adequate diet is not affordable and accessible to all; worldwide, 462 million adults are underweight, and around 45% of deaths among children under 5 years of age are linked to undernutrition [9]. India is one of the nations with a large number of tribal people having malnutrition. This warrants an urgent need to produce maize with sufficient nutrient contents, as maize is a staple and principal food crop for the majority of the tribal people [10]. Various strategies to improve nutritional quality include increasing access to various nutrients, food fortification with supplements, bio-fortification, the introduction of pharmaceutical supplements, and dietary diversification [11,12].

Maize (Zea mays L.) is the most widely cultivated crop in all conditions, ranging from tropical to temperate, and regions of the world, providing nutrients as well as raw materials for biomolecules such as starch, fat, and protein [13]. In India, maize occupies a prominent position, and each part of the maize plant is utilized in one or another way, with nothing going to waste [14,15]. The utilization pattern of maize comprises 59% as feed, 17% for industrial purposes, 10% as food, around 10% for export, and 4% for other purposes, including seed [16]. Among all cereals, maize has the highest growth rate with maximum productivity, and due to it possessing the highest genetic yield potential, maize is known as the "queen of the cereals" [17]. The nutritional composition of normal maize comprises 8–13% protein, 68–73% starch, 2–5% fat, 2–4% sugar, fibers, minerals, etc. [18,19]. However, these nutritional attributes are inter-related, and an increase in one may adversely affect the other such as high-fat maize likely having a lower quantity of starch [20,21]. Keeping in view the inter-relationship of nutritional quality parameters when using the information in hybrid breeding programs, the present research work was undertaken to study the variability, the correlation, and the inter-relationship between the nutrient components of 63 different maize inbred lines. Additionally, the genetic distance between 63 different varieties of maize inbred lines was studied in order to select those that have the potential to be used as donor parents for their respective traits for the development of maize cultivars with enhanced protein, sugar, fat, and starch, with a possible contribution to decreasing malnutrition.

#### 2. Materials and Methods

# 2.1. Plant Materials

The inbred lines were grown in an augmented block design (ABD), with 4 rows per inbred line at 60 cm spacing at a length of 3 m at the Winter Nursery Centre, Hyderabad, India, having a temperature range of 7 to 12 °C, sandy soil, 6.5 pH, and low organic matter, during the rabi season. The plants were selfed, and the selfed seeds were used for the biochemical evaluation. Details of the pedigree of the inbred lines are provided in Table 1.

PEDIGREE	Protein (%)	Fat (%)	Sugar (%)	Starch (%)	100-K wt. (g)	Specific Gravity (g/cm <sup>3</sup> )
DMR WNC NY 396	12.39	3.08	3.58	68.46	14.3	1.19
DMR WNC NY 397	11.56	3.19	3.24	70.60	17.00	1.42
DMR WNC NY 398	12.38	3.05	3.82	68.05	24.50	1.23
DMR WNC NY 399	10.31	3.23	3.71	68.44	19.90	1.67
DMR WNC NY 400	9.86	4.47	3.24	73.39	27.90	1.27
DMR WNC NY 403	15.54	2.41	3.42	67.43	24.40	1.35
DMR WNC NY 404	13.52	2.48	3.55	70.22	26.70	1.34
DMR WNC NY 2430	12.20	3.56	3.08	70.29	25.00	1.39
DMR WNC NY 2392	9.83	3.15	3.44	71.33	23.54	1.47
DMR WNC NY 2393	10.45	3.28	3.14	71.93	20.70	1.15
DMR WNC NY 2394	12.07	2.57	3.56	68.80	26.30	1.10
DMR WNC NY 2395	10.61	2.21	3.47	72.90	26.80	1.41
DMR WNC NY 2396	11.24	2.26	3.38	68.33	23.50	1.10
DMR WNC NY 2397	10.72	2.36	3.90	67.93	24.65	1.17
DMR WNC NY 2398	11.00	2.92	4.27	70.55	21.37	1.17
DMR WNC NY 2399	11.50	2.92	4.04	68.22	22.60	1.19
DMR WNC NY 2431	11.30	2.64	3.23	73.07	20.66	1.15
DMR WNC NY 2430	12.51	2.89	3.08	68.85	27.90	1.27
DMR WNC NY 2400	11.86	3.36	3.65	68.91	24.00	1.26
DMR WNC NY 2401	12.61	2.50	3.51	68.42	21.76	1.21
DMR WNC NY 2402	12.06	3.22	3.06	69.04	27.10	1.13
DMR WNC NY 2403	8.83	2.43	3.74	70.79	28.30	1.18
DMR WNC NY 2404	9.79	2.32	3.86	70.47	26.70	1.21
DMR WNC NY 2405	9.75	2.58	3.28	68.96	26.30	1.20
DMR WNC NY 2432	11.83	3.30	3.33	72.64	22.30	1.17
DMR WNC NY 2433	10.42	2.29	3.13	68.22	21.20	1.12
DMR WNC NY 2406	11.91	2.56	3.01	70.42	29.30	1.13
DMR WNC NY 2407	11.79	2.28	3.64	70.63	20.05	1.22
DMR WNC NY 2408	11.12	2.69	3.28	74.92	25.80	1.17
DMR WNC NY 2434	9.55	3.05	3.65	73.35	23.70	1.25
DMR WNC NY 2409	9.76	2.28	4.34	72.66	25.8	1.17
DMR WNC NY 2410	12.03	2.23	3.71	73.18	29.90	1.15
DMR WNC NY 2435	12.50	2.73	3.68	69.78	35.60	1.19
DMR WNC NY 2436	11.31	3.23	3.75	70.73	17.40	1.16
DMR WNC NY 2412	11.65	2.61	3.10	72.51	24.40	1.22
DMR WNC NY 2414	11.21	2.04	3.01	68.92	30.90	1.14
DMR WNC NY 2415	11.24	3.22	3.45	69.25	27.30	1.14
DMR WNC NY 2416	9.64	2.72	3.56	71.35	27.00	1.17
DMR WNC NY 2417	11.35	2.84	5.37	71.91	26.40	1.20
DMR WNC NY 2418	12.67	3.50	3.68	72.75	19.05	1.90
DMR WNC NY 2419	11.56	2.35	3.17	70.30	21.79	1.21
DMR WNC NY 403	15.54	2.41	3.42	67.43	9.14	0.96
DMR WNC NY 404	13.52	2.48	3.55	70.22	13.63	1.24
DMR WNC NY 2437	11.31	3.18	3.75	74.26	21.94	1.22
DMR WNC NY 2462	12.29	2.23	3.52	70.15	29.98	1.36
DMR WNC NY 2208	12.19	3.01	3.34	71.48	24.33	1.28
DMR WNC NY 2212	12.65	3.21	3.91	68.47	17.26	1.57
DMR WNC NY 2212 DMR WNC NY 2213	10.66	2.45	4.86	71.35	33.30	1.15
DMR WNC NY 2469	10.52	2.43	3.15	70.89	24.74	1.15
DMR WNC NY 2219	11.82	2.47	4.28	74.66	19.61	1.63
DMR WNC NY 2233	12.22	2.82	4.28 4.87	74.66	33.83	1.65
DMR WNC NY 2234	10.12	2.59	4.74	75.31	36.11	1.20
DMR WNC NY 2113	11.08	3.05	4.40	71.72	35.43	1.27
DMR WNC NY 2465	9.93	2.48	3.65	72.53	35.94	1.28
DMR WNC NY 2466	10.00	2.68	3.38	74.76	31.63	1.44
DMR WNC NY2138	11.21	3.41	4.67	68.07	29.23	1.33
DMR WNC NY2143	12.07	2.33	4.61	68.41	24.00	1.20

 Table 1. Nutritional composition of 63 maize inbred lines.

PEDIGREE	Protein (%)	Fat (%)	Sugar (%)	Starch (%)	100-K wt. (g)	Specific Gravity (g/cm <sup>3</sup> )
DMR WNC NY2144	11.66	3.18	4.25	70.87	27.91	1.21
DMR WNC NY 2474	11.54	2.90	4.36	68.88	33.37	1.19
DMR WNC NY 2139	11.59	3.11	4.56	71.25	26.26	1.19
DMR WNC NY 2145	11.93	3.32	5.77	69.82	26.31	1.19
DMR WNC NY 2163	11.66	3.21	4.69	74.09	22.18	1.11
DMR WNC NY 2225	11.52	2.88	3.76	73.38	29.19	1.22

Table 1. Cont.

#### 2.2. Preliminary Analysis

The samples were oven dried at 90  $^{\circ}$ C to reduce the level of moisture of the grains to meet the accuracy of the results. The kernels were ground to powder by course and fine grinding using a pestle and mortar and finally kept in desiccators for analysis of various nutritional quality parameters.

# 2.3. Estimation of Protein, Moisture, Sugar, Starch, 100-Kernel Weight (100-Kernel wt.), Specific Gravity, and Fat Concentration

Protein concentration was determined by the micro-Kjeldahl method of AOAC [22]. In this method, the de-fatted samples were digested until the solution became colorless. Further distillation and titration were conducted by using 8 mL NaOH and 0.02 N HCl. The moisture level of the grains was determined by the oven drying method OAC 934.01 by drying at 135 °C for 2 h [22]. Total sugars were estimated according to the method of Nelson–Somogyi [23]. Starch concentration was determined according to the method of Clegg [24] using Anthrone reagent. After extraction of starch with perchloric acid, it was further hydrolyzed (in an acidic medium) into glucose, which formed a green color compound on reaction with Anthrone reagent. The optical density was recorded against blank at 620 nm. To calculate the 100-kernel weight, one hundred maize kernels were counted manually, and then these were weighed by an electronic weighing balance with 0.01 g accuracy. The specific density of the kernel was determined by the method of Sangamithra [25]. Fat concentration was estimated according to the method of sangamithra [25]. Fat concentration was estimated according to the method of was extracted at 40–60 °C using non-polar solvent petroleum ether.

#### 2.4. Statistical Analysis

## 2.4.1. Analysis of Variance (ANOVA)

ANOVA was performed to study the significance of the genotypic differences and conducted using Statistical Package for the Social Sciences (SPSS) software. All the biochemical evaluations were conducted in duplicate.

### 2.4.2. Univariate and Multivariate Statistics

The results were expressed as univariate and multivariate statistics. Scott–Knott correlations at significance levels of 1% and 5% and hierarchical cluster analysis (HCA) based on the squared Euclidean distance using Ward's method were performed using Statistical Analysis Software (SAS 9.2 English). Factor analysis for variability component loading was conducted using Statistical Package for the Social Sciences (SPSS) software. The principal component analysis (PCA) method was used as the extraction. The loading plot was prepared using SPSS. The Pearson correlation coefficient |r| among 63 maize inbred lines was calculated by the formula given below:

$$r = \frac{\sum XY - \frac{(\sum X)(\sum Y)}{n}}{\sqrt{\left(\sum X^2 - \frac{(\sum X)^2}{n}\right)\left(\sum Y^2 - \frac{(\sum Y)^2}{n}\right)}}$$
(1)

where X and Y are the variables and n is the total number of samples used in the study.

# 3. Results

# 3.1. Variability Analysis and Factor Analysis

The ANOVA indicated that the inbred lines differed significantly for all the nutritional quality traits of maize kernel (Table 2), and a wide variability among these quality traits was also observed.

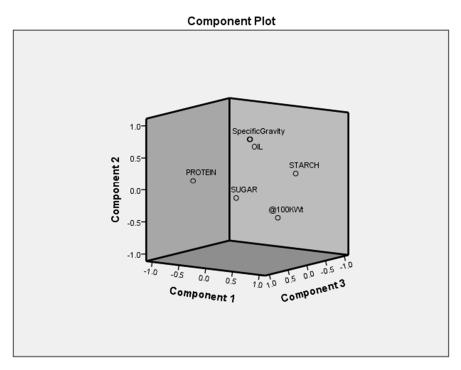
**Table 2.** Mean, standard error, and F-ratio of nutritional quality attributes of 63 maize inbred lines for variability analysis.

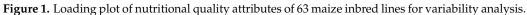
Variables	Ν	Mean	F-Ratio	Minimum	Maximum
Protein	63	$11.34 \pm 0.15$ *	13.61	8.83	15.54
Sugar	63	$3.54\pm0.08$ *	22.07	3.01	5.37
Starch	63	$70.46 \pm 0.04$ **	21.37	67.43	75.31
Fat	63	$2.81 \pm 0.25$ *	4.11	2.04	4.47
Moisture	63	$9.11 \pm 2.0$ *	2.80	9.16	10.49
Specific gravity	63	$1.25\pm0.11$	12.89	0.96	1.90
100-K wt.	63	$25.08\pm0.23$	206.72	9.14	36.11

\* Significant at the 0.05 probability level; \*\* significant at the 0.01 probability level.

The protein concentration in the maize kernels varied from 8.83 to 15.54%. The highest protein concentration was observed in the DMR WNC NY 403 inbred line (Table 2). The sugar concentration ranged from 3.01 to 5.77%, and the highest sugar concentration was observed in the DMR WNC NY 2417 inbred line. The starch concentration of the maize kernels varied from 67.43 to 75.31%. The highest starch concentration was observed in the DMR WNC NY 2408 inbred line. Meanwhile, the fat concentration varied from 2.04 to 4.47%. The highest fat concentration was observed in the DMR WNC NY 2408 inbred line. Meanwhile, the fat concentration varied from 2.04 to 4.47%. The highest fat concentration was observed in the DMR WNC NY 400 inbred line. The 100-kernel wt. varied from 9.14 to 36.11 gm, the highest being exhibited by DMR WNC NY 2234, and specific gravity varied from 0.96 to 1.90 g/cm<sup>3</sup>. Specific gravity data were rightly skewed to their means and contributed towards a high specific gravity. The moisture level of all 63 inbred lines chosen for this study was within acceptable limits.

Factor analysis indicated that the variability among the present inbred lines is mainly contributed by protein and starch concentrations, followed by 100-kernel wt. (Table 3). These three components were extracted based upon principal component analysis, which showed that these alone contribute 68.58% towards kernel variability. The loading plot (Figure 1) revealed that protein, starch concentration, and 100-kernel wt. strongly influence the variability as their loading is close to 1.0. However, the variability does not affect sugar, oil, and specific gravity as their values are below 0.5, i.e., less than 1.0, and as we know, loadings close to -1 or 1 strongly influence the variable, and higher loadings either positively or negatively indicate that the particular variable has a strong effect on the principal component.





**Table 3.** Component matrix, communities, and total variance of nutritional quality attributes of 63 maize inbred lines for variability analysis. (**a**) Component matrix. (**b**) Communities. (**c**) Total variance explained.

			Component				
			1	2		3	
]	Protein			0.073	0	.376	
	Starch			0.243	_	0.272	
100-	100-Kernel wt.			-0.391	0	.168	
	Fat			0.770	0	.107	
Spec	Specific Gravity			0.758	0	.042	
	Sugar			-0.012	0	.865	
			Initial		Extr	Extraction	
]	Protein				0.709		
	Oil			1.000		0.618	
	Sugar			1.000		0.907	
	Starch			1.000		0.643	
100-	100-Kernel wt.			1.000		0.655	
Specific Gravity			1.000		0.583		
Component		Initial Eiger	values		action Sums of Squared Loadings		
component	Total	Variance (%)	Cumulative (%)	Total	Variance (%)	Cumulative (%)	
1	1.725	28.750	28.750	1.725	28.750	28.750	
2	1.384	23.074	51.824	1.384	23.074	51.824	
3	1.006	16.762	68.586	1.006	16.762	68.586	
4	0.745	12.420	81.006				
5	0.627	10.452	91.458				
6	0.513	8.542	100.000				

Extraction method: principal component analysis. Extraction was performed by the principal component analysis method.

#### 3.2. Correlation Analysis

Scott–Knott correlation coefficients among various traits such as protein, fat, starch, sugar, 100-kernel wt., and specific gravity of the grains are provided in Table 4. These results reveal that the protein concentration exhibited a significant negative correlation with starch and 100-kernel weight. Contrary to this, the fat concentration showed a significant low positive relationship with specific gravity. Sugar and starch possess a non-significant positive correlation with all the traits under study, except protein.

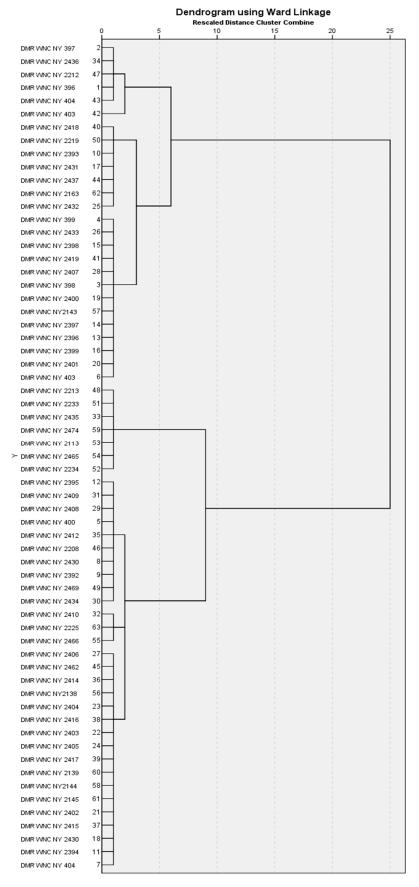
**Parameters** Sugar Starch 100-K wt. Specific Gravity -0.049-0.048-0.392 \*\*-0.351 \*\* 0.031Protein P = 0.702P = 0.711P = 0.001P = 0.005P = 0.8110.076 0.129 0.283 \* -0.140Fat P = 0.553P = 0.315P = 0.272P = 0.0250.101 0.247 0.016 Sugar P = 0.429P = 0.051P = 0.9000.227 0.149 Starch P = 0.074P = 0.245-0.091100-K wt. P = 0.479

Table 4. Coefficients of nutritional quality attributes of 63 maize inbred lines.

\*\* Correlation is significant at the 0.01 level; \* correlation is significant at the 0.05 level.

#### 3.3. Genetic Distance Measurement and Hierarchical Cluster Analysis

The genetic distance relationship of 63 maize inbred lines, depicted by the squared Euclidean distance based on Ward's method of hierarchical clustering, was obtained based on nutrient composition (Figure 2) data. Cluster analysis was used to reveal the association between the inbred lines used in the present study. Cluster analysis provides various clustering algorithms such as "sequential hierarchical and neighbor clustering". Due to the great heterogeneity within groups, hierarchical cluster analysis can be used to cluster maize inbred lines according to their differences and similarities to further investigate chemical compositional relationships between them. A total of two major clusters are formed at a distance of 25, having 26 and 37 inbred lines in cluster I and cluster II, respectively. On further reducing the distance to 9, three clusters were formed in which cluster I remained unaffected, while cluster II was split into two sub-clusters, viz., C II-SC 1 and C II-SC 2, having 7 and 30 maize inbred lines, respectively. On further reducing the distance to 6, cluster I was split into two sub-clusters, viz., C I-SC 1 and C I-SC 2, having 6 and 20 members, respectively. DMR WNC NY 397 and DMR WNC NY 404 are the farthest apart, having major differences in their protein, fat, starch, specific gravity, 100-kernel wt., and sugar concentration, followed by DMR WNC NY 2436 and DMR WNC NY 2394, DMR WNC NY 2212 and DMR WNC NY 2430, DMR WNC NY 396 and DMR WNC NY 2415, DMR WNC NY 404 and DMR WNC NY 2144, and DMR WNC NY403 and DMR WNC NY 2115. This variability can be exploited in crop improvement programs, particularly for grain quality traits.



**Figure 2.** Depicting hierarchical clustering of 63 maize inbred lines by squared Euclidean distance based on Ward's method of hierarchical clustering based on their nutritional composition differences.

### 4. Discussion

Maize kernels contain essential components for plant growth and reproduction [23]. However, the nutritional quality of maize is determined by its genetic background, and hence the inbred varieties with a high concentration of proteins, sugars, and other nutritional attributes are desired to combat malnutrition, particularly in sub-Saharan Africa, Southeast Asia, and Latin America [3].

A significant negative correlation between the protein concentration and starch and 100-kernel wt. indicated that an increase in the protein concentration will down-regulate starch and 100-kernel wt. This might be because protein and starch are both mostly present in the endosperm part of the kernel [26]. Although the endosperm contains lower amounts (8%) of protein than the germ (18.4%), it provides the highest quantity, i.e., 78% as compared to the 18% provided by the kernel germ [27]. Contrary to this, the fat concentration showed a significant low positive relationship with specific gravity, indicating that the specific gravity of maize may be increased with an increase in the fat concentration. Therefore, selecting for high fat and specific gravity altogether will not cause any difficulty during crop improvement programs. Maize starch accounts for about 72% of the dry weight of kernels, and it is located in the endosperm, while sugars such as sucrose, maltose, glucose, fructose, and raffinose constitute about 1–3% of the total dry weight of kernels [28]. The major (75%) portion of these free sugars is located in the germ, with the endosperm constituting only 25% of the total sugars [28]. A negative low correlation between protein and fat revealed that breeding efforts cannot be applied for increasing both energy-rich sources simultaneously, and genetic manipulations to increase one may affect the other. Another result of this study is a non-significant positive correlation of sugar and starch with all the traits under study, except protein, as starch contributes mainly to the grain filling characteristics of maize, hence increasing the 100-kernel wt. and, simultaneously, the density of the kernel. Specific gravity was reported to enhance the viability of the grains through an increase in their quality in soybean [29].

Muhammad Saleem et al. [30] and Chaudhary et al. [21] found a positive correlation between protein and sugars. The protein in maize kernels has been studied extensively because it forms the core of the quality of maize. This might be the reason for the above results because sugars are mainly confined to the germ as discussed earlier as well. Our results agree with some other previous findings of Willmot et al. [31]; Clark et al. [32]; Dudley et al. [33]; Liu et al. [34]; Zhang et al. [35]; and Chaudhary et al. [21]. A negative correlation between protein and moisture concentrations indicates an increased moisture level of the grains would decrease the quality of the grain. Therefore, a balanced moisture level is required for improving the quality as well as the quantity of grains. Chaudhary et al. [21] postulated that in maize, the germ accounts for 8–10% of the total grain weight and may contribute 15–20% of total protein, whereas the endosperm accounts for 80-85% and contributes approximately 80% of protein. Panthee et al. [36] found an inverse relationship between protein and fat concentrations and hence postulated that it will be difficult to improve both attributes simultaneously. Generally, it was observed by many researchers that the protein concentration is negatively and positively correlated with starch and fat concentrations [31,32,34,35]. Similarly, an inverse relation was found between starch and fat [37]. An increase in fat, which might have originated from a bigger germ size, could adversely affect the endosperm volume, thus indirectly contributing towards a lower starch concentration. High-fat maize is usually maize with a higher germ size and a lower endosperm if compared to normal maize genotypes [21]. Few researchers reported that there would be no serious barrier in selecting for both high-protein and high-fat in maize [38,39]. Okporie and Obi [39] and Okporie Oselebe [40] found that sugar also has a very low non-significant positive correlation with starch, which may be attributed to the source and sink relationship. Generally, a high sugar concentration has comparatively low starch synthase activity that may be due to the high Km value of enzymes. Here, both might be there in the equilibrium phase.

Genetic distance analysis presented two main clusters having 26 and 27 clusters, providing the base for selecting parents at a farthest distance in order to attain nutritional variability.

Various strategies for attaining nutritional security can be adopted which are feasible and sustainable as well. Bio-fortification is one of the most important strategies because it is rural-based, where 70% of the resource-poor live, and is cost-effective and sustainable too. Maize provides about 15% of the world's protein (essential amino acids) and 20% of the world's calories [5]. A maize inbred line rich in protein and sugar concentrations has a positive correlation between these nutrients and is highly important for proper human nutrition, growth, health, and immunity and for combating malnutrition.

The outcomes of the present study will help in designing breeding strategies for developing nutritionally improved maize hybrids for the nutritional security of India and the world, and this can answer many of the nutritional quality issues. The authors strongly believe that along with these strategies, bioavailability studies should be carried out to confirm that the targeted nutrient is being readily absorbed in the body and can fulfill the recommended dietary allowance (RDA) because the nutritional quality of the "resource-poor" is much more important than that of the quantity. This approach may help to take a step forward to improve the livelihood security of a nation.

### 5. Conclusions

The results of the present study reveal a wide variability among protein, starch concentration, and 100-kernel weight in maize inbred lines, and these three contribute to 68.58% of the kernel variability. The inbred lines were proposed as donors for the development of high cultivars for their respective traits, viz., high protein (DMR WNC NY 403 and DMR WNC NY 404), starch concentration (DMR WNC NY 2163, DMR WNC NY 2219, DMR WNC NY 2234, DMR WNC NY 2408, DMR WNC NY 2437, and DMR WNC NY 2466), 100-kernel wt. (DMR WNC NY 2113, DMR WNC NY 2213, DMR WNC NY 2233, DMR WNC NY 2234, DMR WNC NY 2414, DMR WNC NY 2435, DMR WNC NY 2465, and DMR WNC NY 2474), sugar (DMR WNC NY 2417), and specific gravity (DMR WNC NY 2418). Nutritional components in maize inbred lines are also highly correlated, and an alteration in one may positively or negatively affect the other. Although all three were the principal components of variability, an increase in protein will lower the starch concentration. Further, an increase in the weight of the kernels might increase the starch concentration rather than lower the protein concentration of the grain. The development of high-protein maize will affect the grain yield to some extent. However, selecting for high fat would increase the specific gravity which in turn enhances the viability of the grain, providing a wide base for maize hybridization programs.

