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Food Safety Economics in the COVID-19 Pandemic

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Abstract

The consequences of the COVID-19 pandemic crisis for both food safety and especially the economic sustainability of food production in Canada and around the globe are explored. A full analysis is made of the nature of the virus, and it is spread as they relate to the forces of globalization which have created a global food supply chain, with a focus on the weaknesses of a global supply chain that fell prey to the COVID-19 virus and its associated economic effects. Comparison was made to past outbreaks of Spanish flu and Ebola, both of which challenged public health, food safety, and food supply systems. A more focused analysis examines how public and private responses to the pandemic create opportunities and challenges for several linkages in the supply chain, including farms, food processing facilities, grocery stores and restaurants. The quarantine procedures put in place around the world to manage the COVID-19 necessitated radical shifts in food production and. Ultimately the response from any individual government is insufficient to weather these events, as the fundamentally international and cross-industry factors involved require a holistic, globally coordinated approach which was not possible with the tools available before these events began.

Keywords: COVID-19, food safety, supply chain management, food economics

1. Introduction

At the tail end of 2019, a virus began to spread from Wuhan, China outward into the surrounding area. Carried by tourists, businesspeople, and innumerable other travellers, the virus which came to be known as COVID-19 was spread not only throughout China, but throughout the entire world, triggering a pandemic on a scale unheard of since the Spanish flu outbreak which occurred in the wake of the end of the First World War (Mallapaty, 2021).

Borders were closed, whole cities were put under quarantine, and a significant percentage of the economy of the entire world ground to a halt as governments, industry, and private individuals rushed to create and enact strategies to slow the spread of the respiratory infection and prevent a soaring death toll from increasing any further (McFadden et al., 2021). Ultimately, citizens around the world were called upon to isolate themselves in their homes to avoid contracting or transmitting the virus and, as a necessary extension, non-essential businesses of all kinds shut down (Deconinck, Avery and Jackson, 2020).

In any viral outbreak and in any natural or man-made disaster of any meaningful scale, there are consequences which extend into the realm of food. The ability of people to get enough food to sustain themselves, the ability of farmers to maintain their productive capacity and their business, the ability of food processors to remain safe and economically viable, and the ability of grocery stores, restaurants, and other purveyors of food to carry out their business are all deeply impacted by the COVID-19 outbreak (Gundersen et al., 2021). Where food intersects with public health, business, trade, and travel, COVID-19 intersects with food.

This paper, written during the ongoing COVID-19 crisis, will provide an in-depth account of the impacts of the outbreak on food through an examination of the origins of the outbreak, its effects on all food-related industries, the health risks at the intersection of the virus and food, and the structural vulnerabilities in the global food distribution system which allowed this outbreak to have such a devastating effect (Curtis et al., 2014). In addition, it will present an analysis considering the consequences of COVID-19 for vulnerable populations around the world and within the North American context as they relate to food from an intersectional perspective, with reference made to historical effects on food safety and availability for such populations during comparable crises (Goddard, 2020; Thilmann et al., 2021).

Given the ongoing nature of the situation, the analysis, and recommendations here cannot necessarily be assumed to describe the complete scope of the situation.

2. Historical Context

To understand the current situation, it is helpful to consider similar situations past and how they may align or differ with the COVID-19 outbreak. The most direct correlation can be found in the Spanish flu, which spread like wildfire in the wake of the First World War. This spread is generally attributed to two factors: First World War (WWI) trench warfare and developments in transportation technology (Ellison et al., 2021).

Trench warfare saw large numbers of people stuck together in close quarters for long periods of time in extremely unsanitary conditions, which provided an ideal environment for the Spanish flu to incubate and spread within the ranks of the opposing forces. It would be when the troops returned home, however, that the flu would begin to spread globally (Dowling, 2020; Rivington et al., 2021).

While air travel was in its infancy in the later days of the First World War, rail travel was already commonplace and was the main mode of transportation for troops entering and leaving the front. Trains once again provided cramped quarters in which the virus could spread from person to person, while the disembarking troops carried the flu out to the surrounding areas where it was rapidly transmitted. The global nature of the conflict meant the flu was carried to all corners of the world. Its movement, however, was relatively slow, as it could only be carried by rail and by slow-moving ships crossing the oceans (Chappell, 2020). The scope of this spread has been mirrored by the global spread of COVID-19, but advances in transportation (notably rapid and regular air transit) have increased the speed. What might have taken months in 1918 takes days or even hours in 2020.

Spanish flu was also like COVID-19 in that it tended to cause its victims to develop pneumonia as their immune systems were compromised. However, this is one area where the current situation is better than the 20th century crisis. COVID-19's mortality rate is significantly lower than that of the Spanish flu (Kitz et al., 2021).

What ultimately ended the Spanish flu epidemic was the development of public health policies which undermined its ability to spread. This included quarantines and more widespread access to medical services. Many of the public health policies put in place during the Spanish flu outbreak formed the base for the present-day responses to COVID-19. The necessity of these health services was largely the result of the new reality of urban life, where people were always living and working in close quarters (Le Valleé and Charlebois, 2015; Dowling, 2020). This situation is only more common