DMR WNC NY 403 and DMR WNC NY 404 are proposed as high-protein, low-sugar, and low-starch materials, DMR WNC NY 2163, DMR WNC NY 2219, DMR WNC NY 2234, DMR WNC NY 2408, DMR WNC NY 2437, and DMR WNC NY 2466 as high-starch and low-protein materials, and DMR WNC NY 2418 for its high specific gravity and fat level. DMR WNC NY 2234 is proposed as a promising material for its starch concentration and 100-K wt., and DMR WNC NY 2163 and DMR WNC NY 2219 are proposed for their protein, sugar, starch, and specific gravity, only needing to improve their 100-K wt. and fat concentration. Further, DMR WNC NY 2113, DMR WNC NY 2408, DMR WNC NY 2417, and DMR WNC NY 2437 are proposed as excellent materials for their protein, sugar, starch, 100-K wt., and specific gravity, only needing to improve their fat content. Next, DMR WNC NY 2213, DMR WNC NY 2465, and DMR WNC NY 2466 are found as promising for all other traits than protein and fat concentration, whereas DMR WNC NY 2414 and DMR WNC NY 2435 need improvement in their fat and starch concentrations only.

Among the various inbred lines, DMR WNC NY 397 and DMR WNC NY 404, followed by DMR WNC NY 2436 and DMR WNC NY 2394, DMR WNC NY 2212 and DMR WNC NY 2430, DMR WNC NY 396 and DMR WNC NY 2415, DMR WNC NY 404 and DMR WNC NY 2144, and DMR WNC NY403 and DMR WNC NY 2115, were genetically the farthest apart inbred lines, having major differences in their protein, fat, starch, 100-kernel weight, specific gravity, and sugar concentration, and arising from two different clusters as well. In brief, there is a high variability for three major nutritional traits as identified by factor analysis; therefore, these inbred lines can be used as potential donors of the respective traits and would be beneficial to be proposed in breeding programs, as combinations of these lines and their crosses would result in hybrids or genetic variability with high values in one or many traits.

The adoption of maize inbred lines possessing higher proteins and amino acids would result in a significant decrease in malnutrition. Development and consumption of nutrientrich maize varieties would help in preventing malnutrition and in achieving nutritional security more holistically.

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## Article Investigating International Students' Perception of Foodservice Attributes in Malaysian Research Universities

Anisa Zahwa Akbara <sup>1</sup>, Bee-Lia Chua <sup>1</sup>,\*<sup>1</sup>, Heesup Han <sup>2</sup>,\*<sup>1</sup> and António Raposo <sup>3</sup>

- <sup>1</sup> Department of Food Service and Management, Faculty of Food Science and Technology, Universiti Putra Malaysia, Serdang 43400, Malaysia; akbarazahwa@gmail.com
- <sup>2</sup> College of Hospitality and Tourism Management, Sejong University, 98 Gunja-Dong, Gwanjin-Gu, Seoul 143-747, Korea
- <sup>3</sup> CBIOS (Research Center for Biosciences and Health Technologies), Universidade Lusófona de Humanidades e Tecnologias, Campo Grande 376, 1749-024 Lisboa, Portugal; antonio.raposo@ulusofona.pt
- \* Correspondence: chuabeelia@upm.edu.my (B.-L.C.); heesup.han@gmail.com (H.H.)

**Abstract:** University foodservice is expected to satisfy students' food needs and is one of the necessities at a university. However, serving a community of international students who are multicultural is not an easy task. Thus, it is necessary to recognize international students' needs and wants in order to increase their satisfaction with the overall on-campus dining experience. This study conducted an importance–performance analysis to examine international students' perceived importance and perceived performance of university foodservice attributes. Using a self-administered questionnaire, a total of 620 international students who were studying in Malaysian research universities comprised the sample of this study. The results showed that food price was the most satisfactory foodservice attribute as perceived by international students. Food quality was deemed unsatisfactory and represented the main weakness. Results from this study can assist in determining how international students perceive the quality of the key attributes of on-campus foodservices and identify fields in which improvements are required.

**Keywords:** university foodservice attributes; importance–performance analysis (IPA); international students; Malaysian research universities

### 1. Introduction

The admissions of global students remain important to the university sector in Malaysia, since Malaysia sought to be acknowledged as a world-class higher education center by the year 2020 [1]. Moreover, foreign students also generate international business possibilities and ties to world trade, become diplomatic allies, and encourage interest in foreign policy [2]. However, to maintain international students in this competitive setting, significant efforts are needed. Efforts were made to attract foreign students by improving the reputation of Malaysian higher education institutions. Therefore, the Malaysian Research Universities (MRU) program has provided Malaysian universities and research institutions with an awareness of the research culture. Among the initiatives are (1) targeting the enrolment of global students, (2) improving Malaysian universities' world ranking, (3) creating more "Malaysian chairs" at universities overseas, and (4) collaborating and cooperating on research and educational issues with world-renowned universities [3]. In addition to these goals, superior service to meet student needs and expectations and maintain student satisfaction and loyalty to their chosen study destinations have become a core objective of these colleges [2,4].

According to Ezeokoli and Ayodele [5], the quality of foodservice is acknowledged as a main academic excellence metric and a significant strategic variable to boost the market share of colleges and service providers, with enduring impacts on the organization and its students. Klassen, Trybus, and Kumar [6] added that foodservice is a fundamental factor

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**Copyright:** © 2021 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). that influences student satisfaction. Furthermore, previous studies have shown various foodservice attributes, such as food quality, price fairness, hygiene, food variety, service quality, food price, and ambience, that could predominantly influence university students' satisfaction [7–14]. The foodservice outlet is the primary form for student foodservice in colleges [15]. Drummond and Brefere [16] noted that food can make students become more focused on academic studies and ultimately can affect their academic performance. Therefore, in order to be a study destination for international students, evaluating the role of on-campus dining service is critically important.

University foodservice is one of the amenities that must be taken into consideration in order to accommodate students [17]. Students' preferences for food and services are changing. Students who are dissatisfied with the service quality of a university cafeteria will choose off-campus dining [18]. Most university foodservice operations are outsourced in this modern age, with independent food operators or food firms given a certain number of years of contract to operate the company on campus [19]. Independent operators also run restaurants, cafeterias, food courts, kiosks, vending machines, dining halls and stalls on university premises. This is also the case for Malaysian university foodservices [20]. Consequently, for university foodservice operators, it is important to understand students' needs and satisfy their overall on-campus dining experience, even though some food operators are independent.

On the other hand, university foodservice operators in Malaysia have the most difficult task of serving a community of students who are multicultural and confined to the university environment [21]. Abdullah, Mansor, and Naba [22] found that students were generally dissatisfied with the food quality and price provided by university food operators. Liang and Zhang [15] discovered that the key factors contributing to student dissatisfaction when patronizing a university cafeteria were food quality, price, and value. Klassen, Trybus, and Kumar [6] identified that the most common complaints regarding university foodservice by international students were poor ambience, lack of resources, price, lack of food choices, and low food quality. In the context of university foodservice in Malaysia, there has been relatively limited empirical studies examining the importance and performance of foodservice attributes among international students in Malaysian research universities. To address this issue, this study analyzed the importance and performance of foodservice attributes perceived by international students in Malaysian research universities by utilizing the importance-performance analysis (IPA) method. This method is popular for evaluating service quality in a multitude of areas. IPA examines the "importance" individuals place on any given product/service attribute. Furthermore, the method has been employed in designing corporate marketing strategies, in guiding government planning choices, and in evaluating the organization and supervision of activities and schemes. The IPA method offers a pragmatic theoretical basis for important evaluation. The results of this study could be used to demonstrate which foodservice attributes need to be strategically planned and improved. The objectives of this study are as follows:

- To identify international students' perceived importance of on-campus dining attributes.
- To examine international students' perceived performance of on-campus dining attributes.

### 2. Literature Review

### 2.1. University Foodservice Attributes

Many studies have emphasized the effect on customer satisfaction of foodservices [17,19,23,24]. According to El-Said and Fathy [12], foodservice is an important component of overall planning that affects quality of life at university. Particularly, foodservice was verified as an important component of a healthy arrangement that affects the personal satisfaction of students and faculty at universities and colleges [25]. Kim, Lee, and Yuan [26] in their study of college students' satisfaction with university also confirmed that foodservice attributes influenced college student satisfaction.

Student–seller interaction is the type of communication that takes place in a university cafeteria. The interactions between the student and the foodservice personnel are important,

as students can be highly sensitive [27]. Moreover, Chang and Suki [28] added that the staff performance was found to have a statistically significant impact on student satisfaction of the experience of student dining in the cafeteria. The performance of cafeteria employees is particularly important for students in terms of customer friendliness and responsiveness. Hence, interactional quality is an important factor for improving college student satisfaction [27]. Concerning the food quality of on-campus foodservice, Andaleeb and Caskey [10] found that most students noted that, were the food quality to improve, they would more often consider on-campus foodservices. Food quality is a feature of foods acceptable to customers and includes the appearance, smell, taste, and texture of foods [29]. In shaping and affecting customer satisfaction, quality is of essential importance. Customers are the primary drivers of customer satisfaction for food quality [30].

Students also consider cost when purchasing of food at the cafeteria as an important aspect of university foodservice. It is well known that students have limited budgets that affect their food choices; they are always looking for reasonable prices [31]. This is the first problem students have in college foodservice, because students buy food with limited resources, as Nadzirah, Ab-Karim, Ghazali, and Othman [21] claimed. The paid price should be appropriate for the amount of food served to students so that they feel they are getting good value the products and services, producing student satisfaction. The dining atmosphere can be regarded, according to Ha and Jang [32], as critical for affecting customer satisfaction levels, mainly because customer reactions to the setting form part of the consumer experience. For university foodservice, the spatial organization of the sitting, various internal decorations, and the suitability of the sound environment, which are essential to enhanced gratification, dictate the cafeteria atmosphere [33]. Chang and Suki [28] also added that cafeteria design affects the students' food decisions and eating behaviors.

In addition, McLachlan and Justice [34] explained that food selection was found to be an important part of the satisfaction of international students. It was defined as food supplied in on-campus foodservice that matches the culture, religion, taste, etc., of international students. Raman and Chinniah [27] also noted that the variety and diversity of food and comfort in university cafeterias gives students a sense of "home" while on campus. Nevertheless, empirical investigations on international students' perception of foodservice attributes in Malaysian research universities are relatively few. Therefore, this study aimed to demonstrate which foodservice attributes need to be strategically planned and improved as perceived by international students. Foodservice attributes in terms of interactional quality, food quality, atmospheric quality, food price, and food selection are employed in this study.

### 2.2. International Students' University Life Adaptation and its Relation to University Foodservice

International students are subjected to numerous changes and can struggle to adjust in a number of ways [35]. According to Andrade [36], international students experience more fear, stress, homesickness, loneliness, and lack of social support than domestic students. Thomas and Althen [37] also added that international students may experience a variety of transition issues, including depression/helplessness, hostility toward the host country, anxiety, over-identification with the home country, isolation, homesickness, and loneliness. Transitioning from parental care to independent living entails many novel decision-making experiences and choices, which can have a long-term influence on eating habits and health condition for students [38]. Lin and Yi [39], in agreement with the literature, reported that many foreign Asian students encounter the following when they study abroad: academic distress, language differences, financial concerns, performance anxiety, depression, loneliness, homesickness, relationship issues, non-assertiveness, individualism and bicultural tension, stereotyping, racism, bigotry, and fear. They may have anxieties about readjustment if they are intending to return to their own country. Specifically, there are several categories summarized by Tseng and Newton [40] as the main challenges encountered by international students: socio-cultural adjustment, personal psychological adjustment, academic adjustment, and general living adjustment. Socio-cultural change leads to culture shock, injustice, and adjusting to unfamiliar traditions, expectations, festivals, and rituals. Personal psychological adjustment is characterized by feelings of depression, solitude, alienation, homesickness, and dissatisfaction. Weather/climate adaptation, transportation systems, bill payment, health care systems, accommodation conditions, and food are examples of general living adjustments.

It has been argued that institutions should play a constructive approach in embracing and supporting global scholars in their academic and social adjustment. The more universities recognize the demands of foreign students, the more effective their students will be [41]. Furthermore, a satisfied student population is a source of favorable outcomes, such as spreading positive word of mouth and recommending the university to others. It can be impossible to obtain overall satisfaction if a negative attitude is formed; this could result in complaints, decreasing loyalty, and negative promotion by word of mouth [42]. Of particular interest to the present study is the fact that international students have discovered that food is a vital aspect of adjusting to Malaysia. International students rely on ethnic dishes from their home countries to alleviate homesickness anxiety [43]. McLachlan and Justice [34] identified food disparities as a component that aids foreign students in dealing with change overload and transformation shock. Furthermore, in order to reach and help international students adapt academically and socially, organizations have to take on a proactive approach. Garg [44] stated that many university administrators are starting to recognize the concept of offering comprehensive campus facilities that are essential to foodservices. Demand for foodservice has steadily increased, especially in universities, as the number of students continues to rise. As a consequence, these higher requirements for college foodservices have placed increasing pressure on operators to satisfy students' requirements [10,45]. One of the primary objectives of universities is student satisfaction. According to Ruetzler, Taylor, and Hertzman [41], campus foodservice has a huge impact on the overall satisfaction of the student population. The canteens provide goods and services that can enhance the life of all those members of today's progressively varied university population with regard to social, financial, educational and personal fulfilment. Students expect greater levels of attention and more friendly service. For most customers, the food quality, cleanliness of the dining area, atmosphere, and freshness of the ingredients are satisfaction factors [46]. Amelia and Garg [9] also stated that the major factors of foodservice satisfaction are first impression, food quality, serving time, and employees. The satisfaction of students in university cafeterias depends entirely on the quality of the food and beverages served in cafeterias, the variety and choices of food and beverages, and fair price and value [9]. Campus administrators have stepped up their efforts to recruit and select only the most talented and experienced foodservice directors in response to this demand.

### 2.3. Importance-Performance Analysis

Initially established by Martilla and James [47], IPA provides ideas on what products or services a company should concentrate on in order to attain customer satisfaction. It examines quality attributes according to two measures: their effectiveness (satisfaction) and their value to customers (importance). The evaluation of these two dimensions is then combined with a matrix to determine the key factors of satisfaction, propose goals for improvement, and recognize areas of over-conformity and appropriate drawbacks [48]. To simplify data analysis, the IPA method incorporates measurement of consumer-perceived performance and importance into a two-dimensional matrix [47]. Figure 1 depicts a matrix that categorizes attributes into four groups or quadrants to establish goals in allocating finite resources.

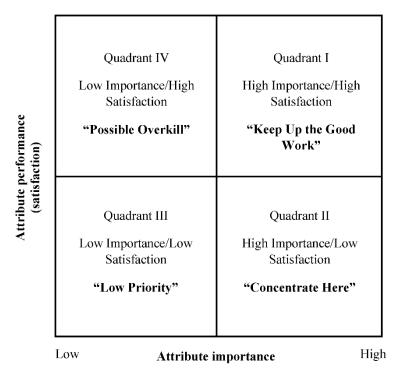


Figure 1. Importance-performance quadrants (Source: Matzlera et al. [48]).

Quadrant I, "keep up the good work", refers to the important strength of a product or service and the prospective competitive benefit. The characteristics in this quadrant are regarded to be successful and require continuous investment. Quadrant II is the "concentrate here" area. The attributes in this quadrant are deemed insufficient, constituting major shortcomings and risks to the product's competitiveness. Attributes in Quadrant III are deemed to be comparatively unimportant to consumers and fall under the "low priority" category. They are not doing exceptionally well or are considered to be relatively unimportant to customers. Quadrant IV is a relatively unimportant section of the matrix [48]. The "possible overkill" region includes characteristics that are of low value to consumers but work well, suggesting a possible excess of scarce resources that are being used inefficiently and should be reallocated elsewhere [48].

Previous studies [49–52] noted that the IPA method is a tool to assess the performance of the service factor level, which measures the importance and satisfaction of the service innovation process. The importance of measuring and managing customer satisfaction in foodservice is widely acknowledged [53]. The critical factors determining satisfaction and importance must be identified. The best way to attain the greatest level of satisfaction is to decide how scarce resources are deployed. The IPA method is seen as an efficient way to set priorities. The tool appears to be an easy and effective instrument in practice [54]. The decision on how best to allocate scarce funds to maximize satisfaction is very helpful. To maximize fulfillment, IPA is very helpful to decide how best to allot scarce resources. For example, Tzeng and Chang [55] used IPA to explore restaurant service quality. The results of their study provided the direction to improve the service quality in the foodservice industry. Gemmel [56] argued that the IPA technique can help administrators with improvements in order to achieve high customer satisfaction with service quality.

### 3. Method

### 3.1. Measures and Development of Survey Instrument

The research instrument of this study was a self-administered questionnaire. The questionnaire consisted of demographic questions and relevant measures. The "interactional quality" variable consisted of seven items, while six items were used to measure the "food quality" variable. The "atmospheric quality" variable was composed of six items. In addition, there were three questions measuring the "food price" variable. The four variables were adapted from El-Said and Fathy [12] and Liang and Zhang [15]. The "food selection" variable consisted of five items, adjusted from Ruetzler, Taylor, and Hertzman [41]. Furthermore, all the measurement items were measured on a 7-point scale, ranging from level 1, which represented "very dissatisfied", to level 7, which signified "very satisfied". Another 7-point scale ranged from 1, which represented "very unimportant", to 7, which signified "very important".

The mean scores of importance and satisfaction for foodservice attributes were obtained. These values then were used to analyze each element in the importance satisfaction grid as a plot. The vertical axis showed satisfaction of international students with the performance of service and service providers on a given attribute, ranging from highly dissatisfied to highly satisfied (7-point Likert scale). The horizontal axis, ranging from very unimportant to very important (7-point Likert scale), represented the importance of the attribute to international students. Satisfaction and importance scores of students were plotted on the IPA grid, which was constructed as the crossing point between mean values of the attribute ratings of importance and satisfaction. Four quadrants were generated by IPA, each with a different marketing strategy. Based on the quadrant in which it fell, each attribute was evaluated.

IPA plot interpretations followed each attribute's composite of importance and satisfaction scores. For instance, attributes high in importance and high in satisfaction proposed that university foodservice was doing a decent task and could continue to assign resources to these attributes. There may be little strategic advantage for additional investment. Particular attention should be paid to attributes of high importance and low satisfaction. A large amount of resources should be invested in improving the efficiency of the attributes. Lastly, low value and high satisfaction attributes could be protected by the university foodservice, but not explicitly allocated any extra funding.

### 3.2. Validity and Reliability

By using SPSS 20 for Windows, the validity analysis of this study was carried out by computing the correlations between individual items and the score sum of their respective construct. All the correlation coefficients between the items and their respective construct were statistically significant (p < 0.05). In this study, after the validity of the instrument was tested, it was distributed to subjects. The test result identified the coefficient of instrument reliability. The reliability coefficient of the instrument in this study was identified using Cronbach's alpha ( $\alpha$ ) coefficient. The reliability analysis results revealed that the measurement items of the instrument were reliable to measure the five key constructs: interactional quality, food quality, atmospheric quality, food price fairness, and food selection. The results defined that all five key constructs were reliable. The values were included in the very high reliability category ( $0.80 < \alpha < 1.00$ ).

### 3.3. Data Collection and Samples

The target population and unit analysis consisted of all full-time international postgraduate and undergraduate students enrolled in five public Malaysian Research Universities (MRUs) in Malaysia. They were Universiti Malaya (UM), Universiti Kebangsaan Malaysia (UKM), Universiti Sains Malaysia (USM), Universiti Putra Malaysia (UPM), and Universiti Teknologi Malaysia (UTM). Two stages of sampling techniques were used in this study: stratified sampling (stage 1) and purposive sampling (stage 2). The reason stratified sampling was chosen is because the population of this study was international students in MRUs. There are five separate research universities in Malaysia, and all have international students. Hence, a sample of international students from each university was taken. Subsequently, purposive sampling was done by searching the members (sample) of a group purposefully. Only subjects fit for the study purpose were selected. The inclusion criteria of the study subjects were (1) full-time undergraduate or postgraduate international student at a research university in Malaysia; (2) eating on campus at least three times at the same foodservice outlet (e.g., cafeteria, food stall) since enrolled at the university; (3) staying in university housing (e.g., hostel, apartment, dorm) at least one semester since enrolled at the university.

The survey was carried out for a 12-week period from 3 October 2018 to 27 December 2018. The questionnaire was distributed by paper-and-pencil and online. The researcher visited the libraries, canteens, and cafeterias of UM, UKM, USM, UPM, and UTM from Monday to Wednesday from October 2018 to December 2018. The researcher was recommended by the librarians to come to the libraries, since many students were available in the libraries from Monday to Wednesday. The subjects were approached in each university's canteen/cafeteria and library and given a piece of paper that contained the online link to the survey. In addition, the researcher attempted to access subjects by seeking assistance from the members of the Indonesian Student Association in each MRU to distribute the questionnaires to international students to distribute the link via WhatsApp groups to their community in UPM and the other four universities.

### 3.4. Demographic Information

A total of 620 international students who were studying in MRUs were selected as the sample of this study. Satisfaction and importance means were used to determine where the coordinates of each item fell according to international student experience. The average level of satisfaction with the different elements of university foodservice attributes and the average importance for the total sample of five constructs in foodservice attributes were measured. These values were then used to analyze each element in the importance satisfaction grid as a plot. To provide an overview of international students regarding their demographics as well as the nature of their dining experiences, descriptive characteristics of the sample were examined. The respondents' demographics were analyzed in terms of their gender, home country, name of university, level of higher education, current academic year, and year enrolled in the university.

In this study, more male respondents (58.4%) participated in the survey research than female (41.6%). The respondents came from various home countries, including Indonesia (29.8%), Iraq (11.6%), Iran (10.3%), China (9.4%), Afghanistan (7.7%), and India (7.2%). In addition, there were also participants who came from Korea (6%), Saudi Arabia (5.3%), the Philippines (3.9%), Vietnam (3.4%), Qatar (1.9%), Somalia (1.1%), Egypt (1%), Sri Lanka (0.9%), Ethiopia (0.3%), and Germany (0.2%). Furthermore, the dominant international student group was from UPM (26%), followed by UM (19.8%), USM (19.4%), UTM (18.4%), and UKM (16.6%). The demographic distribution by higher education level. demonstrated that respondents with a bachelor's degree made up the majority of the total respondents (43%), followed by those who were earning a master's degree (41.6%) and a Ph.D. (15.5%). It was revealed that 30.5% of the respondents were in the second year of their program, whereas those who were in the third year made up 27.6% of the total respondents. The participants who answered "other" were 20.8%, meaning that the respondents had studied more than four years since enrolling in the program. The data showed 16.6% of respondents were in the fourth year of their program, whereas only 4.5% respondents were university students in their first year. In summary, the participants of this study were mainly undergraduate international students who were studying in one of these research universities: UM, UPM, UKM, UTM, and USM.

The on-campus eating behavior of international students was measured for the period since the students enrolled at the university. The measurement items are provided in Table 1. With regard to the frequency of visits to the on-campus foodservice outlet(s), most of the respondents (46.8%) visited the university canteen daily. Daily customers were double those who visited the foodservice outlet(s) monthly (23.2%), and monthly customers were fewer than the respondents who visited the university cafeteria(s) weekly (30%).

Items	Frequency	Percentage (%)
Frequency of visit		
Daily	186	46.8
Weekly	290	30.0
Monthly	144	23.2
Spending for meals per visit		
≤5 MYR	89	14.4
6 MYR-10 MYR	429	69.2
>10 MYR	102	16.4
Dining companions		
Alone	288	46.5
Friends	332	53.5
Reason for dining on campus		
The price is inexpensive	568	63.2
There is no other option	230	25.5
To socialize/meet friends	82	9.1
The location is convenient	11	1.2
The food is good	3	0.3
Other	5	0.6
Type of food purchased when dining on campus		
Snacks	243	30.2
Meat/Fish/Chicken	238	29.6
Noodle dish	86	10.6
Rice dish	73	9.0
Vegetables/Fruits	69	8.6
Dairy products	54	6.7
Other	42	5.2

**Table 1.** International students' on-campus eating behavior (n = 620).

The findings revealed that over half of the participants (69.2%) spent their money for meals in the university cafeteria in the range of 6 MYR to 10 MYR per visit, or approximately US \$1.50 to \$2.50. Respondents who spent more than 10 MYR (US \$2.50) took second place (16.4%) followed by respondents who spent less than/equal to 5 MYR (US \$1.25) per visit. A large group of participants indicated that they dined with friends (53.5%), with fewer dining alone (46.5%). In the section asking the reason why international students eat on campus, the respondents were able to choose more than one answer. Therefore, the most significant reason to eat on campus was found to be the price (63.2%). They chose to eat on campus because the meal price was inexpensive. Another significant reason for the respondents was a lack of other options (25.5%). It is not surprising that most of the students stated that they eat with friends in the university canteen, because 9.1% of the participants selected socializing or meeting with friends in the canteen, while 1.2% of the respondents thought the location of the foodservice outlet was convenient for them. Only 0.3% of the respondents stated that "the food is good" in the university cafeteria. Participants could select the food they normally order, with more than one option for this question. The most common choices were snacks (30.2%) and meat, fish, or chicken dishes (29.6%); 10.6% of the respondents mentioned that they like to eat rice dishes, such as fried rice, at the university canteen. Therefore, in term of respondents' on-campus eating behavior, the majority of the respondents were daily consumers and they mostly came to the university foodservice outlet(s) because the price was inexpensive and there was no other option. An overview of the cafeterias in the five Malaysian Research Universities is provided in Appendix A.

### 4. Results

### 4.1. Overall IPA Grids

The average value of these five constructs was computed. These values were then used to draw each element on the important satisfaction grid as coordinates. Table 2 illustrates the means of importance and satisfaction (performance) from the five foodservice attributes IO

AO

in this study as well as the coordinates of each foodservice attribute in the IPA grid. The variables in the table were based on the order of Quadrant I to Quadrant IV. SPSS 20 for Windows was used as a tool for the analysis.

Labels	Variables	bles Satisfaction Importance			
Labels	vallables	Satisfaction	importance	Quadrant	
FP	Food price	4.71	5.35	1	
FQ	Food quality	4.36	5.41	2	
FS	Food selection	4 24	5.04	3	

4 63

4.55

5.05

5.16

4

4

Table 2. Importance-performance means for five university foodservice variables.

Interactional quality

Atmospheric quality

As presented in Figure 2, the horizontal (*x*-axis) axis shows the importance attributes from low to high perceived by international students (overall importance); whereas the vertical axis (*y*-axis) shows the rate of service satisfaction for international students (overall satisfaction), ranging from low to elevated. The grid is characterized for satisfaction and importance for the five variables. As specified in Table 2, each variable was labeled for easy recognition and to shorten the name while being analyzed in SPSS 20 for Windows. The labels were also applied to each item of the variables, as shown in Table 3.

The Quadrant I component was considered to be highly important and highly satisfied. The main driver of customer satisfaction was the food price (FP), and the management task was to guarantee that college foodservice "keep up the good work". The component was high importance and low satisfaction in Quadrant II. The underperformance of food quality (FQ) located in this quadrant is regarded to reflect the main weaknesses of a product and suggests efforts should be "concentrate here".

Subsequently, the component in Quadrant III was rated of low importance and low satisfaction. Food selection (FS) was the attribute of "low priority". Apart from being comparatively unimportant, its bad performance is obviously not an issue. Efficiency should be enhanced only if Quadrant II (greater priority) does not contain the characteristics and/or if the improvements are not too expensive [35]. Components in Quadrant IV were rated of low importance and high satisfaction. Interactional quality (IQ) and atmospheric quality (AQ) could be viewed as areas of "possible overkill". They were relatively unimportant for the customers, but the service was very good at the university dining facilities.

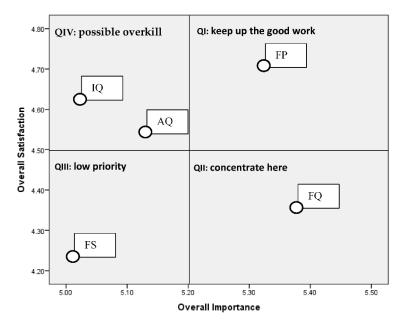


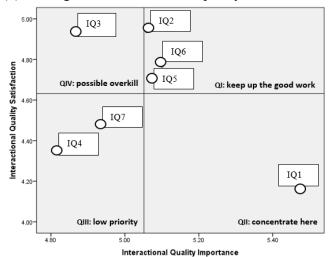
Figure 2. IPA grid for five constructs of university foodservice attributes.

Labels	Items	Satisfaction	Importance	Quadrant
Interactional quality (IQ)				
IQ2	Respectfulness of the foodservice outlet staff	4.94	5.06	1
IQ6	Staff knowledge of the food items sold in foodservice outlet	4.77	5.10	1
IQ5	Speed of service in foodservice outlet	4.72	5.07	1
IQ1	Cleanliness and neatness of staff appearance in foodservice outlet	4.16	5.47	2
IQ7	Easiness talking to staff (able to answer my questions)	4.50	4.95	3
IQ4	Professionalism in service delivery in foodservice outlet	4.38	4.83	3
IQ3	Friendliness of the foodservice outlet staff	4.95	4.88	4
Food quality (FQ)				
FQ4	Freshness of food ingredients	4.38	5.74	1
FQ2	Appropriateness of food flavor	4.36	5.46	2
FQ1	Nutritional benefits of food items	4.36	5.45	2
FQ5	Food hygiene and safety	4.29	5.50	2
FQ6	Appropriateness of food temperature	4.36	5.33	3
FQ3	Attractiveness of food items displayed	4.42	4.97	4
Atmospheric quality (AQ)	1 7			
AQ1	Seat availability	4.99	5.61	1
AQ2	Convenience of service hours	4.94	5.43	1
AQ5	Cleanliness of the foodservice outlet	4.38	5.65	2
AQ3	Decoration of the foodservice outlet	4.51	4.90	3
AQ4	Noise level	4.45	4.89	3
AQ6	Foodservice outlet entertainment, such as music	4.02	4.48	3
Food price (FP)	.,			-
FP1	The announcement of food price changes	4.87	5.45	1
FP2	The reasonableness of the quality of food items provided for the price paid	4.89	5.57	1
FP3	The reasonableness of the quantity of food items provided for the price paid	4.37	5.02	3
Food selection (FS)	1 1 1			
FS2	The variety of food served	4.37	5.23	1
FS3	The choices of food to meet my religious needs	5.26	5.47	1
FS1	The choices of food to meet my cultural preferences	4.16	5.07	2
FS4	The selection of food to make me feel at home	3.63	4.89	3
FS5	The selection of food is similar to that in my home country	3.77	4.56	3

Table 3. Importance-performance means for 27 university foodservice attributes.

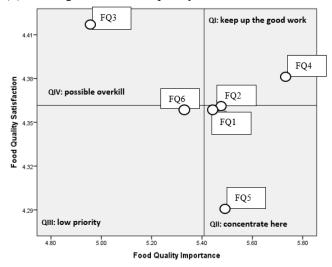
### 4.2. Individual IPA Grids

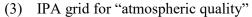
Each of the five characteristics represent the various component elements. The mean value of importance and satisfaction of each attribute for the 27 items is shown in Table 3. The items in Table 3 are based on the order of Quadrant I to Quadrant IV. Five IPA grids were developed in order for the five characteristics of university foodservices to be analyzed and identified in terms of weaknesses and strengths. For each attribute, the mean values of importance and satisfaction were calculated and used as the coordinates for each individual grid. The crosshairs and the positioning of the axis in the grids were developed as excellent means of importance and satisfaction for each element (see Figure 3). Hence, there were different crossing points for the five IPA grids.

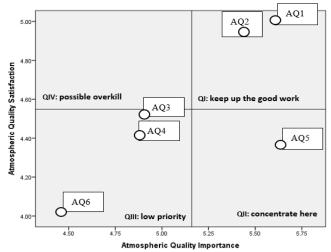


### (1) IPA grid for "interactional quality"

### (2) IPA grid for "food quality"







### Note:

- IQ1: Cleanliness and neatness of staff appearance in foodservice outlet
- IQ2: Respectfulness of the foodservice outlet staff
- IQ3: Friendliness of the foodservice outlet staff
- IQ4: Professionalism in service delivery in foodservice outlet
- IQ5: Speed of service in foodservice outlet
- IQ6: Staff knowledge of the food items sold in foodservice outlet
- IQ7: Ease of talking to staff (able to answer my question)

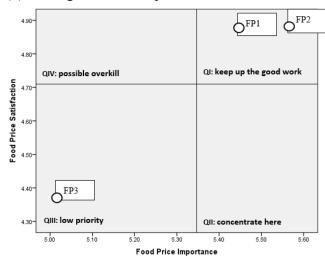
Note:

- FQ1: Nutritional benefits of food items
- FQ2: Appropriateness of food flavor
- FQ3: Attractiveness of food items displayed
- FQ4: Freshness of food ingredients FQ5: Food hygiene and safety
- FQ6: Appropriateness of food temperature

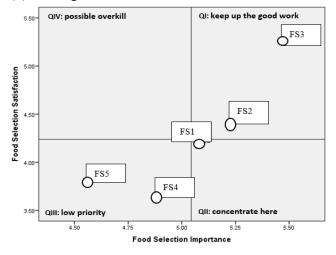
- Note:
- AQ1: Seat availability AQ2: Convenience of service hours
- AQ3: Decoration of the foodservice outlet
- AQ3: Decoration of AQ4: Noise level
- AQ4: Noise level
- AQ5: Cleanliness of the foodservice outlets AQ6: Foodservice outlets entertainment, such as music

Figure 3. Cont.

### (4) IPA grid for "food price"



(5) IPA grid for "food selection"



Note:

FP1: The announcement of food price changes

FP2: The reasonableness of the quality of food items provided for the price paid

FP3: The reasonableness of the quantity of food items provided for the price paid

Note:

- FS1: The choices of food meet my cultural preferences
- FS2: The variety of food served
- FS3: The choices of food meet my religious needs
- FS4: The selection of food makes me feel at home
- FS5: The selection of food is similar to that in my home country

Figure 3. Individual IPA grid for items of university foodservice attributes.

### 4.2.1. Interactional Quality

The international students felt that "cleanliness and neatness of staff appearance in the foodservice outlet" (IQ1) was very important, but indicated low satisfaction with it because this item fell into QII. The respondents indicated that "respectfulness of the foodservice outlet staff" (IQ2), "speed of service in foodservice outlet" (IQ5), and "staff knowledge of the food items sold in foodservice outlet" (IQ6) were very important, and the students were satisfied with the service, as the items fell into QI. If the items in QI are further provided, then the customer can suppose that an organization performs excellent work with a highly significant attribute [57]. The foodservice staff should maintain their excellent job in offering friendly service. "Professionalism in service delivery in foodservice outlet" (IQ4) and "Easiness talking staff (able to answer my question)" (IQ7) indicated no particular intervention was required, because the international students indicated these were of low priority, with the items falling into Quadrant III (QIII). The items appear to be not an issue because their poor performance is comparatively irrelevant. "Friendliness of the foodservice outlet staff" (IQ3) registered in the low importance high satisfaction quadrant (QIV), indicating a potential overuse of efforts and resources expended on this item. In this quadrant, the items were relatively unimportant for international students, but the university foodservice did exceptionally well at providing them. Administrators may choose to reassign resources to Quadrant II. Figure 3 shows the IPA grid of interactional quality items.

### 4.2.2. Food Quality

As seen in Figure 3, three items fell into the "concentrate here" quadrant (QII); "Nutritional benefits of food items" (FQ1), "Appropriateness of food flavor" (FQ2), and "Food hygiene and safety" (FQ5) were crucial but students were comparatively unhappy with these items. It can be suggested that attention is needed to improve the satisfaction of international students with these features. "Freshness of food ingredients" (FQ4), which indicates elevated efficiency and high priorities, was situated in the "keep up the good work" quadrant (QI). "Attractiveness of food items displayed" (FQ3) fell into the "possible overkill" quadrant (QIV), showing high satisfaction but low importance. According to this result, one could consider that too much emphasis is placed on this item, considering it is not important to international students. International students were not satisfied with "Appropriateness of food temperature" (FQ6) in university foodservice. However, it was a low priority, since this was also a relatively low priority for students.

### 4.2.3. Atmospheric Quality

According to Figure 3, international students were less satisfied with "Cleanliness of the foodservice outlets" (AQ5) than with other elements. This dimension was considered by students to be essential, but the output was relatively small. This indicates that efforts for enhancement should be focused on this element. There were three items indicated in "low priority" quadrant (QIII): "Decoration of the foodservice outlet" (AQ3), "Noise level" (AQ4), and "Foodservice outlet entertainment, such as music" (AQ6). Under the "keep up the good work" category were "Seat availability" (AQ1) and "Convenience of service hours" (AQ2). No component was located in the "possible overkill" quadrant in this attribute.

### 4.2.4. Food Price

As shown in Figure 3, two items of food price attributes were in the "keep up the good work" quadrant: "The announcement of food price changes" (FP1) and "The reasonableness of the quality of food items provided for the price paid" (FP2). These characteristics were highly important and satisfactory for global students. While "The reasonableness of the quantity of food items provided for the price paid" (FP3) was rated comparatively poor, satisfaction was also rated insufficient. These characteristics did not involve any extra resources because of the small salience; if resources were limited, they could be used if needed in different fields that were more relevant.

### 4.2.5. Food Selection

As depicted in Figure 3, "The variety of foods served" (FS2) and "The choices of foods permit me to meet my religious needs" (FS3) were located in Quadrant I. International students felt these items were both important and satisfactory; management ensured that these fell into the category of "keep up the good work". "The choices of food meet my cultural preferences" (FS1) was located in Quadrant II. This feature was very important but the respondents felt unsatisfied, which represented a priority for management action. The administrators of university foodservice should concentrate on this point. This food selection item was deemed important, but international students were only slightly satisfied with it. The management therefore required special attention for this point. An additional two items were shown in Quadrant III: "The selection of food makes me feel at home" (FS4) and "The selection of food is similar to that in my home country" (FS5).

### 5. Discussion and Implications

In accordance with the results of this study, food price attribute was the key driver of international student satisfaction, and management's job is to ensure that the university

foodservice "keeps up the good work". Two of three foodservice components, "The announcement of food price changes (FP1)" and "The reasonableness of the quality of food items provided with the price paid (FP3)" emerged as the most satisfactory factors, indicating that international students held highly positive perceptions of university foodservice in these areas. These two components were highly important in attracting international students to eat on campus. Thus, the food prices are critical elements affecting international students' satisfaction. Foodservice operators are advised to focus on these aspects in their promotional efforts of university foodservice in order to capitalize on their importance. Based on the importance-performance analysis, food quality attributes emerged as very important, but international students were not satisfied. Food quality situated in Quadrant II was considered to be underperforming and, as such, represents the product's major weaknesses. Hence, the managers must concentrate on this attribute. "Nutritional benefits of food items (FQ1)", "Appropriateness of food flavor (FQ2)", and "Food hygiene and safety (FQ5)" were three components of food quality that were very important, but international students were less satisfied with these items. It can be suggested that these items should be taken into consideration for cafeteria administrators and can be addressed by providing more vegetables dishes, the availability of juices, etc. University foodservice managers can collaborate with food science and technology departments in their university to create a variety of healthy foods with reasonable prices in the university cafeteria.

On the other hand, "Freshness of food ingredients (FQ4)" was located in "keep up the good work" quadrant, indicating high performance and high priority. The participants felt that this dimension was crucial, and the university operators seemed to perform very effectively in its delivery. The implication is that continued resources should be directed towards improving this item. The college foodservice providers should guarantee that ingredients used for student meals are checked periodically in the college cafeteria for each kitchen counter. The checked components can consist of the expiry date, freshness of the vegetables/spices and herbs, refrigeration methods used in the cafeteria, etc. Meanwhile, two foodservice attributes were relatively unimportant to the international students, but the university foodservice performed very well; these were interactional quality and atmospheric quality. This suggests a possible over-employment of efforts and resources being spent on these attributes. Management may wish to reassign resources to the attributes associated with food quality. In interactional quality items, the foodservice providers must consider the cleanliness and neatness of staff. This item was indicated as very important but had low satisfaction by international students. The findings suggest that special attention is needed for these highly significant performance foodservice components. University foodservice operators need to improve upon these features. The operators can provide training to university foodservice staff in order to increase their knowledge of hygiene and safety. In addition, operators also can conduct audits of the staff.

Meanwhile, in atmospheric quality components, international students deemed "Cleanliness of the foodservice outlets (IQ1)" particularly important yet were less satisfied with it relative to other items. Foodservice managers in the MRUs should pay particular attention to improving international students' satisfaction with this item because it was positioned in the quadrant of "concentrate here". This aspect was regarded as very important for international students, but the production was comparatively low. This should be the focus of improvement initiatives. Foodservice operators should conduct a regular inspection of the appearance of their staff, including the use of gloves when serving meals, the use of safety shoes, the use of aprons, wearing cooking caps to avoid hair falling in the meals, etc. Nevertheless, "keep up the good work" was achieved for "Seat availability" (AQ1) and "Convenience of service hours" (AQ2). The items in Quadrant I are usually concerned with the operation of foodstuffs and should continue to improve the efficiency level. It would therefore be realistic for university foodservice managers to keep on focusing on these attributes in foodservice development on campus. For the food selection components, international students felt they were both important and satisfactory in terms of food variety served and food choices to meet their religious needs. The management should ensure

these items continue to "keep up the good work". Nonetheless, the international students felt that food choices that met their cultural preferences were very important, but they were unsatisfied with the performance. Thus, the university foodservice administrators should focus on this, including increasing the variety of food (e.g., shawarma, takoyaki, sushi, tacos, etc.) to reflect more diversified ethnicity and age groups and to support students with special dietary needs and providing a variety of foods from various countries. The university foodservice operators can collaborate with the international student association and food departments to create, innovate, and provide food choices that can be accepted by international students and be reasonably priced.

The findings are useful in forecasting international student satisfaction and offering good empirical evidence for the importance–performance analysis, which assumes that attribute performance influences international student satisfaction less when the attribute's self-stated significance is poor. If international student satisfaction is highly influenced by attribute performance, the attribute's self-assessed value is high. The findings of this research provide a better understanding of the international students' satisfaction with on-campus foodservice in MRUs. Results from IPA also assist in determining how international students perceive the quality of the key competencies of on-campus foodservices and identify fields in which improvements are further required. The results also are useful in determining how to enhance international students' satisfaction with foodservice attributes in the university.

Overcoming these limitations can provide guidance for future research. First, Indonesian students made up the majority of those who participated in this research. The result could be a non-response factor, which was implemented owing to a lack of representation in the population of these non-respondents. It was possible that most of the perception was based on differences in taste or culture. This may lead to an upward bias in responses. Consequently, the findings of studies on the population of interest should be implemented to counter these limitations. Second, international students in this study came from different cultures around the world. Culture can affect consumer thinking and perceptions, as Li [46] has indicated. For this reason, future research may seek to determine how the relations between the studies differ in terms of service delivery and students of distinct backgrounds and cultural variables. This research is an exciting expansion. Furthermore, the practical data acquired will certainly help food operators in understanding global students' distinctive requirements. Third, the research results may not be generalizable outside this population, because only international students from Malaysian research universities were included in the study population. It is critical that comparative studies be repeated at other Malaysian universities in order to increase generalization. However, the results of the study do not guarantee the same outcomes when various conditions for allocations of different international student population groups are applied. Approved by any clear findings regarding alternative population segmentation criteria, similar assessments must be made for comparative reasons.

### 6. Conclusions

The empirical findings of this research demonstrated that interactional quality, food quality, atmospheric quality, food price, and food selection attributes in each IPA quadrant were positioned by the mean values of importance and performance. The results are effective in predicting international student satisfaction and provide strong empirical support for the importance–performance analysis, which assumes that international student satisfaction will be influenced less by attribute performance when the self-stated importance of the attribute is low. Where international student satisfaction is influenced by attribute performance, the self-stated importance of the attribute is high. According to the results of the importance–performance analysis, food price was the key driver of international student satisfaction, since the students felt that food price attribute was important and they were satisfied with the attribute. Unfortunately, food quality was considered to be underperforming and, as such, represents the product's major weakness. Thus, this attribute should be addressed by foodservice managers. Subsequently, food selection was an attribute of low priority. Interactional quality and atmospheric quality could be viewed as areas of performance that were relatively unimportant to international students; however, the university foodservice performed very well in these areas. The importance–performance analysis technique examined international student satisfaction with on-campus foodservice. The findings of this research provide better understanding of international students' satisfaction with on-campus foodservice in MRUs, which can help to meet university quality standards. Results from the IPA can also help to determine how international students perceive the quality of the key competencies of on-campus foodservices and identify fields in which improvements are further required.

**Author Contributions:** Conceptualization, A.Z.A. and B.-L.C.; methodology, A.Z.A. and B.-L.C.; writing—original draft preparation, A.Z.A.; writing—review and editing, B.-L.C. and H.H.; visualization, A.Z.A.; supervision, H.H. and A.R.; project administration, A.Z.A. and B.-L.C. All authors have read and agreed to the published version of the manuscript.

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**Institutional Review Board Statement:** Because of the observational nature of the study, and in the absence of any involvement of therapeutic medication, no formal approval of the institutional review board of the local ethics committee was required. Nonetheless, all subjects were informed about the study and participation was on a voluntary basis. The study was conducted in accordance with the Declaration of Helsinki.

Informed Consent Statement: Not applicable.

**Conflicts of Interest:** The authors declare no conflict of interest.

### Appendix A

Image 1. Cafeteria of Universiti Malaya (UM)



Source from http://www.ummc.edu.my (accessed on 26 May 2021)



Source from http://www.ummc.edu.my (accessed on 26 May 2021)

### , TokMad Cafeteria



Source from http://www.ukm.edu.my (accessed on 26 May 2021)

# Pendeta Zaba College Cafeteria



Source from http://www.ukm.edu.my (accessed on 26 May 2021)

Subaidah Restaurant

Image 3. Cafeteria of Universiti Sains Malaysia (USM)

Image 2. Cafeteria of Universiti Kebangsaan Malaysia (UKM)



Source from https://news.usm.my (accessed on 26 May 2021)



Source from https://news.usm.my (accessed on 26 May 2021)

Image 4. Cafeteria of Universiti Putra Malaysia (UPM)

Kolej 13 Cafeteria



Source from https://k13.upm.edu.my (accessed on 26 May 2021)

Kolej 16 Cafeteria



Source from https://k16.upm.edu.my (accessed on 26 May 2021)

# Richiamo Cafe

Source from https://www.utm.my (accessed on 26 May 2021)



UTM Cafeteria

Source from https://www.utm.my (accessed on 26 May 2021)

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Image 5. Cafeteria of Universiti Teknologi Malaysia (UTM)

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Article



# A Co-Created Methodological Approach to Address the Relational Dimension of Environmental Challenges: When Critical Legal Analysis Meets Illustrated Storytelling

Margherita Paola Poto <sup>1,2,\*</sup> and Arianna Porrone <sup>3</sup>

- <sup>1</sup> Faculty of Law, UiT The Arctic University of Norway, 9011 Tromsø, Norway
- <sup>2</sup> Department of Management, University of Turin, 10100 Turin, Italy
- <sup>3</sup> Department of Political Science, Communication and International Relations, University of Macerata, 62100 Macerata, Italy; a.porrone@unimc.it
- Correspondence: margherita.p.poto@uit.no

Abstract: Environmental education research needs to take into account the relational dimension of the ecological challenges of our time. It requires the development of methodological techniques that prioritize community concerns, and generally foster positive relational dynamics of the research and study group. This leads to the construction of a research and educational approach around the collective and cocreated interpretation of stories related to ecological bonds and knowledge, and the adoption of illustrations enabling participation, inclusion, and interaction among the parties. Through the lens of critical legal analysis and participatory research, we explore the beneficial effects of cocreating knowledge with the help of a specific learning toolkit (LT), built around storytelling and designed to stimulate respectful relationships between participants. The LT addresses a wide audience of indigenous and local communities, students, and researchers. Founded on participated storytelling, collective interpretation, and illustration, the toolkit includes (1) the project cover, (2) an illustrated handbook based on an indigenous story, and (3) the illustration and conceptualization of a silent book. Through the interpretation of stories on the ecological bonds between humans and nonhumans, we analyze how the process of looking for common solutions to environmental threats makes participants reflect on their relational connection to the theme and each other. We also observe how the discussion generates a sense of responsibility that comes with bringing a new idea into being. The result is that both education and research become part of the solution to the challenge itself in the shape of a harmonious relational and transformative experience. The solution lies in the recognition of the individual and collective capacity to change systems by changing relationships. Only through a collective effort towards a common sense of relational accountability and trust we can heal the wounds of our planet, and our individual and collective wounds.

Keywords: participatory; learning; critical; legal; theory; integral; ecology; relational

### 1. Introduction

"The idea of a system of infinite relationships *between everything and everything else*" (I. Calvino, Six Memos for the Next Millennium, 1988).

Legal, philosophical, and spiritual reflections on environmental ethics converge in the belief that the ecological crisis of our time reflects the dysfunction of the human and nonhuman relationship [1–9]. As Karen O'Brien puts it, 'Adaptation involves more than simply accommodating the impacts of climate change: it is also about confronting the societal context in which these changes are occurring' [5]. This study develops from the premise that a participatory approach in environmental legal studies contributes to the improvement of the societal context in academia and research. Such an improvement is crucial to address environmental challenges, considering the key role played by academia and research as fundamental incubators in the support and advancement of environmental

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**Copyright:** © 2021 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). solutions. Therefore, in the attempt to provide a scientific response to ecological relational dysfunctions, this study explores the applications of a novel methodological approach that combines critical legal thinking, participatory learning and action (PLA), and art-based (ARB) research. The interdisciplinary study of the ecological crisis applies a lens that brings together legal reflections, indigenous models of research and knowledge, and the narrative of ecofeminism, united in the aim of 'changing the system, not the climate' [10]. The system can be changed precisely by starting from the production, research, and teaching of knowledge, and thus from the world of research and education.

The novel combination of critical legal analysis with PLA [11–14] and ARB [15,16] is relevant in light of Leavy's argument [16], according to which scientific and artistic methodologies bear intrinsic similarities since they both attempt to illuminate aspects of the human condition. The participatory and artistic inquiry is structured through workshops that involve communities of researchers and students, and foster their interaction.

The workshops are further embedded in the context of integral ecology (IE), intended as the integrated framework encompassing visible and nonvisible aspects of natural and social systems, which allows for decentering from the human dimension and focusing on the relational one [17,18]. In the world of research and scientific methodologies, the IE perspective translates exactly in the need for employing participatory processes aimed at cocreating solutions to current ecological challenges, which result in a relational problem or dysfunction. In this system, where 'the whole is greater than the part' [18]: (i) ecological solutions transcend disciplinary and sectoral boundaries; (ii) the subject–object dichotomy of research is critically revisited and overcome; and (iii) hierarchical power structures that are established in the scientific community are generally rethought, reread, and reinterpreted in the light of the values of care, relational accountability, respect, reciprocity, and responsibility.

An artistic and participatory process that looks into the environmental threats of our times through the perspective of IE may be more likely to find fertile terrain for an effective cocreation of the solution to the challenges insofar as IE unifies life as an intersubjective system [17,18]. IE looks into the ecological challenges to detect the symptoms of a relationship problem in the planetary wounds [19]. The transformational change that an artistic participatory approach of knowledge cocreation generates is expected to advance the body of solutions to the relational problems in the intersubjective system that is the IE.

### 2. Materials and Methods

### 2.1. From Indigenous Methodology and Legal Design (LD) to the Learning Toolkit (LT)

Our understanding of the role of cocreation of knowledge in the relational study of ecological issues is supported by theoretical and empirical approaches, both inspired by the (1) indigenous methodology [20] and (2) legal design. First, we developed our approach to relational research thanks to the training and the experience gained from the Indigenous Law Research Unit (ILRU), Victoria, British Columbia, Canada [21]. The ontological set of values that indigenous methodology brings to the table of a scientific approach to law and social studies is rooted in the idea that a continuously informed consciousness is generated within the community of research participants, where the relation of researcher–researched becomes irrelevant if not highly questionable, and where the ultimate goal is to explore how to change and improve the relational flow between participants and reality. In such a realization, there are numerous angles from which we draw insights, all valid and including ecofeminism, critical analysis, storytelling, and other multisensory experiences such as visual and illustrated narratives.

Second, we also developed our materials around the study of the communicative and evocative power of images in legal communication. The use of illustrations is a relatively new technique in legal research. Thanks to the research carried out by the Legal Design Lab at Standford University, coordinated by Margaret Hagan, in collaboration with the Faculty of Law and the Faculty of Design, illustrated language and legal research are also gradually establishing themselves as a discipline of study and an object of research [22–25].

Margaret Hagan, in particular, studied the stages of design thinking, observing how illustrations can complement the process of creating legal solutions centered on human needs. The researcher used the expression 'human-centred design approach' to define a methodological approach that aims to generate new forms of intervention and solutions to complex problems [25]. Using the technique of human-centredness, the needs of the parties are identified and graphically represented. This approach, according to Margaret Hagan, fits perfectly with PLA.

A final related field is legal participatory action research. Like a human-centered design approach, this form of action research also involves close work with the stakeholders in a given challenge area, and it encourages researchers to use interactions and creative work to produce new insights. Legal design borrows from the social science participatory action research approach. It involves researchers working alongside people whom they are studying or in the context that they want to understand [25].

Another cluster of excellence in the study of legal design, which integrates aspects of visual law and illustrated legal research on environmental issues, is being consolidated in Brazil, at the Universidade Federal do Estado do Rio de Janeiro, thanks to the research team coordinated by Giulia Parola, which sees the collaboration of a law firm, young researchers belonging to the indigenous Chiquitano people from Mato Grosso (in Brazil), and a group of legal design consultants [26–28].

Against this background, our methodology consists of a three-step process conveying into our LT that includes the following materials: (1) The ideation of the concept idea or project cover (2019); (2) development and testing of a handbook (2020); (3) illustration and conceptualization of a silent book (2021).

The concept idea of the LT was first launched with project cover An Illustrated Storybook on Indigenous Stories (Step 1, Figure 1) representing the interaction between two shape-shifter feminine spirits that voice (indigenous) stories. Both stories and storytellers mirror the fluid and immanent governing water and earth in such a way that can be understood by students, indigenous and nonindigenous researchers, and community members at large. The two spirits of water and earth intend to accompany the overall project as if it was an illustrated story of stories in itself, collecting narratives, ideas, and observations on the symbiotic relationship that governs communities and nature. The cover representing the idea was presented in 2019 during international workshop Kjønnsforskning NÅ!, endorsed by the Gender Research Group, at the UiT The Arctic University of Norway [29]. The idea to build bridges between different legal cultures and orders with the help of illustrations led to a successful and well-received first project outcome, and the cover was published as a final scientific product. Hence, the inspiration to continue developing the idea to open creative venues for legal reflections.

Step 2 (Figure 2) of our LT was largely inspired by this work, with the creation and publication of handbook A Story About Knowledge, inspired by an Arctic story (based on the learning materials of the University of the Arctic, training module 4, edited by Gord Bruyere and Einar Bergland), and specifically rooted around an illustrated episode of the story [30].

The story was chosen for the focus on the search for the best place where to find and guard knowledge. The search is triggered by the Creator's request addressed to a trickster spirit, to find that place, and it is delegated from the trickster spirit to the animals of Earth (symbolically represented by a bear, an eagle, a salmon, and a mole). The search becomes an interactive, situated, and yet delocalized thought-provoking process. One of the protagonists of the story is the mole, gifted without apparent eyesight and with great vision. Knowledge is found and guarded in the heart of Earth, and it is the mole that, at a first sight, seems to solve the enigma. At a deeper level, though, the audience realizes that the solution is the result of a collective sum of apparently vain individual efforts of the other animals. The illustration, the narration of the story based on the illustration, and a series of lessons are the three core elements of the handbook. The lessons are developed



around a character of the story, and are intended to stimulate reflections and debate around the roles of the characters in the unfolding of the story.

**Figure 1.** Concept idea. Illustrated storybook of indigenous stories as a learning tool for scholars and communities; illustration by Valentina Russo, Mucho Amor, 2019, published in https://doi.org/10.7 557/5.5040.



**Figure 2.** Handbook, colored version of the official book cover. A Story About Knowledge, illustrated by Valentina Russo, Mucho Amor, 2020.

Step 3 (Figure 3), or the silent book [31], continues along the line of the conceptual idea (working on the relational aspect of ecology), this time situated in geographical and colored settings (the mountainside, airspace, the waterside, the underground). Five art prototypes display the five crucial moments of the animals' search for knowledge, and consequent full immersion in and interaction with their own environment. The key episodes were developed in the form of an illustrated book for kids of all ages, and ultimately as a silent book. The book aims to become eligible for the program of Silent Books: Final Destination Lampedusa, The IBBY Network [32], a book network that donates wordless books to a newly created library in Lampedusa. Our LT addresses a composite audience of university students, researchers, and the community at large, in person and face to face, and with digital research or facilitation.



**Figure 3.** Image 4, excerpt, Russo, V. (illustrator). *A Story About Knowledge. Illustrated version*. ed. by A. Porrone and M. P. Poto, Aracne: Roma, forthcoming 2021.

### 2.2. Testing the Foundations with Communities of Students and Researchers

Enhancing Environmental Reflections through a Consolidated Classroom Practice

In mid-2020, we brought into play the LT through a set of seminars and a series of academic lectures that we held for young researchers and students in the fields of global studies, law, economics, and interdisciplinarity.

In particular, we integrated our Step 2 into university curricula: in an elective undergraduate course on administrative law and the agenda 2030, and on public law for economics (University of Turin, Italy, Department of Management; academic years: 2019– 2022); in a module of the master's program on food security and safety (University of Turin, Italy, academic year: 2020). Moreover, we used the teaching material to lead a seminar on the theme of situated knowledges as part of the core activities complementing Year I and II students' doctoral training in Global Studies. Justice, Rights, Politics (University of Macerata, Italy, Department of Political Science, Communication and International Relations, Academic year: 2019–2020). Eventually, the teaching methodology was adopted in an interdisciplinary master's program on global health with a specific focus on Arctic governance (McMaster University, Hamilton, Ontario, Canada 2021).

Our approach to Step 2 of the LT steers the process of learning and environmental problem solving through a consolidated practice. Seminars and classes were designed to deepen the individual and collective awareness of the root causes of ecological wounds, and the role that participatory research and education can play in the wound-healing

process. Before the seminar or class starts, we send an invitation to the participants to come prepared on a general reflection about their understanding of why their environment is degraded, and the role that they and other members of their communities and society at large play in this process. Where possible, we send the text of the story to the participants before the session with instructions for a preliminary reading.

Engagement with Step 2 of the LT follows a protocol that contains care and gratitude at its core. We thank the audience, the territory that embraces us (especially in cases of occupied lands), and the virtual space that hosts our meeting. At times, we initiate the conversation by reading the text of the story out loud, respecting breaks and silences in the text to slowly accompany readers into the story. Every time, we experience the power of reading out loud, the fully engaging exercise where all our energy as readers–narrators is put into the task without any distraction. It is a multisensory activity (engaging voice, hear, sight) that builds a strong connection between the mind and voice of the readers and the listeners. It seems comparable to the work of a solo player who interacts with an orchestra. The spoken word helps to strengthen our minds and take ownership of our ideas, filling in the room with sound and meaning, building bridges and connections between participants.

When our time is limited, after giving attendees time to read and reflect, we summarize the story's main points, focusing on the physical and psychological features of the animals and the symbolic integration in their environment.

After the story reading or telling, we listen to the audience's impressions and report back preliminary impressions (first reactions to an unconventional academic text, potential common themes, summaries of previous experiences with the same story or with illustrated storytelling) before initiating a deeper conversation around the subject matter. When we use the learning toolkit in teaching environments, we tend to ask our audience to work as a team to provide feedback, identify common key ideas that require refining, changing, or additions, and emphasize themes or ideas that resonate with them. Each animal's action or decision making is associated with a specific lesson, composed of an objective, keywords, questions, and an interactive activity.

Before, during, and after the sessions, participants are encouraged to engage with the handbook by providing written answers and their creativity. Creative answers by designing and coloring journaling prompts are highly encouraged and recommended. Multisensory experiences are part of the learning process and a proactive way to respond to the sequence of questions specifically designed for the project. Engaging with the story lesson through journaling exercises and prompts is part of a collective therapeutic process to bring about awareness on the need to heal the planet by healing our relationships and improving our overall health conditions by creatively engaging with inner and outer conflicts. The work outside the seminar room contributes to spur new conversations, allowing for us to discuss key issues in greater depth, elevating the richness and complexity of mutual understanding. Through these continued conversations, the key underlying principles forming a common conceptual framework of integral ecology were identified, for instance, in how the relationship with the environment and the peoples emerges from narratives, stories, and dreams, and what it entails in terms of rules and obligations for the communities.

Our sessions aim to self-empower and motivate participants to take action, develop relational strength through empathy, compassion, and care for each other and for the environment that surrounds them.

A typical structure for the conduction of activity through Step 2 of our LT is provided in Table 1.

**Table 1.** Example of participatory techniques of self- and collective reflection through storytelling applied to education and research.

### Reading of the Story—A Story About Knowledge

Brainstorming—participants are asked to think about the role played by one of the characters of the story, e.g., in the case of the bear, we ask participants to reflect on the role of leaders and decision makers. This process can be developed through different steps, involving the intellectual ability to:

- think about that role;
- write down some of the characteristics of that given role;
- draw a mind map, a matrix, a blueprint of the activities, decisions, responsibilities of the given role

Sharing session—participants are asked to work in a group:

- In the case of in-person meetings with a concept poster (flip chart papers, a blackboard), participants are asked to complete with drawings, words, sticky notes, index charts, task lists.
- In the case of virtual meetings, by preparing a presentation (we experienced a range of presentations from conventional ppt to Prezi, and to videos).

Ranking engagement: participants are asked to:

 Share in words and pictures how they felt during the workshop, and how it felt to work through a story and illustrations.

### 3. Results and Discussion

The research results of the cocreated approach include the organization of a workshop on Donna Haraway [33], with a focus on the need to rethink and reframe current ecological challenges through new stories with research group Social Dynamics in Marine and Coastal Areas (Kiel University, Germany, Department of Geography, Academic year: 2020–2021).

In particular, the workshop was held on a digital platform (Zoom) and accompanied by an interactive ppt (Canva) with research group Social Dynamics in Marine and Coastal Areas (Kiel University, Germany, Department of Geography), and benefited from a relaxed, friendly, and open atmosphere. Engagement with the story led the participants to unexpected scenarios. Reflecting with the help of an illustrated story is an unconventional method, especially in Western-based research contexts. The session facilitator played a key role by creating the rules of the game and the atmosphere to engage all participants in the common working space. The session opened with the story reading and the assignment of an active role to the participants. Participants were asked to reflect on the main research question with the help of the story, compare the story with other resources, share their opinions, and synthesize the results in a common conceptual framework. Results were then collectively reassessed, and rereading the story ends the workshop. In this case, it was the illustration of the mole and her underground world, filled with important questions (Porrone, Poto, 2021, such as 'How is wisdom related to the senses?', 'How is it related to individual and collective efforts?', and 'What does the earth teach to the researchers?') that guided the meditation.

Take-away reflections included questions on epistemology and methodology (e.g., on the actors involved in knowledge production, on the way knowledge is achieved, and on the purpose of such an achievement). Such issues and many others were discussed in a final group session, collectively and in a nonjudgmental space. All participants contribute to the cocreation of a healthy and respectful environment. A participant referred to this as a form of 'collective intelligence', open to unpredictable possibilities, a process that should also translate into the vocabulary of law and global studies. A research environment based on a healthy relationship between researchers has beneficial effects on the quality of the research content and ultimately on a healthy planet. The experience of delving into collaborative educational and research activities, and thus engaging in a reciprocal effort of cocreating and looking for solutions, shifts the conversation towards purpose, intention, and the consciousness of a common interconnected world. Discussing concerns for the natural environment, increased climate change threats, economic and social disparity, or inadequacy of the international legal tools to overcome the fragmentation of the actors and solutions, led us to reflective questions (e.g., "What is the ontological structure of nature that law should mimic?" and "How can we make the international community shift towards a novel conceptual structure?") or evaluative statements about action (e.g., "It will be the next generations who will suffer the most, which is why we have to build a sustainable model for future generations"). In a sense, these reflections develop a sense of relational accountability, as knowledge exchange and collective search for solutions are understood to be part of a ceremony [34], which raises the level of consciousness of our reciprocal duties to respect, honor, and care for each other and our environment.

Overall, the positive impact that the research has on the principal investigators is part of the success of the project. Following a protocol of respect and care for the place that is hosting gatherings teaches the coordinators the value of intellectual humility by the act of establishing a connection between people and the physical space that hosts them [35].

Moreover, erasing the divide of researchers and researched gives a new dimension to equality, where barriers between teacher and learner vanish, and the relational flow does not encounter hierarchical obstacles. Dealing with problems and solutions with the help of nonverbal languages, such as illustrations, silences, and sounds, provides a richer experience of the diverse approaches and relational attitudes towards a problem.

### 4. Conclusions

This paper analyzed the conceptually and methodologically rigorous approach to developing effective cocreated participatory and inclusive methods and techniques to deal with the challenges presented to the world of research and education by the ecological crisis. The cocreation of participatory and artistic approaches to research and teaching is configured as a methodological response to ecological challenges, through a perspective that takes into account the relational aspect that emerges from the ecological dysfunctionality. The theoretical approach to IE needs to be complemented with participatory empirical research that places the relationship between subject and object of research at the center of the investigation, suggesting a *modus operandi* that reconciles and ultimately overcomes the divide between researchers and object of research.

The new conceptual and methodological framework described here is extremely demanding for the communities of participants, researchers, and learners: it requires a considerable time commitment and engages participants' involvement in defining the relational dynamics between them, around the research question they are asked to answer. In these ways, our approach creates opportunities for transformative research and education to be propagated around inclusive and collective research and education spaces.

To gain further empirical evidence to support our approach, we are extending our participatory approach to focus groups from nature-connected community members, such as the indigenous river communities of Brazil and Northern Norway [36–38]. Along this path, future research could explore how this community-based approach could support environmental justice research, where researchers and learners could become actors of change and paladins of environmental justice [39,40].

The whole process of cocreated methodological approaches to compelling legal questions on the environmental challenges contributes to an active and continuous process of knowledge shaping. Moreover, through the collective reflections of the participants, the approach contributes to the building of a new collective narrative and visualization of ecology, as a space where legal and global reflections are accompanied by the development of strong relational bonds among the parties involved in the research and education pat. The collective sharing of concerns for the natural environment, increased climate change threats, and the inadequacy of international legal tools to overcome the fragmentation of the actors and solutions develop a sense of relational accountability, as knowledge exchange and collective search for solutions are understood to be part of a ceremony [34,41] that raises the level of consciousness of our duties to respect, honor, and care for each other and our environment.

By raising the environmental consciousness through collective research, it is possible to heal relational wounds and in this sense offer a solution to the environmental challenges, which are also and above all relational challenges, of our time. When brought into practice, the idea of a system of infinite relationships between everything and everything else becomes the collective solution to the ecological challenges of our times.

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# Article A Study on the Sustainable Use Intention of Restaurant Companies Using the Information Attributes of SNS: The Dual Process Theory

Sangmook Lee<sup>1</sup>, Gumkwang Bae<sup>2</sup> and Hyojin Kim<sup>3,\*</sup>

- <sup>1</sup> Department of Hospitality and Tourism Management, Kyungsung University, Busan 48434, Korea; mookvndn@ks.ac.kr
- <sup>2</sup> Division of Hotel, Tourism and Foodservice Management, Dong-Eui University, Busan 47340, Korea; 14116@deu.ac.kr
- <sup>3</sup> Department of Tourism Management, Mokpo National University, Mokpo 58554, Korea
  - Correspondence: hyojin1126@hotmail.com

**Abstract**: This study was conducted to define detailed factors by combining the factors of SNS (social network services) information attributes and dual processing process theory and to investigate the relationship between customer satisfaction, brand attitude, and sustainable use intention. An empirical analysis was conducted using data collected from 361 participants who have experience in SNSs for searching for restaurant information. The major results are as follows: First, two intuitive SNS information attributes (reliability and liveliness) and two analytical attributes (usefulness and conciseness) have a significant positive influence on customer satisfaction to use an SNS. In particular, the reliability was the most significant antecedent of the SNS information attributes in this study. In addition, the current study verified the significant relationships among customer satisfaction, brand attitude, and sustainable use intention. Based on these research results, it was verified that SNS information characteristics are important attributes in eliciting customer satisfaction, attitude toward the brand, and sustainable use intention from customers. This study will contribute to providing various practical implications to establish valuable marketing strategies.

**Keywords:** dual processing theory; SNS information attribute; satisfaction; brand attitude; sustainable use intention

### 1. Introduction

With the development of SNS, human lifestyles and consumption behaviors have also brought about great changes, and this is directly affecting the PR and marketing strategies of corporate management. Currently, most companies do not use SNSs simply to promote their products and services, but to communicate with customers and utilize customer characteristics through SNSs. In particular, these approaches can develop customer trust and increase purchase intention through customer relationship management, along with analyzing their products and services [1,2]. In the food service industry, SNSs create several channels and generate revenue through service functions that connect customers and restaurant companies, and most of the consumers who have purchased or used food services through SNS information are affected by the SNS [3–5] (Cho an Shin, 2020; Chun, Lee, and Park, 2020; Kim and Lee, 2017). According to the Korea Rural Economic Research Institute (2019), online food purchases are rapidly increasing, and the online sales of fresh foods (concentrated and marine products) also increased steadily from KRW 315.7 billion in the first quarter of 2014 to KRW 518.7 billion in the fourth quarter of 2017.

Currently, research related to SNS is being actively reported in previous studies related to the food service industry. Ji, Hu, and Byun (2013) [6] analyzed the influence relationships among the efficiency of promotion, user satisfaction, and impulse purchases of the SNS information characteristics of catering companies, and the study verified the positive



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**Copyright:** © 2021 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). relationship between SNS information characteristics and customer satisfaction. Joung and Kim (2015) [7] classified the characteristics of SNS information into playfulness, neutrality, and reliability of information and verified that the site characteristics of SNSs directly affect the intention to visit a restaurant. In addition, Kim, Hwang, and Park (2018) [8] identified the structural relationship between word-of-mouth information characteristics (consensuality, vividness, neutrality, timeliness), trust, purchase intention, and word-of-mouth intention of restaurant consumers on SNSs, and the results confirmed that when the perceived benefits of dining-out products on SNSs are high, word-of-mouth or recommending information about dining products to others was increased.

Dual processing theory is actively used in various fields from cognitive psychology to behavioral economics to consumer studies by understanding how individuals process information according to the characteristics of thought processing and the decision-making process through analysis of these processes [9]. In particular, the dual processing theory understands the consumer from the perspective of two types of human thought processing: the intuitive part and the analytical part [9,10]. Previous studies applying this dual processing theory mainly explain the decision-making process that pursues persuasion communication and information effectiveness, so it is useful to explain the information processing process through recommended online information and persuasion in the information system field. Therefore, dual-processing theory is a theoretical foundation for the better understanding of consumers because the foodservice business can make decisions based on various information provided online through SNSs and directly affect sustainable purchase intentions.

The theoretical contribution of this study lies in its examination of six elements of restaurant SNS information attributes that sustainably influence customer satisfaction, brand attributes, and intention to use services. First, the present study draws lessons from the dual processing theory model, and it focus on the information characteristics of individual SNS users. In addition, the present research considers the impact of the SNS information based on the intuitive and analytical concept of the dual processing theory, and this study applied the theory to restaurant consumers. Second, this study considered the customer satisfaction and brand attitude responding to sustainable use intention. There are enough studies related to use intention in foodservice, while there are only a few studies combining the sustainable use intention of restaurants through attributes of information on SNSs, satisfaction, and brand attitude. In addition, in previous studies, research related to SNS has been actively conducted, but most of the research has focused on the information characteristics and quality of SNSs without theoretical support. Recently, as the proportion of dining out has increased due to the influence of COVID-19, consumers' interest in dining-out information on SNSs such as Instagram, Facebook, and blogs is increasing, and it is considered to be important to prepare a differentiation strategy to build and develop a marketing strategy using a blog or SNS app. Therefore, this study proposed to define the detailed factors by combining the factors of SNS information characteristics based on the dual processing process theory and to investigate the relationships between brand attitudes, customer satisfaction, and sustainable purchase intention by using empirical analysis. It has academic originality in that it understands the sustainable behavior of restaurant consumers through SNS information attributes based on logical theory. Furthermore, this study has academic originality in that it predicts the future behavior of restaurant consumers through various SNS information provided by restaurant companies.

# 2. Literature Review

# 2.1. Theoretical Framework

The Ministry of Agriculture, Food, and Rural Affairs and the Korea Agro-Fisheries and Food Trade Corporation selected "Multi-streaming Consumption" as the key word for the restaurant trend in 2020. This trend is a major phenomenon that stimulates the emotions of dining out, and it is actively promoted through SNSs such as YouTube, Facebook, and Instagram. Even in the current COVID-19 situation, in particular, the food and dining economy reported that the restaurant market has expanded from offline (stores) to online after COVID-19. In addition, the concept of physical space is disappearing, and e-commerce, home shopping, distribution companies (convenience stores, marts), and shared kitchens are establishing themselves as new dining trends. In a situation where the flow of consumption through the Internetis changing as such, the increase in sales using online spaces such as SNSs continues, and the importance of online marketing is emerging in an age of continuous dining out.

Chaiken's (1980) [11] heuristic systematic model (HSM) is the most widely used in dual process theory. HSM explains the information processing process by dividing it into heuristic information processing and systematic information processing. In particular, the peripheral-systematic model is a suitable theory for explaining the personal information processing process through the Internet community, SNSs, etc., and is used as a theory specialized in online information technology [12]. Specifically, systematic information processing is a process similar to the central route concept asserted in the elaboration and possibility model and refers to the process in which the user analytically processes a large amount of information. On the other hand, the heuristic information processing process is considered to be similar to the peripheral route of the elaboration possibility model, and it is information processing that puts weight on the peripheral information related to itself rather than the quality of the information [13]. In other words, in the case of online information exchange, trust in the person delivering the information is required, and source credibility is an important factor in the process of peripheral information processing since it is not a face-to-face process [14]. In addition, the consumer accepts the information after judging the usefulness of the information based on the trust, and it is effective in individual decision-making and problem solving because it influences behavior by acquiring knowledge [12].

Previous studies applying the dual processing theory to SNSs are as follows. First, Hu, Ji, and Byun (2012) [15] said that the dual processing theory is the result of the continuous development of a change in attitude as a kind of information processing through a traditional attitude. Ji, Hu, and Byun (2013) [6] extended the existing research and verified the dual process theory as a theory that specialized in information reliability pursuit, persuasion communication, and online information technology. Seok (2020) [16] applies the dual process theory to consumer product purchase decision making, which occurs when a consumer purchases a product according to two ways of thinking: intuitive and analytical. The previous studies employed a dual processing theory that has focused on the psychological aspect so far, but this study expands and applies the theory to the consumer's psychology through the SNS information characteristics of the restaurant business. Hence, the current study intends to classify SNS information characteristics as detailed factors based on the heuristic and systematic viewpoint of the dual processing theory.

### 2.2. SNS Information Attributes

SNS is an abbreviation for social network service, and an SNS has a unique characteristic in that users form a social network and disclose it to others, rather than simply providing a connection with people [17]. An SNS is an online system that forms and strengthens social relationships through free communication, information sharing, delivery, and expansion of personal connections between users [18]. Information attributes of SNSs are classified into various factors. Choi and Kim (2012) [19] found that consumers generally search online information to reduce decision-making time and make better purchase decisions, and the SNS information characteristics were divided into broadness, storability, accessibility, anonymity, transcendence of space and time, and rapid dissemination. Hong (2011) [20] classified SNS information characteristics into interactivity, accessibility, information provision, reliability, and up-to-dateness, and Kwok (2013) [21] classified the SNS characteristics of catering companies into informational, up-to-date, lively, concise, and playful for a study on the effect of SNS characteristics on technology acceptance attitudes and behavioral intentions. In addition, Bae, Yang, and Park (2018) [22] classified the information characteristics of SNS into information provision, reliability, liveliness, and interactivity and studied consumers' attitudes toward snack products, word of mouth, and purchase intentions. Therefore, this study intends to classify SNS information characteristics that consumers can recognize through food service companies based on previous studies into consent, reliability, liveliness, up-to-dateness, usefulness, and conciseness. First, according to Kelly's (1973) [23] attribution theory, people become convinced when experiencing the same effect for the same entity, and information with such consent has a greater and stronger communication effect than information without consent. Accordingly, consent can be defined as the degree to which two or more individuals agree on the performance of a product and a plurality of information recipients agree on the performance of the product [24]. Second, reliability can be defined as information perceived by the recipient that the source will provide unbiased opinions and objective information with knowledge, skills, and experience related to the subject of communication [25]. Third, the vitality of information is easily remembered and exerts a relatively greater influence than other factors, and detailed information, personal experiences, and advice or aphorisms to others play a very effective role in the diffusion of information [26]. Fourth, up-to-dateness is the degree to which various contents used by users are generated and provided in time [27]. Fifth, usability can be defined as the usefulness and usable value, and generally refers to the degree to which the usefulness of information satisfies a need for information that users can use and obtain [28].Last, conciseness means that the content the user is looking for should be provided in an appropriate amount at the right time and place, and although it may be considered that it does not contain a sufficient amount of information, it is rather an attribute that excludes unnecessary content and facilitates intensive understanding [29].

# 2.3. Customer Satisfaction

Satisfaction has been considered as an important variable for predicting behavioral attitudes in the marketing field, because it is possible to evaluate customer needs or expectations and predict the evaluation after use [30]. Hoffman and Bateson (1997) [31] defined satisfaction as a customer's perceived response that satisfies or exceeds customer expectations through a service provider. Satisfaction on SNSs is an important factor in the evaluation process. It refers to the emotional state that users feel when the provided product or service meets their expectations and is also defined as the degree of satisfaction experienced by using an SNS [32]. Im (2012) [33] reported that enhancing a customer relationship through customer satisfaction and customer loyalty can increase the reuse of products and services. This study found that the SNS activity of a restaurant company had a significant effect on customer value as well as customer satisfaction and behavioral intention. Jo (2017) [34] divided the SNS quality of travel companies into information quality, interface quality, and system quality and demonstrated that service quality directly affects customer satisfaction and purchase intention. In addition, Ahn and Go (2017) [35] analyzed the correlation between customer satisfaction and purchase intention in the SNS characteristics of restaurant companies and verified that the most important key factor in predicting and understanding customer behavior is SNS information along with satisfaction. Hence, previous studies confirmed that the characteristics of various information provided by SNSs and customer satisfaction can be closely linked in the restaurant business. Therefore, in this study, the variable of customer satisfaction is considered as a factor that plays a mediating role with the outcome variables such as SNS information characteristics and continuous use intention.

#### 2.4. Brand Attitude

Attitude is a consistent reaction and psychological response that appears favorably or unfavorably to an object through learning and experience, and this attitude can be determined according to the importance of major attributes of an object [36]. Fishbein (1963) [37] stated that brand attitude refers to emotions such as positive or negative and favorable or unfavorable, which are specific individual feelings toward a brand. In addition,

Keller (1993) [38] reported that a brand attitude is an individual's consistently favorable or unfavorable response to a specific brand and that it is the highest level of an abstract and powerful form of association. Similarly, Grace and O'Cass (2005) [39] identified that brand attitude is the sum of the positive or negative tendencies that appear as a result of perception and satisfaction with the experience of service brand elements in the past. In previous studies, brand attitude was set as an important outcome variable or a parameter between key factors such as customer satisfaction and loyalty. For example, Kwon (2016) [40] studied the correlation between blog immersion and such brand attitudes and intentions to visit and verified that they have a significant positive (+) influence relationship with each other. Jo (2017) [34] verified how a hotel company's SNS characteristics affect brand attitudes and purchase intentions. The study defined the brand attitude as the positive or negative attitude of consumers toward the brand and the position in which the attitude appears. Due to its characteristic of being stable and consistent, it can be said that brand attitude is an important variable for understanding and predicting consumer behavior [41–43]. In addition, Byun, Kim, and Lee (2013) [41] studied the causal relationship between brand information and consumer attitudes targeting family restaurant users. As a result of the study, it was verified that positive or negative information about the brand of a family restaurant directly affects the brand image, brand attitude, and purchase intention. Based on previous studies, therefore, the present study considered that various SNS information attributes of restaurants have a causal relationship directly or indirectly on the brand attitude.

#### 2.5. Sustainable Use Intention

The intention of continuous use online can be interpreted in more various forms than offline, and it is characterized by being very free of time and place restrictions [43]. In previous studies, continuous use intention has been defined as a concept explaining a consumer's plan to continue to use a service or product [44]. A prior study found that consumers who are satisfied about a specific service or product have driven the continuous use of branded content and reuse intention [44]. In recent years, the issue related to sustainable consumption of a branded product has been reported by only a few studies [45–47]. For instance, Hong (2018) [45] performed a study to identify the predictors of the sustainable intention to use Facebook in Korea and showed that the attitude factor has a direct influence on the behavioral intention to use an SNS. In addition, Kim and Lee (2021) [46] conducted a study to verify how motivation affects sustainable use intention for webtoon brands. More specifically, the study demonstrated that the attitude towards the webtoon brand was a critical antecedent of sustainable use intention, and the attitude was influenced by motivation factors.

Therefore, in the context of the foodservice business, understanding the concept of sustainable use intention through various elements that can influence the sustainable use intention from consumers is a major point. However, very little research has sought to examine the concept of sustainable use intention along with satisfaction and brand attitude in the context of the foodservice sector.

#### 2.6. Hypothesis Development

### 2.6.1. Relationships between SNS Information Attributes and Customer Satisfaction

Satisfaction is used as a variable to measure performance or efficiency after users actually use the system in the field of information systems [48]. The satisfaction that consumers feel after using online services such as SNSs can be a major variable to explain the influence of online services, and the properties of information systems can be used as important measurement variables to evaluate satisfaction. Ahn and Ko (2017) [35] classified the characteristics of SNS into interactivity, information availability, and convenience of access and verified the significant relationships among the SNS characteristics of catering companies, customer satisfaction, and purchase intention. In addition, Sung, Ko, and Kim (2012) [49] demonstrated that that users' satisfaction with SNS information had a

positive effect on their continued use intention and recommendation intention in retrieving dining-out information. Therefore, this study divided SNS information characteristics into intuitive elements (synonymy, reliability, and liveliness) and analytical elements (up-to-dateness, usefulness, and conciseness) based on the dual-processing process theory and assumed that the characteristics of SNS information would have a positive effect on user satisfaction in terms of the following hypotheses.

Hypothesis 1a (H1a). SNS information attributes will have a positive effect on customer satisfaction.

Hypothesis 1b (H1b). Consent will have a positive effect on customer satisfaction.

Hypothesis 1c (H1c). Reliability will have a positive effect on customer satisfaction.

**Hypothesis 1d (H1d).** *Liveliness will have a positive effect on customer satisfaction.* 

**Hypothesis 1e (H1e).** *Up-to-dateness will have a positive effect on customer satisfaction.* 

Hypothesis 1f (H1f). Usefulness will have a positive effect on customer satisfaction.

**Hypothesis 1g (H1g).** Conciseness will have a positive effect on customer satisfaction.

2.6.2. Relationships between Customer Satisfaction, Brand Attitude, and Sustainable Use Intention

Brand attitude is a stable and continuous characteristic for predicting and understanding consumer behavior such as choosing a brand or motivating purchase behavior. Previous studies verified that customer satisfaction can directly influence brand attitude as well as purchase intention. For example, Byun, Kim, and Lee (2013) [41] verified the significant relationship between information factors and brand attitude from family restaurant consumers. Taylor and Hunter (2014) [50] identified the direct relationship between e-satisfaction and loyalty from consumers, and the e-satisfaction loyalty relationship appears to be mediated by brand attitudes in this study. In addition, Hwang, Choe, Kim, and Kim (2021) [51] investigated the antecedents and consequences of satisfaction as perceived by customers who used a coffee shop operated by robot baristas and customers who used a coffee shop operated by human baristas. The study demonstrated that consumer satisfaction has a significant impact on bran attitude as well as loyalty.As such, studies on the relationship between customer satisfaction and brand attitude by applying SNSs were insufficient, but several previous studies assumed that customer satisfaction could have a direct effect on brand attitude. Therefore, the current study established the following hypothesis.

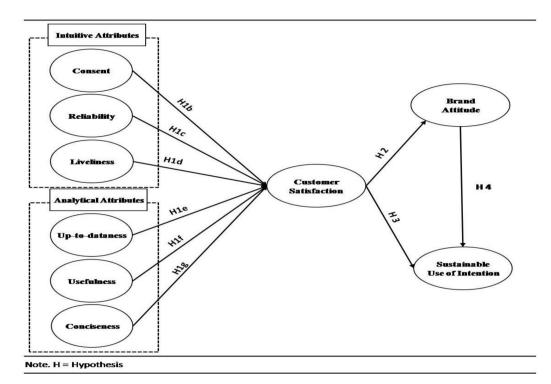
**Hypothesis 2 (H2).** *Customer satisfaction will have a positive effect on brand attitude.* 

In previous studies, a significant causal relationship between customer satisfaction and continuous use intention was verified in various fields. For example, Hossain and Kim (2018) [44] verified the relationship between satisfaction and sustainable use intention as perceived by Facebook users. Therefore, the present study established the following hypothesis. In addition, previous studies confirmed the important about the SNS factor as a predictor of behavioral intention [52,53]. For example, Suh, Won, and Hong (2010) [52] empirically investigated the effects of six SNS website quality factors (interface quality, system quality, information quality, service quality, emotion quality, and authoring environment quality) on user satisfaction and the intention of continuous use as well as word-of-mouth. Their study found that the system quality, emotion quality, and authoring environment quality were critical antecedents of customer satisfaction, and the customer satisfaction influenced the intention of continuous use as well as word-of-mouth.

Hypothesis 3 (H3). Customer satisfaction will have a positive effect on sustainable use intention.

Prior studies have verified the significant relationships between brand attitude and loyalty or behavioral intention in various sectors. For example, Kim and Lee (2021) [46] identified that the attitude towards a webtoon brand can directly influence sustainable use intention. Similarly, Hossain and Kim (2018) [44] found that consumers who are satisfied with Facebook's website have sustainable use intention about the site. In addition, Hwang, Choe, Kim, and Kim (2021) [51] verified that brand attitude has a significant influence on brand loyalty from coffee shop visitors. Therefore, this study developed the following hypothesis based on the previous research.

#### Hypothesis 4 (H4). Brand attitude will have a positive effect on sustainable use intention.



Based on above hypotheses we suggested the following research model on Figure 1.

Figure 1. Research frame.

# 3. Methodology

# 3.1. Measurement of Variables and Survey Method

In order to conduct this study, a preliminary survey was conducted with50 customers who used a restaurant service through an SNS in 2020. Reliability verification and validity verification of the questionnaire items were performed through a preliminary survey, and the items with low reliability were corrected and supplemented with relevant professors and graduate students to create the final questionnaire. After modifications from the pilot test, the developed survey was conducted in February 2021 through the online survey method. The online survey was conducted for consumers who searched for and used SNSbased dining-out information, and it was commissioned by a consumer panel survey expert company (MicromillEmbrain). A self-administrated questionnaire was designed, and the questionnaire comprised three sections. The first part included six constructs related to SNS information attributes: synonymy, conciseness, up-to-dateness, reliability, usefulness, and liveness. The second part included customer satisfaction, brand attitude, and sustainable use intention. All questions from the sections included three to four items using a 7-point Likert-type scale (1: strongly disagree to 7: strongly agree) for the following: "Please indicate your level of agreement with the following statement". The final section of the questionnaire included participants' personal information, such as age, gender, education

level, frequency of visiting the SNS for choosing a restaurant, and so on. To measure the attributes of SNS information on restaurant businesses, our study adopted 20 items from Bae et al. (2018) [22] and Lim (2019) [54]. The four items for customer satisfaction were based on earlier research by Ahn and Go (2017) [35], Hellier, et al (2003) [55], Oliver (1980) [56] and Kim et al (2009) [57]. Brand attitude was measured using four items that were modified from Fishbein (1963) [37]. In addition, the sustainable use intention was measured using four items that were identified by Hossainand Kim (2018) [44]. The final section of the questionnaire included respondents' relevant personal information, such as age, gender, household income, and dining motivation.

# 3.2. Data Collection and Statistical Analyses

Distribution of the questionnaire encompassed 500 restaurant customers who were using SNSs for searching for a restaurant. From these samples, 450consumers participated in the survey (90% response rate). There were 35 questionnaires with missing values, and 29 disqualifications were due to the last visit on any SNS site for finding information about a restaurant being more than one month earlier. In addition, multivariate and univariate outliers found 33 outliers, which were also excluded. After all checks for sample validity, 361 responses from participants remained for hypotheses testing. Statistical analysis used SPSS25.0 and AMOS 23.0 programs. Specifically, this study performed the data analysis method by Anderson and Gerbing (1988) [58]. First, a confirmatory factor analysis (CFA) tested whether or not the observed variables reflected the hypothesized latent constructs using a covariance matrix, and composite reliability confirmed the measurement reliability. Second, validity tests (convergent and discriminate) were conducted using factor loading and average variance extracted (AVE). After verifying the measurement model, structural equation modeling (SEM) examined the overall fit of the proposed model along with assessing the hypotheses.

#### 4. Results

#### 4.1. Demographic Chatacteristics

Table 1 shows the participants' characteristics. Among the 361 valid questionnaires, 165 were male (45.7%) and 196 were female (54.3%). The occupations of respondents were office worker (34.1%), student (33.0%), professional worker (10.5%), personal business (10.2%), government officer (7.5%), and others (4.7%). The result of the age group shows that 20~29 was the highest (32.4%), followed by 40~49(24.1%), 30–39(22.7%), and over 50(20.8%). The results of education level found that university and graduate school graduates accounted for more than 70% of the respondents. Last, the results of the frequency of SNS use for dining out indicated that 3~4 times per week was the highest (47.1%), followed by everyday (17.2%), 5~6 times per week (16.1%), and 1~2 times per week (13.0%).

Table 1. Results of respondent characteristics.

	Variables	Ν	%
C l.	Male	165	45.7
Gender	Female	196	54.3
	Everyday	62	17.2
Erroquances of	1~2 times per week	47	13.0
Frequency of	3~4 times per week	170	47.1
SNS using for	5~6 times per week	58	16.1
Dining-out	3~4 times per month	20	5.5
	1~2 times per month	4	1.1

Variables		Ν	%
	Student	119	33.0
	Government officer	27	7.5
Occupation	Office worker	123	34.1
Occupation	Personal business	37	10.2
	Professional worker	38	10.5
	Others	17	4.7
	20~29	117	32.4
A ===	30~39	82	22.7
Age	40–49	87	24.1
	Over 50 y $\leq$	75	20.8
	High school graduate	16	4.4
	Technical college graduate	43	12.1
Education Level	University graduate	114	31.6
	Graduate degree	148	41.0
	Others	39	10.8
	Total	361	100.0

Table 1. Cont.

# 4.2. Reliability Verification of Variables

In this study, the attributes of SNS information characteristics were measured with a total of six variables (synonymy, conciseness, up-to-dateness, reliability, usefulness, liveness) based on previous studies. The measurement items for each factor consisted of a total of 20 items: three items of synonymy, conciseness, reliability, and liveliness, and four items of up-to-dateness and usefulness. Customer satisfaction consisted of three items, and brand attitude and sustainable use of intention consisted of four items each. Using Cronbach's alpha value, it was verified whether the measurement factors consisted of homogeneous variables. According to previous studies, a Cronbach's alpha value of 0.6 or higher for exploratory research and of 0.7 or higher for confirmatory research is considered appropriate. All of Cronbach's alpha values for six variables related to SNS information attributes exceeded the standard value of 0.6 (synonymy: 0.799; conciseness: 0.860; up-to-dateness: 0.875; reliability: 0.860; usefulness: 0.907; liveness: 0.875), so the reliability of information characteristic factors could be verified. In addition, the Cronbach's alpha value of use of intention was 0.942. Therefore, the reliability of all variables was verified (Table 2).

Variables	Measurement Items	Mean	Cronbach'sα
	There are many positive comments on the SNS information.	5.25	
Synonymy	A lot of comments with the same tendencies depend on various people on the SNS information.	5.16	0.799
	There is a lot of sympathy for the SNS information.	5.25	-
	The SNS information is concise	4.89	
Conciseness	The SNS information is well summarized.	5.02	0.860
	The SNS information is easy to read.	5.12	-
	The SNS information provides the latest information.	5.33	
Up-to	The SNS information is continuously updated.	5.11	- 0.875
dateness	The SNS information was most recently updated.	4.79	- 0.875
	The SNS information contains the latest trends.	5.12	-

Table 2. Results of demographic characteristic.

Variables	Measurement Items	Mean	Cronbach's a
	The SNS information is reliable.	4.89	
Reliability	The SNS information has expertise.	4.52	0.860
	The SNS information can be trusted and used.	4.75	-
	After using the SNS information, it actually helped me.	5.45	
	The SNS information actually existed.	5.52	
Usefulness	I made an economical consumption choice due to the SNS information.	5.49	0.907
	The SNS information I searched for will be helpful in the future.	5.46	-
	The SNS information is very specific.	5.07	
Liveliness	The SNS information evokes a feeling as if I have actually experienced it.	5.02	0.875
	The SNS information is very realistic.	4.96	-
Customer Satisfaction	Overall, I am satisfied with the SNS information of the restaurant I used.	5.10	
	I am satisfied with the promotions and events through the SNS information of the restaurant I used.	4.93	0.860
	The evaluation through the SNS information of the restaurant I used was satisfactory.	4.59	
	I had a good impression of the brand by using the SNS information of the restaurant that I used.	5.03	
Brand Attituda	I was satisfied with the brand by using the SNS information of the restaurant I used.	5.12	0.898
Brand Attitude	Using the SNS information of the restaurant I used, the brand feels familiar.	4.99	0.090
	I can trust the brand by using the SNS information of the restaurant I used.	5.03	
	I will use the SNS to find information about the restaurant in the future.	5.16	
Sustainable use	I would use the SNS more than any other information.	5.02	0.942
intention	I will visit the SNS to find restaurant information as often as I can.	5.10	-
	I usually visit the SNS when I am online.	5.08	

Table 2. Cont.

#### 4.3. Confirmatory Factor Analysis

In this study, structural equation modeling (SEM) was employed to more specifically verify the causal relationship and direction between measurement variables. Structural equations can be analyzed in consideration of measurement errors of independent and dependent variables, and parameters can be measured at the same time as setting a system of equations, so more complex and step-by-step theoretical structural analysis can be effectively performed [59,60]. In this study, the overall fit of the model was measured and verified using both the absolute fit index and the incremental fit index [60,61]. The results of the confirmatory factor analysis for the validation of the validity and fit of the measurement variables and the validation of the relationship between the constructs were presented in more detail. First, the  $x^2$  value was 815.448, the degree of freedom (df) was 394, the CMIN/DF was 2.07(p = 0.000), the GFI was 0.877, the AGFI was 0.845, the RMR value was 0.046, and the RMSEA value was 0.055. Based on these results, this study verified that the degree of absolute fit was acceptable [60,62]. Next, the NFI value was 0.916, the IFI value was 0.955, the TLI value was 0.946, and the CFI value

was 0.954 to verify the intermediate fit [60,62]. Checking factor loading and average variance extracted assessed convergent validity in this study. Composite reliability for all elements was calculated for assessing the instrument's reliability. As shown in Table 3, all constructs represented desirable levels of CR (composite reliability), ranging from 0.800 to 0.937, so the current study satisfies convergent validity [61]. In addition, AVE values of all measurement factors exceeded the reference values of 0.7 and 0.5, except for five relationships (up-to-dateness and usefulness, up-to-dateness and brand attitude, satisfaction and brand attitude, satisfaction and sustainable use intention, brand attitude and sustainable use intention) [60,61]. To identify the discriminant validity, therefore, the variables combined and compared the constrained model and unconstrained model using the  $x^2$  differences. The results show that all paired comparisons of the constructs have significant  $x^2$  differences between the measurement model and alternative model (up-to-dateness and usefulness:  $\Delta \chi^2 = 126.316$ ,  $\Delta df = 8$ ; up-to-dateness and brand attitude:  $\Delta \chi^2 = 133.291$ ,  $\Delta df = 8$ ; satisfaction and brand attitude:  $\Delta \chi^2 = 69.922$ ,  $\Delta df = 8$ ; brand attitude and sustainable use intention: $\Delta \chi^2 = 221.255$ ,  $\Delta df = 8$ ). Therefore, the discriminant validity of the designed research model was also verified [60,62] (see Tables 3 and 4).

Table 3. Confirmatory factor analysis for the measurement model.

Constructs and Indicators	Standardized Factor Loadings	Composit Reliability	AVE
Synonymy		0.800	0.572
There are many positive comments on the SNS information.	0.728		
A lot of comments with the same tendencies depend on various people on the SNS information.	0.722		
There is a lot of sympathy for the SNS information.	0.815		
Conciseness		0.859	0.670
The SNS information is concise.	0.793		
The SNS information is well summarized.	0.860		
The SNS information is easy to read.	0.801		
Up-to-dateness		0.860	0.606
The SNS information provides the latest information.	0.725		
The SNS information is continuously updated.	0.791		
The SNS information was most recently updated.	0.820		
The SNS information contains the latest trends.	0.776		
Reliability		0.860	0.673
The SNS information is reliable.	0.826		
The SNS information has expertise.	0.801		
The SNS information can be trusted and used.	0.834		
Usefulness		0.907	0.710
After using the SNS information, it actually helped me.	0.842		
The SNS information actually existed.	0.812		
I made an economical consumption choice due to the SNS information.	0.884		
The SNS information I searched for will be helpful in the future.	0.831		
Liveliness		0.875	0.700
The SNS information is very specific.	0.850		
The SNS information evokes a feeling as if I have actually experienced it.	0.857		

Constructs and Indicators	Standardized Factor Loadings	Composit Reliability	AVE
The SNS information is very realistic.	0.802		
Customer Satisfaction		0.902	0.698
Overall, I am satisfied with the SNS information of the restaurant I used.	0.774		
I am satisfied with the promotions and events through the SNS information of the restaurant I used.	0.816		
The evaluation through the SNS information of the restaurant I used was satisfactory.	0.843		
Brand Attitude		0.852	0.659
I had a good impression of the brand by using the SNS information of the restaurant that I used.	0.838		
I was satisfied with the brand by using the SNS information of the restaurant I used.	0.807		
Using the SNS information of the restaurant I used, the brand feels familiar.	0.856		
I can trust the brand by using the SNS information of the restaurant I used.	0.839		
Sustainable use of intention		0.937	0.787
I will use the SNS to find information about the restaurant in the future.	0.875		
I would use the SNS more than any other information.	0.886		
I would visit the SNS to find restaurant information as often as I can.	0.898		
I usually visit the SNS when I am online.	0.889		

Table 3. Cont.

Table 4. Comparison of AVE and squared correlations of paired constructs.

Constructs	SN	CC	UL	RL	UF	LV	CS	BA	SI
Synonymy (SN)	0.572								
Conciseness (CC)	0.343	0.670							
Up-to-dateness (UL)	0.392	0.500	0.606						
Reliability (RL)	0.392	0.396	0.551	0.673					
Usefulness (UF)	0.484	0.433	0.692	0.446	0.710				
Liveliness (LV)	0.413	0.355	0.546	0.494	0.619	0.700			
Customer Satisfaction (CS)	0.473	0.564	0.588	0.555	0.635	0.582	0.698		
Brand Attitude (BA)	0.442	0.540	0.684	0.661	0.658	0.605	0.812	0.659	
Sustainable Use Intention (SI)	0.433	0.441	0.479	0.452	0.610	0.442	0.863	0.717	0.787

Note. AVE is on the diagonal. Squared correlations of paired constructs are on the off-diagonal.

# 4.4. Result of Structural Equation Model

Measuring the fit of the designed model was confirmed before the validation of the causal relationship for hypothesis testing. Results show that the  $x^2$  was 863.618, the degree of freedom (df) was 405, p = 0.000, and the GFI was 0.871. The AGFI was 0.842, the sRMR value was 0.035, and the RMSEA value was 0.056, confirming the absolute fit of the measurement factors. In addition, the NFI value was 0.911, the IFI value was 0.951, the TLI value was 0.943, and the CFI value was 0.951, so the intermediate fit of the research model for hypothesis testing was also verified. All but two of the path coefficients in the model were positive and significant. Therefore, empirical support accrues to all the hypotheses

except for Hypotheses 1-1 and 1-4, which referred to the path from synonymy to customer satisfaction and up-to-dateness to customer satisfaction. In other words, four elements of SNS information attributes (conciseness, reliability, usefulness, and liveliness) have significantly influenced customer satisfaction. Furthermore, the relationships between customer satisfaction, brand attribute, and sustainable use intention were statistically significant. The more specific hypothesis test results are as follows. The results confirm the proposed effects of intuitive SNS information attributes on customer satisfaction, reliability (Hypothesis 1–2:  $\beta = 0.28$ ; t = 5.098 \*\*\*), and liveliness (Hypothesis 1–3:  $\beta = 0.12$ ; t = 2.146 \*). In addition, usefulness (Hypothesis 1–5:  $\beta = 0.26$ ; t = 3.534 \*\*\*) and conciseness (Hypothesis 1–6:  $\beta = 0.22$ ;  $t = 4.149^{***}$  among the analytical attributes of SNS information positively influenced customer satisfaction. Among all the predictors of SNS information attributes, reliability (t = 5.098 \*\*\*) has the strongest effect on customer satisfaction followed by conciseness (t = 4.149 \*\*\*), usefulness (t = 3.534 \*\*\*), and liveliness (t = 2.146 \*). In addition, customer satisfaction was a very critical antecedent of the brand attitude (Hypothesis 2:  $\beta = 0.95$ ; t = 17.578 \*\*\*), and the customer satisfaction (Hypothesis 3:  $\beta = 0.26$ ; t = 2.201 \*) and brand attribute (Hypothesis 4:  $\beta = 0.77$ ; t = 6.528 \*\*\*) have a direct effect on sustainable use intention. Overall, the study produces significant support for the notion that SNS information attributes of foodservice businesses, as perceived by customers during their consumption experience in a restaurant after finding information about food, positively affects their perceptions of satisfaction and, in turn, has a positive effect on the brand attitude and sustainable use intention with regard to that restaurant (see Table 5, Figure 2).

Table 5. Results of structural parameter estimates.

Hypothesized Path	Coefficient	S.E.	t-Value	Results
Intuitive A	ttributes $\rightarrow$ Custor	ner satisfa	ction	
Hypothesis 1-a:				
Synonymy $\rightarrow$	0.039	0.059	0.668	Not Supported
Customer satisfaction				
Hypothesis 1-b:				
$Conciseness \rightarrow$	0.277	0.054	5.098 ***	Supported
Customer satisfaction				
Hypothesis 1-c:				
Up-to-dateness $\rightarrow$	0.119	0.055	2.146 *	Supported
Customer satisfaction				
Analytical A	Attributes→Custo	mer satisf	action	
Hypothesis 1-d:				
Reliability $\rightarrow$	0.115	0.082	1.407	Not Supported
Customer satisfaction				••
Hypothesis 1-e:				
$Usefulness \to$	0.257	0.073	3.534 ***	Supported
Customer satisfaction				
Hypothesis 1-f:				
$Liveliness \rightarrow$	0.215	0.052	4.149 ***	Supported
Customer satisfaction				
Hypothesis 2:				
Customer Satisfaction $\rightarrow$	0.951	0.054	17.578 ***	Supported
Brand Attitude				
Hypothesis 3:				
Customer Satisfaction $\rightarrow$	0.258	0.117	2.201 *	Supported
Sustainable use of intention				
Hypothesis 4:				
Brand Attitude $\rightarrow$	0.768	0.118	6.528 ***	Supported
Sustainable use of intention				11

Note. \* *p* < 0.05; \*\*\* *p* < 0.001.

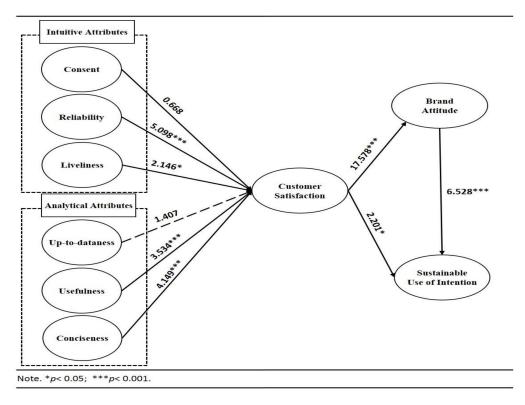


Figure 2. Result of hypothesis test.

# 5. Conclusions and Limitations

# 5.1. Conclusions

This study was conducted to verify the influence relationship between SNS information attributes perceived by restaurant SNS users, customer satisfaction, brand attitude, and sustainable use intention. Through this hypothesis test, we intend to present various practical marketing implications not only for academia but also for the food service industry, which is facing many difficulties due to the COVID-19 crisis. More specific academic and practical implications are as follows.

First, this study applied the attribute of SNS information characteristics to the dual processing theory that verified the causal relationship between satisfaction, brand attitude, and sustainable use intention through the SNS information perceived by restaurant visitors. In particular, previous studies that used dual processing theory mainly applied the concept to identify the effectiveness of SNS information in the engineering field [9,12] or in studies on psychological aspects to understand the psychology of consumers [16]. Therefore, this study identifies the important elements of SNS information attributes as an important marketing promotion strategy by considering various intuitive (consent, reliability, live-liness) attributes and analytical (up-to-dateness, usefulness, conciseness) attributes that consumers can recognize through SNSs in the restaurant.

Second, this study verified the influence relationship between SNS information characteristics and customer satisfaction, and it was verified that reliability, liveliness, usefulness, and conciseness, among the six attributes, had a direct effect on customer satisfaction. According to the results of previous studies that employed the SNS information attributes as a major variable, it was confirmed once again that the influence relationship between perceived SNS information characteristics and customer satisfaction may depend on the research subject or research field.

Third, the present study found that reliability is the most significant factor that can influence customer satisfaction among restaurant SNS information attributes. In addition, conciseness, usefulness, and liveliness also had a directly positive (+) effect on customer satisfaction. Therefore, foodservice companies or restaurants should deliver concise infor-

mation, including meaningful information that is provided on SNSs, and provide reliable information based on their expertise in the provided information. In addition, the information provider needs to provide well-organized and accurate information, including store name, location, menu, and price, to improve the usefulness of SNS information. For example, restaurant-related companies are promoting information on their SNS accounts based on consumers' visiting experiences by using experience groups on Instagram or blogs.

Fourth, it was verified that conciseness and usefulness among the analytical attributes of SNS information characteristics had a significant effect on customer satisfaction. Therefore, in order to increase customer satisfaction and further increase positive sustainable use intention, the concise and essential content-oriented information provided will have a positive effect on the consumers who view the information. Therefore, food service companies should accurately deliver the store name, location, menu, and price to searchbased services.

Last, this study verified that consumers who were satisfied with the information provided by SNSs also increased their positive attitude toward the brand and sustainable use intention. This result re-verified that there is a significant causal relationship between satisfaction, attitude, and continuous use intention, and this can be applied to the food service industry from previous studies [44,45,51,52].

Recently, the proportion of dining out has increased due to the influence of COVID-19 [63,64], and consumers' interest in dining-out information on SNSs such as Instagram, Facebook, and blogs has also increased. Therefore, this study is considered to prepare a differentiated strategy to improve and develop a marketing strategy using the SNS information attributes by considering other critical elements such as satisfaction, brand attitude, and sustainable use intention. These findings are expected to help establish marketing strategies for restaurant business and develop academic grounds.

#### 5.2. Limitations

This research conducted a meaningful study based on the information characteristics of SNSs (Instagram, Facebook, etc.) that, recently, consumers mainly visit to find information on dining out, but the following limitations were not overcome. First, the residence of the survey respondents was not investigated in this study, and data were collected in South Korea only. Therefore, the SNS information can be different based on the size of the city, country, or area of residence. Therefore, it is difficult to generalize the research results. Thus, it is necessary to overcome the limitations of the study subject by considering or comparing various areas.

Second, this study was conducted only focusing on a general SNS setting, such as Instagram, Facebook users, and blog users, among various dining-related SNS websites. However, this study did not distinguish the type of SNS setting, and the results can be different based on the SNS setting. In future research, therefore, a broader investigation of more diverse SNS websites is needed to overcome these limitations.

Third, this study requested a single response rather than multiple responses, for the main purpose of using SNSs to search for information on dining out. This was a single inquiry to check which reason is the most important in searching for dining-out information through SNSs. Therefore, it will be meaningful to inquire with multiple responses in future studies.

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# Article Exploring Consumers' Attitudes towards Food Products Derived by New Plant Breeding Techniques

Gabriella Vindigni, Iuri Peri 🖻, Federica Consentino, Roberta Selvaggi ២ and Daniela Spina \*

Department of Agriculture, Food and Environment, University of Catania, Via Santa Sofia 100, 95123 Catania, Italy; vindigni@unict.it (G.V.); peri@unict.it (I.P.); federica.consentino@outlook.it (F.C.); roberta.selvaggi@unict.it (R.S.)

\* Correspondence: daniela.spina@unict.it

Abstract: New plant breeding techniques (NPBTs) are seen as promising and innovative tools to achieve food security and food safety. Biotechnological innovations have great potential to address sustainable food development, and they are expected in the near future to play a critical role in feeding a growing population without exerting added pressure on the environment. There is, however, a considerable debate as to how these new techniques should be regulated and whether some or all of them should fall within the scope of EU legislation on genetically modified organisms (GMOs), despite the product obtained being free from genes foreign to the species. In the EU, the adoption of these methods does not rely only on the scientific community but requires social acceptance and a political process that leads to an improved regulatory framework. In this paper, we present the results of an online survey carried out in Italy with 700 randomly selected participants on consumer attitudes towards food obtained by NPBTs. By applying the decision tree machine learning algorithm J48 to our dataset, we identified significant attributes to predict the main drivers of purchasing such products. A classification model accuracy assessment has also been developed to evaluate the overall performance of the classifier. The result of the model highlighted the role of consumers' self-perceived knowledge and their trust in the European approval process for NPBT, as well as the need for a detailed label. Our findings may support decision makers and underpin the development of NPBT products in the market.

**Keywords:** agricultural biotechnology; new plant breeding technique; NPBT; consumers' attitude; food safety; machine learning; data mining

#### 1. Introduction

The selection of more efficient and productive varieties is a practice that began with the birth of agriculture itself when farmers chose the best plants from the wild for domestication [1]. For a long time, conventional breeding techniques have been the only methods to improve cultivated plants [2], requiring many generations to achieve the intended results [3]. In the past, the need to increase yields and obtain quality products has usually been addressed with the use of inputs such as chemical fertilizers, pesticides, and irrigation water, in some cases raising environmental concerns [4].

Scientific advances in molecular biology over the past 50 years have contributed to significant progress in plant breeding and the establishment of genetics as a science [5] with a great potential to increase the resilience of food systems and guarantee food security and food safety. Genetic improvement may provide tools able to stabilize yields, increase resistance to biotic or abiotic stresses, increase drought tolerance, reduce adverse effects of climate change, improve nutritional quality, increase shelf life and reduce allergenicity [6]. Despite these advantages, gene editing may cause unintentional implications and genetic errors, and the effect on human health and the environment has still to be proven.

The recombinant DNA technique has been the most innovative method introduced thus far. It has made it possible to insert genes into organisms to encode desirable traits [7],

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**Copyright:** © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). reducing the time required to achieve varieties with improved agronomic and nutritional characteristics [8]. These kinds of organisms have been designated genetically modified organisms (GMOs) and are often considered transgenic cultures because of the introduction of foreign genes [9].

The new genome editing methodologies fall into a broader category of techniques defined as new plant breeding techniques or NPBTs, which have aroused great interest in the scientific community [10–14]. They have been developed to improve the accuracy and speed of breeding [15,16] and to modify existing genes rather than adding genes from other species [17], a technique used in many GMO technologies. According to some experts, this makes it difficult to determine the difference between varieties obtained from NPBT and varieties obtained from conventional breeding since identical point mutations could also occur naturally [18]. The main applications of NPBT are cisgenesis and intragenesis, direct mutagenesis by oligonucleotides, independent DNA and RNA methylation, reverse varietal selection, and agroinfiltration [19].

Notwithstanding the positive opinion of many experts in the field of molecular biology and the interest shown by stakeholders, many policymakers and lobby groups are unconvinced. The differing opinions on safety and related regulatory policies have led to substantial controversy.

Today, European legislation appears to be stringent, generating a debate regarding Directive 2001/18 EC, which categorizes NPBT products as GMOs and, therefore, are subject to a risk assessment to obtain EU authorization [12]. This discourages breeders and biotech companies, which instead pursue possibilities in other countries where plant breeding regulations are less rigorous.

The agricultural biotech industry and connected groups encourage the use of new genetic modification techniques, claiming that they are precise, safe, and controlled and provide us the tools to meet the challenges of environmental overexploitation and climate change.

However, these claims are used to assert that these techniques should be exempted from the EU's GMO regulations and not subject to safety and traceability rules or GMO labeling since they are essentially traditionally bred varieties. On the other side, environmental groups, food retailers, small farmers, and the organic industry criticize this position, insisting that these techniques are fundamentally different from natural processes and that there may be unintentional negative effects. The issue has, therefore, wide implications for investments in plant breeding and trade in agricultural products [20].

In light of this debate, this research aimed to explore consumers' opinions and attitudes toward food derived from NPBTs. It reports the results of a survey conducted in Italy in which respondents had the possibility to express their potential intentions to purchase products derived from NPBTs. To this purpose, we have applied a data mining methodology to discover the relation among attributes, applying a classification algorithm (J48) to predict consumers' behavior.

#### 2. EU Regulation of NPBTs and the Ongoing Debate

The varieties obtained from NPBTs are regulated as GMOs, according to Directive 2001/18 EC [12]. Article 2 defines GMOs as "an organism, except humans, in which the genetic material has been altered in a way that does not occur naturally by mating and/or natural recombination". The Directive aims to protect human health and the environment [12]. The Directive does not apply directly to approvals for the import and processing of genetically modified food and feed, which are governed by Regulation 1829/2003 (EC 2003a) and are defined as "containing, consisting of or produced from GMOs" [21]. It is worth noting that all food and feed covered by Regulation 1829/2003 are also subject to labeling and traceability requirements (EC 2003b) [22].

European Union decides whether to authorize the release of new varieties based on the scientific opinion of the European Food Safety Authority (EFSA), which assesses plant varieties resulting from genetic techniques based on compositional analysis, molecular characteristics, mode of action of the protein expressed by the newly introduced gene, changes in metabolic pathways, and environmental exposure [23]. The process of obtaining the authorization demands high costs and long waiting times for breeders who instead find more possibilities for growth in other countries. In the European Union, approval of a GM crop costs between 11 and 17 million euros and takes, on average, 6 years [24].

On 25 July 2018, the Court of Justice of the European Union (CJEU), upon the request of the French Council of State, has further confirmed that organisms obtained by mutagenesis are GMOs, therefore subject to the requirements of the EU GMO legislation and the obligations of EU-wide authorization processes, traceability, and labeling rules [25,26]. This judgment discouraged European breeders, scientists, and stakeholders, stirring up debate on how the new techniques should be regulated. The focal point is whether the regulation of NPBTs should be product-based or process-based [2].

There are two main points of view: The first is that of advocates for unregulated use and approval of these techniques, which call for an evidence-based approach to proving an organism's harm to human health and the environment [27]. On the opposite side are proponents of a regulatory approach, favoring a comprehensive risk evaluation of GMOs. Advocates of this point of view believe that in the absence of scientific data regarding the probability that an organism will cause harm, products should be removed from the market until they are proven safe [28].

On 29 April 2021, the European Commission published a new study on NPBTs based on the opinions of EFSA and main stakeholders in the member countries of the EU. The study reaffirmed that organisms obtained through NPBTs are considered GMOs, but it also expressed concerns about the current legislation, whose lack of definitions or clarity on the meanings of the key terms causes ambiguity. As NPBTs constitute a heterogeneous group of techniques, EFSA has identified some techniques that have no new hazards compared to conventional techniques [13].

The report stressed the need to develop specific risk assessment procedures for NPBTs. Moreover, the study highlighted the possibility that the EU could encounter problems in international trade relations with countries that approve and use the new genetic engineering techniques. It is extremely complex to distinguish varieties of genetic techniques derived from natural or induced mutants, with consequent implications on world trade, such as a substantial decrease in the number of raw materials imported from third countries, on which the European Union and Italy, in particular, rely.

Therefore, in light of the different regulatory frameworks for NPBTs in other countries, such as the United States and Brazil, which do not specifically regulate genome edited crops, the EU could run into commercial limitations and confusion and thus put European stakeholders at a competitive disadvantage [29]. As a consequence, plant breeding companies have stronger motivations to relocate their research to other countries.

The GMO regulatory process is seen as time consuming and very costly, especially for small–medium enterprises (SMEs) that may lack the know-how and the financial power to face this challenge [30].

Furthermore, EFSA confirms that many of the plant products obtained from NPBTs have the potential to contribute to the Sustainable Development Goals through the EU's Green Deal goals and "Farm to Fork" strategy. The study highlights "the need to make legislation more resilient, future-proof and uniformly applied". The current European regulatory system on NPBTs still remains unclear in its scope and implementation, ill-suited to the advances of the scientific community regarding rapid developments in genetics and genome editing, and poorly harmonized with equivalent systems.

During the development of the study, the issue of consumer perspective remains the key point to consider, as they remain the most important players in influencing the trajectories of agricultural biotechnology innovation.

#### 3. Research Design and Data Analysis

#### 3.1. Data Collection

An online survey was carried out in Italy from March 2021 to June 2021 and disseminated via social media channels. A snowball sampling technique was used to gather responses to our survey [31]. It was adopted to generate a pool of participants for our study through referrals made by individuals to recruit people who have heard of genetic improvement techniques, although they were not well informed. The final sample size was made up of 700 respondents.

The study was grounded in literature concerning consumers' attitudes towards food purchasing habits with the aim to further validate previous research and address the research questions. The questionnaire included only closed questions. It consisted of three parts: the first part focused on the socio-demographic profile of the respondents; the second part explored consumers' food purchasing behavior; the third part addressed the respondents' acceptance and intention to purchase products derived from new breeding techniques. The surveys provided fundamental information, starting with the definition of new plant breeding techniques. The difference between NPBTs and GMOs was highlighted since, in common perception, the two terms often overlap. Participants were also informed that to date, the cultivation of plants and the marketing of products derived from NPBTs is not allowed in Italy, as well as in most European countries, and as such, the products fall under the regulation of GMOs.

#### 3.2. Socio-demographic Characteristics of the Respondents

The socio-demographic profile of the participants is presented in Table 1. Most of the respondents were women (65%), with 39% of all respondents aged under 25, 38% between 26 and 49, and 23% over 50. The respondents' average level of education is high: most have a high school degree (42.6%) or a university degree (43%). The respondents' level of income corresponds to the average distribution of the Italian population: 46.1% have an income between 20,000 € and 50,000 € and 42.3% less than 20,000 €. Considering the country in terms of Northern, Central, and Southern Italy, as conventionally used in official Italian statistics, most of the responses were from southern Italy (77.6%) and a smaller percentage from northern Italy (17%) and central Italy (5.4%).

Male	241	34.4
Female	459	65.6
<25	275	39.3
26–49	263	37.6
>50	162	23.1
South Italy	534	77.6
North Italy	119	17.0
Center Italy	38	5.4
Middle school	62	8.9
High school	298	42.6
University degree	301	43.0
Doctorate/maste	39	5.6
	Female <25 26–49 >50 South Italy North Italy Center Italy Middle school High school University degree	Female       459         <25

Table 1. Demographic distribution of survey participants (number and percentage of responses).

	Student	287	41.0
Occupation	Employment	331	47.3
	Unemployment	82	11.7
	<20,000€	296	42.3
Income	20,000€-50,000€	323	46.1
_	>50,000€	81	11.6

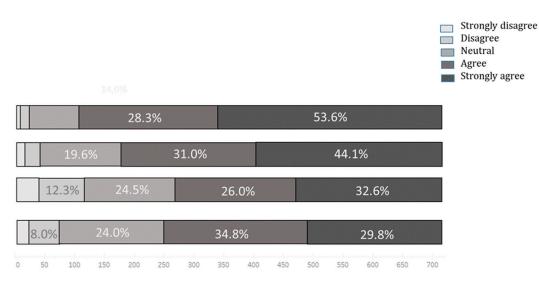
Table 1. Cont.

#### 3.3. Consumers' Food Purchasing Behaviour

We adopted a 5-point Likert scale (1 = Strongly Disagree, 2 = Disagree, 3 = Uncertain, 4 = Agree, 5 = Strongly Agree) to analyze consumers' food purchasing behavior and to measure the intensity of respondents' opinions [32], thereby collecting more detailed information than a dichotomous survey [33]. We proposed some topics of interest to respondent consumers to evaluate to what extent these issues guide consumers' purchasing choices.

The software Tableau was used to create a graph of the survey results. Tableau provides multiple tools such as analytics, data mining, data visualization, and data infrastructure, allowing the user to visualize a large amount of information [34].

From Figure 1, it is possible to observe the aspects which drive consumer purchasing choices.



Attention to nutritional and health quality of food

Attention to environmental issues

Importance of food production methods

Attention to detailed information on food label

**Figure 1.** Survey results on factors driving consumers' choice of food. Source: our elaboration with Tableau Software.

The most important aspect for respondents is health qualities of the product (53.6%). Moreover, consumers pay attention to environmental issues. Hence they may be likely to purchase "green" products (44.1%).

The consideration of the process used to obtain the product is more diversified among the respondents. The highest percentage (32.6%) consider this aspect very important or somewhat important (26%), but a relatively high percentage is indifferent to the topic (24.5%).

The importance of the product label is also, in this case, diverse: the respondents seem split between those who pay a lot of attention to the label (29.8%) and those who do not pay particular attention to its content (24%).

# 3.4. Prediction of Acceptance and Intention to buy NPBTs: A Decision Tree Classification

In recent years there has been a growing interest in the application of artificial intelligence (AI) tools for the identification of regularity phenomena in a set of complex data. Data extraction using specific AI algorithms allow the user to obtain higher levels of information synthesis and to study the possible cause-effect relationships among the available data [35,36]. This highlights the strategic role of some information and the irrelevance of others. This process is known as data mining, which is only one of the phases of a larger interactive process called knowledge discovery in database (Kdd).

Data mining techniques are mainly used to build predictive models for determining the future behavior of some relevant attributes. Classification, one of the major aims of data mining, is used to discover the relationship between the class attribute and other attributes. This knowledge can be utilized to predict the class label, which is not known in advance. The decision tree, a supervised machine learning algorithm, is a multidimensional classification method that is widely adopted for classification purposes [37]. This method predicts class membership by recursively splitting the dataset into smaller subsets for each branch [38] based on the "divide and conquer strategy" often applied in data analysis [39]. This process is then repeated at each node on the branch until a leaf node is reached. The output is a hierarchical decision tree structure where instances are ordered down the tree from the root node to a leaf node, which provides the classification of the instances. Basically, a decision tree defines a set of paths from the root node to the leaf nodes by running a series of tests. Based on information theory approach, the algorithm employs entropy in relation to the information contained in a probability distribution. The goal is to select the attribute that is most useful for classifying instances according to the so-called information gain, a measure that reveals how much information a feature provides about a class. Information gain helps to determine the order of attributes in the nodes of a decision tree [40].

In our study, we adopted decision tree J48, one of the best machine learning algorithms for classification of data [41]. It is an improved version of C4.5 algorithms developed by Quinlan and implemented in Weka, an open-source machine learning software. Weka contains a collection of visualization tools and algorithms for data analysis and predictive modeling, together with graphical user interfaces for easy access to these functions. The software supports several standard data mining tasks, more specifically, data preprocessing, clustering, classification, regression, visualization, and feature selection. In this research, the J48 decision tree has proved to be a suitable method to explore consumers' attitudes towards NPBT products since it is an exploratory analysis process in which we gather to predict a future outcome. Decision tree is a data mining technique for solving classification and prediction problems. Data mining consists of different methods and algorithms used for discovering knowledge from large datasets.

For this purpose, the classification attribute considered was intention to purchase.

Based on literature analysis [42–46], we have identified a set of attributes that have been included in the database: conditions of purchasing, concerns, barriers, degree of self-perceived knowledge, detailed label, and trust in EU food safety. All the information included in the model is shown in Table 2. For each attribute, the respective items, response count, and response percentage are shown.

Based on our set of attributes, a decision tree was induced to predict the relationship of each attribute to consumers' intention to purchase. Table 3 shows the main branches of the tree for predicting consumers' attitudes toward NPBT products. The table reports the decision rules that can be read as a simple IF-THEN statement, consisting of a condition and a prediction. For example: IF the knowledge is high AND the products are perceived as environmentally friendly (condition), THEN the consumer is willing to buy it (prediction).

Attributes	Number of Items	Items	Count	%
		Useful for human health (enhanced with nutrient attributes)	487	69.6
Conditions of purchasing	3	Lower price compared to the conventional breeding product	183	21.1
		Beneficial for environmental sustainability (reduced pesticides, water use and food waste, resistence to pests and diseases)	30	4.3
		No concerns	129	18.4
		Potential risks for human health	219	31.3
Consumers' concerns on NPBT food products	4	Ethical concerns (over exploitation of these techniques)	93	13.3
		Potential negative impact on made in Italy conventional food products	31	4.4
		Costs of the regulatory adoption	286	40.9
Barriers to the diffusion of NPBT products in	3	Costs to develop new varieties	164	23.4
EU market	5	Lack of consumers trust in engineering genetic techniques	209	29.9
		High knowledge	128	18.3
Level of self-perceived knowledge	3	Low knowledge	499	71.3
sen perceivea morrieage		No knowledge	69	9.9
		Important	579	82.7
Detailed label for risk mitigation	3	Not important	24	3.4
		Maybe important	89	12.7
		High trust	365	52.1
Trust in EU food safety authorities	3	Medium trust	231	33.0
		No trust	101	14.4

# Table 2. Model information.

 Table 3. J48 Decision tree model for consumers' attitude towards NPBT products.

Knowledge = LOW	
Detailed label = Maybe important: Maybe (27.04/0.04)	
Detailed label = Important	
Factors affecting purchase = cheaper: Maybe (117.0/6.0)	
<pre>Factors affecting purchase = environmental_friendly</pre>	
Trust in EU Food safety = low: Yes (0.0)	
Trust in EU Food safety = high: Yes (8.14/2.0)	
Trust in EU Food safety = medium: Maybe (5.09/1.09)	
Factors affecting purchase = healthier: Maybe (33.0/5.0)	
Detailed label = Not important: No (19.03/0.03)	
Knowledge = HIGH	
Factors affecting purchase = cheaper: Maybe (33.0/15.0)	
Factors affecting purchase = environmental_friendly: Yes (164.66/1.0)	

 Table 3. Cont.

Factors affecting purchase = healthier: Yes (268.0/2.0)
Detailed label = Maybe important: Maybe (3.0/0.0)
Detailed label = Important: Maybe (21.03/0.03)
Detailed label = Not important: No (2.0/0.0)
Knowledge = NO
Detailed label = Maybe important: Maybe (3.0/0.0)
Detailed label = Important: Maybe (21.03/0.03)
Detailed label = Not important: No (2.0/0.0)

Data visualization (Figure 2) provides clear information efficiently and in an understandable way.

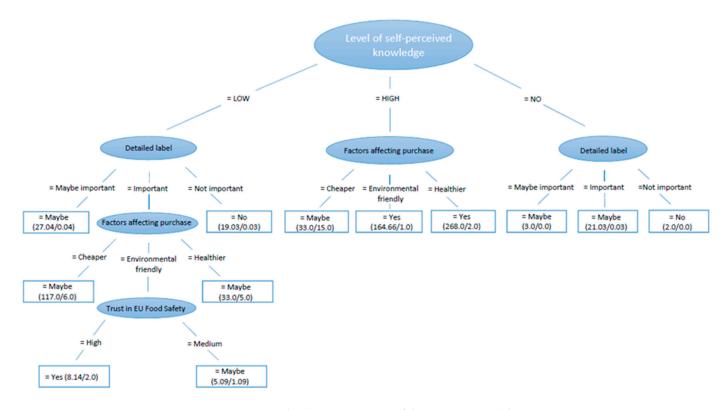


Figure 2. J48 Graphical representation of decision tree model.

Weka software applies the conventional 10-fold cross-validation to estimate the skill of a machine learning model to uncover hidden patterns. Often the procedure has a single parameter k that refers to the number of groups into which a provided data sample is to be split. Therefore, it is called k-fold cross-validation. In our case study, we have chosen a specific value for k, i.e., k = 10, becoming 10-fold cross-validation. Cross-validation is a resampling procedure used to evaluate machine learning models on a limited data sample. During this process, the software trains and evaluates 10 subsets to estimate how useful the learned model is for prediction. In Table 3 and in Figure 2, the statistic in brackets summarizes the performance of the classification. The first value is the total number of instances in each leaf. The second value shows the number of instances incorrectly classified in that leaf. When a value of the attribute in a tree is not known, the system splits the case and sends a fraction down each branch. The three important attributes according to the model are: level of self-perceived knowledge, factors affecting purchasing, and detailed label. The "level of self-perceived knowledge" appears as the first splitting attribute in

the decision tree, i.e., the attribute that can best discriminate among the others. These are reasonable results, considering the well-established axiom that lack of information can generate skepticism and mistrust towards specific scientific and technological innovations.

The model shows that if the degree of self-perceived knowledge is high, respondents who have an adequate or very good understanding of the issue are willing to buy these products when there is a strong association with specific benefits for human health and for the environment, whereas the price does not seem to be such a relevant driver of motivation to purchase. If the degree of knowledge is low, the model predicts that a detailed label is crucial if it is associated with a collective benefit. This occurs in the presence of a high level of trust in European food safety authorities. Even if there is a lack of knowledge, a detailed label plays an important role, although consumers still seem to be undecided.

#### 3.5. Assessing Classifier Performance

We have used the classification accuracy and confusion matrix in order to analyze how predictive our model is. In the present work, the accuracy is estimated as 94.3%. Classification accuracy by class (Table 4) summarizes the performance of a classification model as the number of correct predictions divided by the total number of predictions. However, using accuracy as a performance measure assumes that the class distribution is known and, more importantly, that the errors of incorrectly classified instances are equal. Accuracy may be particularly problematic as a performance measure when the dataset studied is biased in favor of a majority class [47]. In addition, we have used the statistical metrics Precision (P), Recall (R), and F-Measure (the harmonic mean of precision and recall values, it allows us to evaluate P and R together). Specifically, recall is the ability of a model to find all the relevant cases within a dataset. It is defined as the number of true positives divided by the number of true positives plus the number of false negatives. Precision quantifies the number of positive class predictions that rightfully belong to the positive class. As precision increases, recall decreases and vice-versa. Matthews correlation coefficient (MCC) is a robust metric that summarizes the classifier performance in a single value if positive and negative cases are of equal importance.

TP Rate	Fp Rate	Precision	Recall	F-Measure	MCC	ROC Area	PRC Area	Class
0.977	0.728	0.858	0.977	0.914	0.875	0.959	0.924	Maybe
0.945	0.020	0.989	0.945	0.966	0.909	0.971	0.963	Yes
0.655	0.000	1000	0.655	0.792	0.803	0.803	0.716	No
0.943	0.036	0.949	0.943	0.943	0.895	0.966	0.941	Weighted Avg.

Table 4. Detailed accuracy by class.

In machine learning, performance can also be calculated using the AUC (Area Under the Curve) and ROC (Receiver Operating Characteristics) area to summarize the overall accuracy of the classifier. It takes values from 0, which indicates a perfectly inaccurate classification, to 1, which reflects a perfectly accurate test. The precision–recall curve (PRC) can be interpreted as the relationship between precision and recall (sensitivity) and is considered to be an appropriate measure for unbalanced datasets.

An alternative method to gain better insight into the classification and misclassification distribution is the confusion matrix (Table 5). It contains information about actual and predicted classifications made by a classification system [47]. It shows the correct classification against the predicted classification for each class. The number of correct predictions can be found on the diagonal of the matrix. All other numbers represent the numbers of misclassification errors. Misclassifications occur when the row and column classes of a cell do not match. If the intersection across predicted and actual classes of different levels is empty (or zero), then no misclassification has occurred. In our case, the matrix can be

interpreted as 212 instances correctly classified in class "a" (Maybe), 5 instances incorrectly classified in class "b" (Yes), and 0 incorrectly classified in class "c" (No).

Table 5. Confusion Matrix.

a	b	c	
212	5	0	a = Maybe b = Yes
25	430	0	b = Yes
10	0	19	c = No

#### 4. Results and Discussion

Data analysis has shown how the degree of self-perceived knowledge affects the consumers' intention to purchase food obtained by NPBTs. Results of the study demonstrate that perceived knowledge is the most relevant driver of people's risk, benefit, and value perception.

Evidence from previous studies confirms that higher levels of knowledge promote positive acceptance to purchase [48,49], especially when consumers perceive benefits for human health and for the environment, which are issues considered by consumers during their purchasing decisions. This is confirmed by the increasing attention to sustainable food consumption and by people's awareness of their role and responsibilities towards the environment, individual and public health, habitat and biodiversity, social cohesion, and economy [50]. This consciousness leads to a change in consumer attitudes towards a "green" lifestyle, starting with everyday consumption choices [51].

Our results show that consumers who are familiar with NPBTs are more positive toward and more willing to buy such products, especially in relation to their impact on reducing inputs such as chemical fertilizers and pesticides to mitigate greenhouse gas emissions and improve water use efficiency [52]. Health considerations are also crucial drivers in food purchasing decisions. Respondents who consider themselves informed about genetic techniques would be willing to buy NPBT products if they enhance the food's nutritional and health benefits. Despite that, in economic literature, price is usually considered one of the main drivers of food consumer behavior [53], in the case of NPBT products, it appears to be not so relevant. Our findings show that consumers do not pay as much attention to economic convenience as they are interested in products with specific characteristics such as health and environmental aspects [54].

However, several studies underline the differences between stated and revealed preferences, finding that consumers tend to overestimate their valuation of a particular good, service, or outcome, which can lead to misleading estimates of relative value [55]. Therefore, in our study, individuals' stated preferences may not correspond closely to their actual preferences, and this can be considered a drawback of the results obtained.

The model has also revealed that consumers' concerns about NPBT food products are not seen as a threat to the type made in Italy's agri-food system, which is strongly linked in local production with certification labels [56].

When consumers' self-perceived knowledge is low, our model highlights the importance of a detailed label. Hence, a poor self-perceived knowledge does not result in the intention to purchase if not supported by clear and understandable information on the product characteristics [57]. Literature about consumption stresses the key role that the label plays in communicating information about the improved characteristics of novel food and how the food was produced [45]. Consumers' interest in the characteristics of the process makes label essential to learn more about the new food and how it is derived. This output is in line with the notion that consumers routinely rely on experts in the case of complex decisions, which is an admission of knowledge inadequacy [55].

For this group of respondents, the degree of confidence in EU food security is a discriminating factor: if trust is high, consumers are more confident in buying food derived from agricultural biotechnology. This suggests that if the European food safety authorities approved the diffusion of NPBT products, consumers would feel more comfortable buying them.

The lack of knowledge about agricultural biotechnologies brings out a psychological bias derived from the perceived distance between these products and the conventional ones. Consumers that consider NPBT unfamiliar mistrust and fear these products [58–61]. Indeed, familiarity with the product usually leads to a different perception of uncertainty. Consumers who are not familiar with a product tend to believe that they are a higher risk [62]. This behavior in food consumption is called neophobia, the reluctance of individuals to try novel food [63], and can also be seen as the averseness toward new methods of production [64–66].

Our model shows that this gap in knowledge may be compensated for with a detailed label. Indeed, in an increasingly complex food system, consumers need to have accurate information on the characteristics of the food purchased [67,68]. This is in line with the overall EU regulations on labeling, traceability, and quality assurance systems which offer extensive and accessible information to the consumer [69].

The results of the study highlighted that attitude and acceptance change with knowledge. Therefore, in the framework of consumers' concerns arises the need for balanced information and the importance of translating science into laymen's language, which can help informed decisions of consumers.

#### 5. Concluding Remarks

Few studies have investigated the attitude of Italian consumers toward NPBT foods products, and this work tried to contribute to filling this gap in the literature. In this paper, we have used machine learning to classify potential consumers and to acquire efficient information on the attributes that are most important in predicting their behavior towards NPBT products. This study confirms that J48 is a useful tool for the construction of a hierarchical decision support model. The study revealed that consumers are still fearful and uncertain but somehow positive, especially those concerned about the environment and human health. Our study attempted to go beyond a binary "for" or "against" genetic techniques to provide more nuanced data about consumer attitudes that depend on a hierarchy of attributes.

We have seen how the viewpoint of the consumer changes in relation to their level of self-perceived knowledge on the topic. In general terms, being informed greatly reduces the fear and the perception of the risk consumers associate with the product. Knowledge helps consumers understand and, therefore, not reject a priori possibilities that may be advantageous. Mandatory labeling is probably the way to promote consistent decisions. However, current EU regulations do not allow consumers to distinguish NBTs from transgenic products, as the European Court of Justice has ruled that NBTs must fall under the GMO Directive. Policymakers should address advancements in genome editing technologies with proper regulation.

Moreover, there is not an information strategy that may change the trend and empower consumers to deliberately choose among different food options without diffidence. To reverse consumer uncertainty toward NPBT foods, targeted communication campaigns to disseminate their benefits may have major implications for further development and commercialization in the European market. Additionally, a full understanding of the diffusion of NPBT technologies would require further research work. In particular, it would be interesting to analyze the pressure of interest groups in contributing to the social construction of risk.

This study presents some limitations. Firstly, the analysis of the potential willingness to buy intention to purchase new products was carried out in the absence of a real market, hence, stated preference survey responses may not predict actual behavior, leading to hypothetical bias. Future validation of these findings will be possible once NPBT foods are widely available in the EU market. In addition, the sample size is not representative of the overall Italian population, and therefore the quantitative outcomes should not be interpreted as such. However, we have accepted this biased sample since our goal was to survey opinion at an informative level and not for a study of a target population. From our point of view, the outcome does not affect the validity of the results, and they can be accepted because, in this study, consumers' opinion is generalizable across a population in the same geographical area.

Finally, although online surveys are recognized as valid methods that have quickly gained popularity in research due to their low cost and time savings [70,71], they could present a lack of potential depth and suffer correct guessing.

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# Article The Impact of Environmental and Anthropogenic Factors on the Migration of the Rural Arctic Population of Western Siberia

Elena Bogdanova <sup>1,2,\*</sup>, Konstantin Filant <sup>3</sup>, Ekaterina Sukhova <sup>3</sup>, Maria Zabolotnikova <sup>4</sup>, Praskovia Filant <sup>5</sup>, Dele Raheem <sup>6</sup>, Olga Shaduyko <sup>2</sup>, Sergei Andronov <sup>2,7</sup> and Andrey Lobanov <sup>2,7</sup>

- <sup>1</sup> Department of Economics and Management, Northern Arctic Federal University, 163002 Arkhangelsk, Russia
- <sup>2</sup> Excellence Support Unit, Tomsk State University, 634050 Tomsk, Russia; dolcezzamia@mail.ru (O.S.); sergius198010@mail.ru (S.A.); alobanov89@gmail.com (A.L.)
- <sup>3</sup> Arctic Scientific Research Centre of Yamal-Nenets Autonomous Okrug, 629008 Salekhard, Russia; fkg@rambler.ru (K.F.); easukhova@rambler.ru (E.S.)
- <sup>4</sup> Department of National Policy, Government of Yamal-Nenets Autonomous Okrug, 629008 Salekhard, Russia; zabolotnikovamv@gmail.com
- <sup>5</sup> Association of Reindeer Herders in YNAO, 629000 Salekhard, Russia; filant76@mail.ru
- <sup>6</sup> Northern Institute of Environmental and Minority Law, Arctic Center of the Lapland University, 96101 Rovaniemi, Finland; bamidele.raheem@ulapland.fi
- <sup>7</sup> Laboratory for Studying the Mechanisms of Physical Factors Action, Centre for Testing and Examination of Natural Healing Resources, National Medical Research Centre for Rehabilitation and Balneology, Ministry of Health of the Russian Federation, 121099 Moscow, Russia; andronovsv@nmicrk.ru (S.A.); lobanovaa@nmicrk.ru (A.L.)
- Correspondence: e.n.bogdanova@narfu.ru

Abstract: Environmental and anthropogenic factors represent challenges impacting the lifestyle and demographic rural population's behaviour in the Russian Arctic that threaten its social and food security. We aim to explore (1) which key "push" factors are jeopardising social sustainability and increasing migration outflows in the Arctic rural communities of Western Siberia (2) and how the Siberian population's sustainable development could be secured. The methodology and analysis were based on Lee's theory of migration factors with the main focus on the "push" factors forcing people to migrate to other Arctic and non-Arctic territories. The primary sources included fieldwork data and interviews collected during expeditions to the Arctic zone of Western Siberia between 2000 and 2021. Both men and women confirmed the insignificant impact of environmental factors on their emigration plans. However, they signified social and personal motives related to low standards of living that threatened their social and food security. The rural Siberian population's migration strategies could be re-evaluated only by increasing the physical availability of food products and developing the social infrastructure of the settlements as either "models of rural cities" or "service centres for nomadic and rural population".

**Keywords:** migration; rural arctic population; sustainable development; food security; Yamal–Nenets Autonomous Okrug

# 1. Introduction

Human migration in the Arctic is a complex socioeconomic phenomenon driven by historical, geographical, cultural, economic, and political factors [1–4]. It is also accompanied by the need to adapt to environmental shifts including climate change [5–8]. Rural to urban migration, or "outmigration", is particularly disruptive to many Arctic local communities [9–12]. For example, over the last decades, nine of the twenty-four Alaskan census regions in the state experienced population loss more than twice that of the United States national average in rural areas [13].

The main drivers of the outmigration in the Arctic are the many environmental and social risks [14,15] faced by the population due to harsh climatic conditions, extremely

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**Copyright:** © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). low population density, insufficient transport logistics, and social infrastructures, fragile ecological systems, climate change, disproportionate industrial and economic development in the Arctic territories. High costs of living result in challenges for the social and food security of local communities.

Heleniak (2021) predicted little change in the total population of the global Arctic but considerable variations in growth rates among Arctic regions up to 2055: a 10% increase in Alaska, Iceland, Nunavut, Troms, Yukon, the Khanty—Mansiy Okrug, and Chukotka; 5% and 10% growth in Finnmark, Nordland, North Ostrobothnia, and Nenets Autonomous Okrug; over 5% decline in Finland, Arkhangelsk, Karelia, Komi, Magadan, and Murmansk in Russia [16]. In nearly all of the Arctic regions, the common demographic trends are "aging populations, more balanced gender ratios between men and women, increased population concentration into larger urban settlements, and the depopulation of smaller settlements" [16].

Environmental changes will drive consequences for individuals, communities, and populations [17]. Climate change is one of the future threats that jeopardise the sustainability of the lifestyles, and livelihoods of the inhabitants of the Arctic. As pointed out by the Secretary-General of the UN António Guterres, "the climate emergency is a race we are losing, but it is a race we can win" [18]. The Arctic ecosystems are suffering from the devastating consequences of climate change: rising temperatures and environmental degradation, increased natural disasters, and weather extremes, resulting in food and water insecurity, and economic disruption [19]. Growing climate change [20–24] encouraged people to "climigration" caused by "immediate threats from erosion and flooding associated with thawing permafrost, increasing river flows, and reduced sea ice protection of shorelines" [5] (p. 115), which local communities are facing nowadays. Harsh cold climatic conditions and "remoteness between population settlements in the circumpolar North" make people leave their homelands and intensify migration outflow in the Arctic [25].

However, the primary critical drivers for "fast society-based and tangible shifts" [26] (p. 9) impacting migration in the Arctic region are geopolitical and socio-economic factors [27–30]. Migration affects the quality of life and lifestyles resulting in a significant impact on Arctic sustainability and food security locally and globally [31]. Food security strategies should be based on the premise that food insecurity and famine derive from the failure of access to food rather than global food shortage [32].

It was shown that there were three major "waves" of population decline in the Arctic: (1) between 1900 and 1919, migration outflow in the North American Arctic caused by the end of the "Klondike Gold Rush" (41% of the population of Alaska and the Canadian Arctic left); (2) in the 1960s, 2.3% of the population of Finland and Sweden moved to central regions and neighbouring countries; (3) since 1990, the migration outflow from the Russian Arctic due to recession in the economy [33]. The Russian Arctic was no longer associated with high living standards due to insufficient social and engineering infrastructure [34,35]. By 2019, the decline was 1046 thousand people, or 30% of the total population in 1989 [36]. The demographic trends, complicated by the increased outmigration mobility, were jeopardising the sustainable development of local Arctic communities: for example, a high degree of urbanisation with a low population density [37], gender imbalance with a higher number of men, increasing proportion of the elderly population, and a high birth rate accompanied by a low life expectancy [33].

Over the last three decades, the population shift in the Russian Arctic due to natural increases was almost covered by net migration. However, Heleniak et al. proved population decline in 139 studied Russian Arctic settlements due to outmigration [38–40]. From 1990 to 2019, the population of the Murmansk region, the Kamchatka region, the Taymyr Autonomous Okrug, and the Komi Republic decreased by one-third, the population of the Chukotka Autonomous Okrug, and the Magadan region declined by nearly 70 percent. There was not such a significant decline of population (13–20%) in the Nenets Autonomous Okrug, the Republic of Karelia, and the Republic of Sakha (Yakutia). In contrast, Siberian areas showed population growth: 29.9% in the Khanty–Mansi Autonomous Okrug, and

15% in the Yamal–Nenets Autonomous Okrug (YNAO) [41]. However, there was high migration outflow during the last decades [42]. It signifies the need to analyse the critical environmental and anthropogenic factors impacting outmigration, and search for possible solutions for updating migration policy in YNAO.

The research question of our study is: "Which environmental and anthropogenic factors threatening social and food security are the key drivers of rural outmigration in Western Siberia?" The significance of this research is to reflect on appropriate policies for strengthening resilience and reducing migration outflows in the Arctic Siberian population.

#### 2. Materials and Methods

# 2.1. Settings—The Yamal–Nenets Autonomous Okrug: Geography, Population, and Ethnic Structure

YNAO, the geographic focus of our research, is an important region for the Indigenous Peoples of Russia, and it is located in the circumpolar northwest of Western Siberia (Figure 1). It has a population of 547,010 [43] who live in an area of 769,250 square kilometres [44] with a population density of 0.71 people per square kilometre. The population is mainly concentrated in the urban areas of YNAO (84%) [43]. Almost half of the Indigenous small-numbered population of the Russian Arctic (48,932 people) reside there [45].

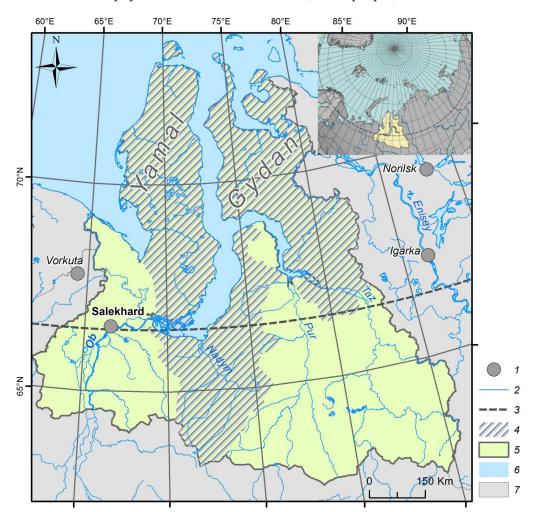


Figure 1. The map of the Yamal–Nenets Autonomous Okrug.

#### 2.2. Study Design

In this paper, we presented the results of a quantitative and qualitative analysis of the impacts of environmental and anthropogenic factors on migration outflow in YNAO. The objectives of our study were to analyse (1) which key "push" factors were jeopardising

social sustainability and increasing migration outflows in the Arctic rural communities of Western Siberia; (2) how social, food security, and sustainable development of the Siberian population could be secured.

# 2.3. Measurement Tools, Methodology, and Study Population

This study applied a multidisciplinary approach based on socioeconomic analysis. The primary sources included demographic data on the longitude dynamics of migration flows of different population groups (2000–2021), fieldwork data, and interviews collected during expeditions to the Arctic zone of Western Siberia between 2012 and 2021.

The data on population, natural increase, and migration for the period from 2000 to 2021 were obtained from the open-source platforms (Rosstat [43], Unified information system for modelling and forecasting the socioeconomic development of the Indigenous Peoples of the North of the Yamal–Nenets Autonomous Okrug [45], Russian Fertility and Mortality Database [46], and Database of Indicators of Municipalities [47]). We used the data collected from the following locations in YNAO: the Nadymsky, Yamalsky, Tazovsky, Krasnoselkupsky, Shuryshkarsky, Priuralsky, and the Purovsky districts. The number of Indigenous small-numbered Peoples in YNAO was received from the Department of National Policy of YNAO [48].

The data on socio-demographic characteristics of the Siberian population were collected during expeditions to the Arctic zone of Western Siberia from 2012 to 2021. The fieldwork was conducted by researchers of the YNAO Arctic Scientific Research Centre, Northern Arctic Federal University, and the Association of Reindeer Herders in YNAO (two of the researchers were Indigenous).

Semi-structured interviews with the participation of rural residents of the Arctic zone in Western Siberia were collected to study the reasons for migration expectations of the population in YNAO. The inclusion criteria for the respondents were as follows: be over 18 years of age, have resided in the tundra, or the settlements of the Arctic zone of Western Siberia for over five years. Data collection was performed in the Russian language with the assistance of Indigenous Peoples. Participants filled out a confidential paper. The reasons for migration outflow and migration expectations were analysed according to the following questions: "Where do you live now?", "Are you going to move to a new place of residence?", "What key factors (environmental, geopolitical, technological, social, and personal) can make you migrate out from your current place of residence?".

The survey sequence was as follows: during an expedition to the settlement between 2012 and 2021, respondents were invited to participate in the survey, and received information about the programme, both verbally and in writing. The written consent form stated that participation was voluntary and that their confidentiality was assured. Participants' personal data and their answers were anonymised, numbered, and entered into de-identified databases.

Statistical analyses were performed using Microsoft Excel 2016 and Statistica for Windows, v. 8.0 (StatSoft Inc., Tulsa, OK, USA). Significant differences were defined at a p-value < 0.05.

# Theoretical Approaches to Policy Analysis

There were different factors that impact migration processes, and the factors influencing outmigration were identified by various theoretical approaches. In 1966, the American demographer E. Lee developed the theory of migration factors [49]. He described it as a gravitational model for explaining the "pull" and "push" factors of migration and presented the factors influencing the decision to migrate in the following ways: factors correlated with the territory of departure of migrants; factors associated with the territory of the arrival of migrants who "work" in the territories of potential arrival migrants; interfering circumstances; factors related to the structural characteristics of migrants. Different factors of migration operate in each of the territories. Some factors are "pull", others are «push» ones. Speaking of "push" factors, he means such qualities of the territories of disposal as political or religious persecution, economic crises, civil wars, military conflicts, natural disasters, and others. Attractive factors may be the following characteristics of migrant entry areas: political stability, freedom of religion, economic well-being, better working conditions, and the possibility of higher wages [49]. In our study, following Lee's approach, we have mainly focused on the "push" factors forcing people to migrate to other territories.

Based on Lee's theoretical approach, L.L. Rybakovsky posits that migration decisions influenced by several factors can be divided into two main groups: objective ("permanent"—environmental; "temporary"—socio-economic infrastructure, gender imbalance, the ethnic composition of the population, and others; "current regulation"—employment opportunities, income rates, national policy, and others) and subjective (personal motivation) [50,51]. V.A. Iontsev differentiated economic, social, cultural, political, ethnic (national), religious, racial, military, and demographic factors of migration [52]. S.V. Ryazantsev identified the following main "push" factors of migration: "ecological and geographical", "ethnic", "military and political", "economic", "social", "demographic, and psychological" [53]. V.I. Perevedentsev offered natural (climatic, orographic, hydrological, and others), and social migration factors (demographic, economic, social, and others) [54]. V.V. Fauser divides all factors of outmigration into constant (climate, geographic location), slowly changing (economic structure, development of the territory, and others), and variables (legal, and others) [28].

With a special emphasis on the Arctic, A.V. Ukhanova et al. specified the list of the critical factors of outmigration: (1) economic (income rates, unemployment, economic, infrastructural development, and investment attractiveness of the region); (2) social (housing, education, healthcare system); (3) demographic (yield of unemployed, yield of youth population); (4) ethnic (ethnic conflicts, customs, and traditions); (5) political, and legal (legislative regulation of migration, instability of the political situation in the region); (6) climatic, and environmental (harsh climate, climate change, ecological situation in the region); and (7) personal psychological (family and historical ties, level of education, qualifications, profession) [55]. In our study, we analysed the mixture of environmental and anthropogenic "push" factors of outmigration in the rural population of Western Siberia.

#### 2.4. Ethics Approval

The study was approved by the Ethics Committee of the Arctic Scientific Research Centre of YNAO, Salekhard, Russian Federation, on 16 January 2012 (approval protocol No. 01/1-13). The research has been done following ethical concerns of working with the Indigenous Peoples in the Russian Federation (Constitution of the Russian Federation, Article 69. 14 March 2020). Communication was initiated with the Associations of the Indigenous Peoples and with representatives from national Indigenous communities in the Nadymsky, Yamalsky, Tazovsky, Krasnoselkupsky, Shuryshkarsky, Priuralsky, and the Purovsky districts of YNAO early in research planning. This resulted in an expression of interest from their representatives in conducting the research in their communities.

# 3. Results and Discussion

#### 3.1. Migration Trends in the Yamal–Nenets Autonomous Okrug

From 2000 to 2020, the total population of YNAO increased by 48,438 (9.7%) (Table 1), which differs from the demographic situation in other Arctic regions of Russia [26].

The interregional migration showed the tendency of moving from rural to urban areas (urban population increased by 43,836, or 10.6%). The previous research proved that "the residents of small settlements replace the migrated population of the municipalities, while the last ones migrate to big cities and other regions of Russia . . . There are similar migration processes in the Priuralsky district: the residents of small settlements migrate to the regional administrative centre, the village of Aksarka, whose residents move to the city of Salekhard" [56] (p. 54). The Arctic researchers, K. Filant and G. Detter, evaluated the impact of depopulation processes in the rural areas of YNAO on the increasing economic burden for municipal authorities: "Migration processes in rural areas have hypertrophied negative

consequences due to the small size of the population, the dispersion of settlement and the insufficient economics. Thus, with a reduction of the population, budget expenditures for maintaining the infrastructure of a settlement will not decrease but will grow per capita" [57] (p. 28). The migration from the rural (especially small settlements) to urban areas became the trend for the Arctic region globally. In Alaska, the population decline in smaller settlements located far from Anchorage and Fairbanks was a result of outmigration, which has cancelled out positive natural population growth. There was the same dominant pattern in Fennoscandia: population growth in larger settlements was accompanied by population decline in surrounding smaller settlements. This was similar to the pattern observed in North Atlantic countries (Iceland, Greenland, and the Faroe Islands) [40]. The Indigenous Sami population inhabited rural northern areas of Norway, Sweden, Finland, and the Kola Peninsula in Russia also partly moved to the urban areas [11].

Table 1. Demographic Statistics in the Yamal-Nenets Autonomous Okrug \*.

Indicator	2000	2010	2015	2016	2017	2018	2019	2020	2020/2000 **
Population, total	497,385	524,630	537,130	535,462	537,472	540,109	543,055	545,823	48,438
Urban population, total	414,288	444,464	449,780	447,916	450,164	452,879	455,712	458,124	43,836
Men	209,485	219,676	223,957	221,687	222,777	224,277	225,725	226,438	16,953
Women	204,803	224,788	225,823	226,229	227,387	228,602	229,987	231,686	26,883
Rural population, total	83,097	80,166	87,350	87,546	87,308	87,230	87,343	87,699	4602
Men	42,261	40,354	43,888	43,888	43,612	43,579	43,726	43,931	1670
Women	40,836	39,812	43,462	43,658	43,696	43,651	43,617	43,768	2932
Indigenous small-numbered Peoples	36,992	41,249	47,541	48,106	48,735	48,606	48,932	N/A	-
Nomadic Indigenous small-numbered Peoples	N/A	N/A	13,978	13,921	13,876	13,365	13,079	N/A	-
Natural increase	3076	5390	6091	5436	4916	4667	4283	3744	668
Birth rate	5839	8263	8913	8251	7530	7214	6836	7037	1198
Death rate	2763	2873	2822	2815	2614	2547	2553	3293	530
Migration outflow	17,055	17,874	47,807	39,774	37,581	37,787	34,334	26,427	9372
Net migration	-1309	-4953	-11,972	-3491	-2418	-1735	-1318	-1071	171
Net migration (from YNAO to the Tyumen region)	-224	-1617	-2980	-2730	-2652	-2526	-2035	-1629	
Net migration (from YNAO to other regions of Russia)	-2398	-4391	-5152	-1950	-1636	-1136	-1229	691	
Net migration (from YNAO to foreign countries)	1313	1055	-3840	1189	1870	1927	1946	-133	
Urban population			-8395	-1495	-426	-473	166	-1465	-
Men			-6337	-864	-9	118	196	-1433	-
15–24 years old			-1037	-116	-112	-91	82	24	-
Women			-2058	-631	-417	-591	-30	-32	-
15–24 years old			-517	-189	56	-62	156	241	-
Rural population			-3577	-1996	-1992	-1262	-1484	394	-
Men			-2160	-943	-872	-395	-566	318	-
15–24 years old			-132	-152	-118	-128	35	53	-
Women			-1417	-1053	-1120	-867	-918	76	-
15–24 years old			-82	-115	-130	-139	-63	60	-

\* Sources of the data: [43,45–48]. \*\* Difference between year 2020 and 2000.

In YNAO, the female population of the urban territories increased faster (by 26,883 women, or 13.1%) than the rural population (by 2932 women, or 7.2%). In contrast, the male population was increasing slowly: urban—by 16,953, or 8.1%; rural—by 1670, or 4.0%. However, the previous research proved the opposite trend among one of the demographic groups in YNAO, nomadic Indigenous Peoples: a catastrophic decrease in the number of women. This resulted in the "shortage of brides in the tundra" [58]. A similar consequential pattern was observed in northern Alaska and Greenland: disproportionate outmigration by locally-born women [29]. Emigration of the Indigenous women from small, isolated communities in Alaska and Greenland was seen as a pattern of female flight that resulted from modernization and decreased the sustainability of such locales [59].

From 2000 to 2020, the migration outflow had doubled (by 9372, or 55%), and it finally overwhelmed the immigration flow resulting in decreased net migration at 171 (-13.1%). Until now, the high birth rate (increased by 1198, or 20.5%), and labour immigrants covered the impact of migration outflow on the total population of YNAO. Sokolova and Choi (2019) noticed that this balance of natural increase and net migration maintaining a sustainable increase in the total population is partly the result of initiated fertility support programmes, and the development of the Arctic areas [60]. Additionally, Lazhentsev (2015) mentioned that negative net migration rates reduce the chances for the intensive exploration of the north [61].

However, jeopardising tendencies are associated with intensive outmigration and increased outflow of well-educated population, and highly qualified specialists from the Arctic region. The previous research showed that the share of emigrants with university or vocational schools education leaving YNAO was 52.6% in 2013, 53.8%, in 2015, and 84.6% in 2017 [62]. Moreover, the migration expectations and intentions of the YNAO residents depend on their level of education, and well-being; the higher level of education people have, the more eager they are to emigrate from the region, and better well-being is associated with less intention to migrate to other regions [56]. Ivanter (2016), Selin (2016), Fauzer (2017), et al. noticed that qualified human resources are replaced with temporary and often low-qualified personnel from Russia's southern regions, or other countries [63–65] due to migration outflow from the Russian Arctic. This impacts "deintellectualisation" of the Arctic zone of the Russian Federation, slowdown of technological development, and losing of the cultural identity of the Arctic population" [63] (p. 597). Along with it, Pilyasov (2009) signified disappearing cultural and intellectual diversity in the settlements since "decreasing total population means decreasing the number of talented and creative people" [66] (p. 294).

To cover migration loss, the Russian government developed programmes of "attracting skilled labour to the North" [67]. The intensified migration outflow was partly initiated by the government, which developed state programmes for the resettlement of the population to other Russian regions [68–71]. These political initiatives impacted the sustainable growth of the population of YNAO and could finally move the depopulation of some local communities.

The threatening migration trends among the youth and economically active population groups revealed the tendency of an "ageing population" in YNAO that had already become the tendency in other Arctic regions in Russia [33,34,72–74]. Common trends seen in nearly all Arctic regions in the future are aging populations, more balanced gender ratios between men and women, increased population concentration into larger urban settlements, and the depopulation of smaller settlements [16]. Rozanova–Smith (2021) noted that "as young professionals continue to leave, resettling compatriots and hiring shift labour may contribute to the vitality of more resilient regions" [75]. Contrariwise, youth's migration outflow from the region would impact the demographic imbalance social and food security of the region due to the increased economic burden of the government for supporting depopulated communities with a high yield of an ageing population, and finally, jeopardise demographic sustainability of YNAO.

## 3.2. "Push" Factors of Migration Outflow in YNAO: The Survey Results

A total of 843 residents of the rural areas in YNAO were interviewed, including 297 men and 546 women. Five hundred seventy-seven of the respondents were Indigenous. The age of the interviewees ranged from 18 to 69 years old; the mean age was 45.6 (95% CI 32.8–58.4).

Only 9.0% of the interviewed (24 men, 52 women) declared their intention to move to a new place of residence (primarily urban areas) this year, 11.0% (31 men, 62 women)—in one to three years, and 37.0% (97 men, 215 women) in five years or later. These migration strategies of the rural residents in YNAO became a sustainable trend over the last decade. For example, previous research also proved the migration expectations in the villages of YNAO. In 2015, 84.5% of rural respondents were planning to change their place of residence; among those, 15.5% were going to move to another location (a city or a village) in YNAO, 47.8% to the Tyumen region, 21.2% to other areas of Russia [57].

All of those interviewed (100%) prioritised the impact of social factors on their migration strategies: low quality of life, insufficient healthcare systems, social and food security, low level of education, unemployment, bad working conditions, and uncomfortable housing conditions. The key "push" factors were "the shortage of job positions in the villages" or "lack of work at all", "dilapidated, emergency and uncomfortable housing", or "the lack of housing". Both female and male respondents were concerned about the lack of prospects for the youth in the villages, and were sure that the best strategy for young people was to move to the urban areas and search for jobs there: "Even if young people graduate from colleges, where they will go? They don't have a job here. What are they going to do here? What's for should they come back to a village?"; "At least, there is some kind of prospect for young people in a city. They can have a choice and find a job there"; "What kind of job is here in a village? At school, a work of a janitor, and that's all..."; "Limited choice of jobs", "Low salaries"; "High cost of living"; "High prices for services, products, housing, and electricity". These are common trends in other Arctic regions. For example, in Canadian Arctic, the research on policy food programmes indicated the presence of chronically food insecure groups who had not benefited from the economic development, and job opportunities offered in larger regional centres of the Canadian Arctic, and for whom traditional kinship-based food sharing networks had been unable to fully meet their needs [76]. Indigenous Peoples in northern Canada experience food insecurity at a rate that is more than double that of all Canadian households [77]. The population of "crowded" settlements suffered from an increased risk of food security. For example, nearly 62% of Inuit families in the Canadian Arctic resided in such households, placing them at risk of food insecurity [78]. Therefore, moving rural Siberian populations to big settlements and urban areas could not be the only solution to provide their social and food security.

Men were worried about losing their traditional lifestyle. However, they did not insist on young people staying in the tundra since there was a low level of salaries and challenging life. Only those who were enthusiastic about nomadic reindeer herding could enjoy this lifestyle. In contrast, others had no other choice but to leave a village for a city: "Young people do not want to go to the tundra. No land where to herd a reindeer. No salaries. No jobs. So, the youth is escaping". Assimilation of the Indigenous population due to changing traditional lifestyles and globalisation processes is replacing traditional livelihoods with industries. The previous research proved that "traditional economic activities of Indigenous Peoples are not currently dominant in the structure of employment of the population in small settlements ... only 15% of the total rural population is employed in traditional Indigenous "industries" [79]. The lack of work in their settlements encourages people to search for employment in nearby regions and extends the daily commute to work. Currently, the municipal statistics of YNAO show that 56% are employed in big settlements or urban areas [80]. This is one of the reasons for the depopulation of small villages. However, other Arctic regions (for example, Canadian Arctic) demonstrated opposite trends; most Inuit families complained about reducing the size of their children's meals because of a lack of money [78].

Previous surveys in other Arctic regions in 2008 [81], 2012 [82], and 2016 [83] also assessed the impact of unemployment and low income on migration outflow. Along with it, high salaries in some Arctic regions (for example, in the Tyumen region [84], and the Murmansk region [85]) do not change youth migration strategies. Ivanova (2017), et al. explain this intensive migration outflow with limited possibility of professional careers for talented young people in the Murmansk region [85]. The common trends of the youth migration aspiration were observed in Alaska. About 50% of the rural students expected to migrate permanently away from their home region. The likelihood of expecting migration increased curvilinearly with community size. Young women and college-aspiring students disproportionately expected outmigration [86]. However, some in Finnish Lapland, demonstrated the phenomenon of an increasing number of young people who, despite the general tendency towards youth outmigration in rural areas, decided to stay in their hometowns [87].

The problem of increasing unemployment is mainly rooted in the limited approaches to the economic development strategies of the rural areas in YNAO and the lack of economic specialisation of the settlements. This formed unique "ecosystems" in the rural areas, where people adapted to their sustainable lifestyle with minimum needs and expectations, and low requirements for modern social and technological infrastructure [80]. Rural residents' households are mainly based on a subsistence economy, and they are not focused on producing goods and increasing their income [88]. Therefore, finally, the life-system support of these settlements, social benefits, and insurance for the population to maintain minimum living standards are budgeted by regional governments. Over 60% of residents of these settlements are employed in educational organisations (schools, kindergartens), housing and utilities, and local governments, while only about 40% are working in agriculture [80].

A low density of population and insufficient transport logistics resulted in limited access to medical services. Most respondents mentioned that the most painful issues are the lack of hospitals, medical doctors, and insufficient quality of medical help: "Healthcare and roads. That is the essential basis for everyday life"; "Severe climate results in worsening health. However, we have no chance to visit medical doctors. We need to go to the hospitals in the big settlements for that."

Over 70% of those interviewed confirmed that one of the critical drivers of emigration is insufficient housing and educational infrastructure: "*All housing is old*", "*Houses are almost destroyed*"; "*Most buildings need capital repair*"; "*They lack central heating, hot and cold water, electricity in the houses*." However, we disagree with Shelomentsev (2018), et al. [27], who noticed that the most significant factor impacting migration outflow in YNAO is "price of houses". This factor was not mentioned by any of our respondents, they primarily worried about bad conditions, and the lack of modern conveniences in the houses.

Over 90% of women signified the critical factor of low level of education, and the lack of secondary schools in many settlements: "I have to send my kids to school to another big settlement since we have an only primary school here. I can't see my children for several months while studying and living there. I want a better life for my kids. Maybe, we will have to move somewhere else." The interviewees mostly did not complain about the quality of the education. However, they highlighted the importance of the communication services in their settlements (sustainable Internet and mobile connection). It was especially significant for the youth respondents: "The quality of the Internet is low, that does not allow me to participate in the social life of other districts and regions, to receive information and knowledge, and study online".

Intensive educational migration in YNAO since 2010 can be explained by the closing of the branches of universities, and increased unemployment rates in the region. So, young people finish school and leave for other cities outside YNAO to get higher education and find better jobs. All interviewed respondents (100%) aged 18–24 years old were not interested in using the opportunities of getting higher education online based on the Internet distance-learning platforms, and they plan to move to other regions. Over 90% of people aged 18–24 years old leave their residence and study in different places [80]. However, the rates of "educational migration" are also high in those Arctic regions of Russia which

have universities. The surveys conducted by Rosstat (2005, 2008) [89], and by Arkhangelsk Scientific Center of the Ural Branch of the Russian Academy of Sciences [83] confirmed that this is one of four key drivers of youth migration outflow in the Russian Arctic.

Most respondents (over 90%) were not satisfied with the social infrastructure, and the quality of social services, which provides their personal motivation to emigrate from the region. Some of them decided to move to a new place of residence following their families or close relatives (14%), and others hoped to get married in a new location (primarily women). Some people were forced to migrate due to limited access to medical services since they have chronic diseases and need regular doctor visits. Over 70% (mostly women) do not see any changes or prospects for the life of their children in the region and are ready to emigrate with them. The respondents mentioned: "The kids at school are not motivated to stay. They just don't see their future here"; "What will happen to Nyda? I don't think that anything will change for the better in 5–15 years"; "It's better moving than losing everything here"; "I don't particularly believe that something will happen in the future. People have no hope"; "If you would come in the spring when our children go to school in swamps"; "In 15 years, only people who were born here will stay here. Our children and grandchildren will move to the city". The interviewed did not believe that any changes could happen since "The system will never change. So, everything will stay the same". Furthermore, personal motives were always supported by other factors (primarily social and technogenic) since the desire to change a life, lifestyle, or location is always rooted in some objective factors. Parlee (2012) studying Canadian Arctic mentioned that people can easily face the challenges of food insecurity since their individual views of their own well-being contradict regional statistics on quality of life [90].

All respondents could clearly explain the reasons for their migration expectations (what exactly they were not satisfied with, in their current residence) and listed the number of factors impacting their decision (why they want to leave for another location). It was noticed that "A great dissonance in subjective perceptions of the quality of life is a result of the comparison of the level and quality of life in urban or large rural areas while people are travelling, and the media (television, through information channels on the Internet) also has important influence" [80]. When making a final decision on moving to the new location, people are comparing the "losses" and "benefits" of this change. The researchers Beglova and Musin (2017) mentioned that "A migrant's decision about possible relocation is formed under the influence of a rational comparison of the actual standard of living with its potentially improved option, predetermined by the migrant's resettlement. A migration flow occurs when a non-disabled household member prefers the "move" option to the "stay" option, with the costs of moving being offset by expected future benefits." [91]. The priority impact of personal factors on migration strategies of the Russian Arctic population was also confirmed in the previous surveys conducted by Rosstat (2005, 2008) [89], Kola Scientific Research Centre (2008) [81], and the All-Russian Center for the Study of Public Opinion (2012) [82]. Similar results were received in Alaska where personal rural residents' outmigration motives were also dominant [92].

The respondents considered another group of factors referred to so-called "technogenic factors" as one more critical driver of migration. Over 50% were not satisfied with logistics infrastructure since it limited access to social infrastructure and jeopardised their food security. The interviewee responses signified the correlation between insufficient logistics, high prices, and low quality of products in the local shops: "*The products are delivered here by helicopters in summer, autumn and winter (if not bad weather), or we have to wait until winter when the rivers are frozen, and we can deliver what we need by "zimnik (frozen river or lake used as a logistic route—E.B.)*", "If there were a good road, probably the products would be cheaper. Because *it would be more accessible if there are good roads. We have to buy everything in the city and it deliver here*". The shortage of vegetable food and dairy products, high food prices, and limited access to food products strongly impact women's migration expectations: "Marketplaces are almost empty, there are no deliveries here. We depend on deliveries by helicopters". One woman complained that her little child aged seven years old had never tried ice cream there, and

they could not buy fresh milk in their settlement but only dried milk due to complicated logistics. Transportation, distribution, and handling services have direct impacts on food security as exemplified by other authors on the Arctic food system [93,94].

In contrast, the problem of transport accessibility of the settlement was not so significant for men, and it slightly affected their migration strategies since they considered it an employment opportunity. Some remote settlements can be reached only by helicopters and boats, and the products are delivered there once a month: "*If you need to buy some products or goods you can either wait until the helicopter arrives in a month or go by boat to the big settlement. However, it is very costly, and if there is no urgent need, it is better to delay and wait*".

High costs of products and services in YNAO (as well as in other Arctic regions) can be explained by high production and logistic costs. Due to rising inflation and political and economic crises, these costs tend to grow. Vasiliev and Selin (2017) noticed that the coefficients of increasing production costs would change in the interval of 1.3–3.0 in the north; these values are higher in the north and northeast of Russia. The increase in production costs in the north varies from 30–35% in the European North to 60% in the Far Northeast of Russia [95] (p. 24). However, the researchers signified that an increase in these production costs should not be "compensated" and "subsidised" only by the government. Instead, the enterprises are to focus on reducing costs to increase their products' competitiveness [95] (p. 24). The same issue with high costs of living is faced by other Arctic global communities. For example, current food systems within the northern subarctic and arctic regions of Canada rely heavily on imported foods that are expensive (when available) and are environmentally unsustainable [22].

The insufficient logistics also impact the shortage of medical personnel and medicines in the pharmacy, the lack of a general practitioner, a dentist, and other doctors in the remote settlements [23] that "determines public health in the Arctic areas" [24]. The interviewee mentioned: "*Health care and roads. We all depend on them. We have helicopter service. We can't go by car and get to the city quickly*". The situation is complicated by the fact that the transport accessibility of small settlements totally depends on weather conditions and seasonality [58] since the main transports are water (in summer), and air (in winter). Using automobile transportation is possible only in winter. Several remote settlements in YNAO are accessible only by snowmobiles in winter, and during the off-season, logistic routes are closed due to weather conditions, and distant geographic locations.

One more component of the technogenic factors, digitalisation, and the insufficient innovative technologies, was more relevant for the youth respondents since they all complained about "bad Internet", and "limited choice of mobile phone service". They noticed that "there is no ATM in the village, which worsens already the low quality of life", "It is difficult to buy the tickets for a helicopter that is the only regular transport here". However, in total less than 20% of the interviewed mentioned this factor as significant for their migration strategies. Lazhentsev (2013) prioritises the role of digitalisation since it is the critical instrument of innovative development of the Arctic region: "The localisation of human, intellectual, industrial, and other resources for the north for the Arctic is necessary ... It is possible only due to high-speed transport, and a fibre-optic network with its connection to educational and scientific centres in Russia and foreign countries." [82].

Geopolitical factors associated with ethnic conflicts, the criminal situation, and the COVID-19 pandemic did not strongly impact the migration strategies of YNAO residents. Over 60% of respondents did not mention these factors, while about 40% of them noticed that this is important without giving any additional explanations. In 2016, a survey of the Arctic regions' residents showed that one of the significant reasons for outmigration in the Russian Arctic was the "insecure criminal situation" (yield was 17.4%) [96]. However, Shelomentsev (2018), et al. [27] considered this data to be unreliable and considered that geopolitical factors did not have a relevant impact on migration expectations of the Arctic population.

Significant warming trends have already affected the Arctic Peoples' traditional lifestyle, well-being, and health in Scandinavia and the Russian Arctic [97–100]. Climate

change represents a global challenge that impacts the Siberian population's environment, traditional lifestyle, and health and threatens its food security [19]. This requires increased adaptive capacity [101,102], and coping strategies [103] for changes in the terrestrial ecosystem caused by climate change are urgently needed [104,105]. However, climatic factors were not signified as the critical drivers for migration strategies of the interviewed participants (less than 5%). The respondents mentioned that the climate is changing worldwide but did not specify any significant impact on their lives. The same results were received by the researchers in the Canadian Arctic: significant changes in climate being observed were not currently affecting female food security, with socio-economic-cultural factors as the primary determinants of food security [106].

More important for the respondents were environmental pollution (15%) and limited access to natural resources (hunting and fishing) relevant to food security. In contrast, the survey conducted by Goncharenko, et al. (2008) [81] in another Russian Arctic region, the Komi Republic, showed that one of the critical drivers of their migration strategies was the intention to change to a milder climate. This was also confirmed later by the results of a survey initiated by the All-Russian Center for the Study of Public Opinion (2012) [61].

Finally, none of the respondents offered the only key factor that impacted their migration strategy; all the interviewees listed at least 3–to 4 most relevant factors. The social, technogenic, and personal factors dominated, and they were closely linked.

The main strength of our study was using the unique data of quantitative and qualitative research collected from residents of the local rural communities during the expeditions that took place over ten years (2012–2021). Most similar studies remain fragmentary and are often hard to access. However, our study had several limitations. We interviewed only a part of the population in different districts of YNAO. Participation was voluntary and did not include all representatives of all local communities of the studied territories, limiting the generalizability of our findings. Future research could also benefit from exploring the outcomes of migration outflow from the region (with a particular focus on the social security of the local communities), and "pull" factors of migration. Nevertheless, this analysis can give precious results for improving and updating governmental programmes of socio-economic policy in YNAO.

## 4. Conclusions

Our findings showed that migration expectations are a complex phenomenon, and critical drivers for the human adaptation strategies to the changing Arctic ecosystems. Both men and women confirmed that environmental (with emphasis on climatic) factors did not significantly impact their emigration plans. However, both of them signified the impact of social and personal motives: low-quality housing, uncomfortable living conditions, high costs of living, limited access to medical care, lack of life prospects in the settlement, desire for changes and a better life for their children, the decision of family members to move to a new place of residence. They also considered migration to other settlements or cities to meet new people, make new friends, and obtain access to active social activities.

As the critical factors for their migration strategies, women signified social (limited access and insufficient quality of medical care, education, unemployment, high prices for food products and goods, food security issues, the lack of conditions and social support for disabled people) and personal factors (health issues, the lack of prospects of living in the rural areas, desire to "give a better life for their children"). Women were also more motivated to migrate to improve their social status. Conversely, men consider the more significant influence of the following factors: changing traditional lifestyle, environmental risks, soil degradation, and transformation of natural landscapes, political situation, the insufficient introduction of innovative technologies in the social sphere (medicine, housing construction), low income, and personal motivation (for example, desire to change their lives and become independent from relatives).

The influence of a group of factors threatening social and food security and impacting migration outflow from the rural areas of YNAO differed, with the prevalence of a group

of social factors and other factors directly related to low standards of living. At the same time, none of the factors was the critical driver influencing migration strategies of the rural Siberian population. The combination of factors was individual and depended both on the objective socio-economic conditions in the settlement, and on the subjective perception by the residents of acceptable, sufficient, and desirable living standards, as well as on the ideas about the perspectives for sustainable development of the Arctic territories. Residents consider the logistical inaccessibility of YNAO settlements as a factor that primarily affects the availability of social services, health services, and food security (physical and economic). Along with it, it is also one of the factors increasing migration opportunities of the population.

We offer short-, medium-, and long-term measures to support the sustainable development of the local communities that can improve quality of life in the rural areas of YNAO with consequences on migration strategies of the population:

*Short-term measures:* 

- To develop state programmes for subsidising and compensation of logistics costs of delivery and storage of goods and food in the rural areas of YNAO;
- To introduce state programmes for digitalisation of educational, medical, and social services;

Medium-term measures:

- To improve the quality of housing (primarily, providing houses with a water supply and sewerage systems);
- To introduce the programmes for developing social infrastructure (for example, increase in the number of feldsher-midwife stations in remote settlements);
- To improve the logistics infrastructure of rural areas (enhance the logistics between the settlements);
- To develop innovative high-tech bioproduction industries in the region (livestock, poultry, aquaculture), including traditional economic activities of local communities;
   Long-term measures:
- To identify and develop the settlements that can become the "models of rural cities".

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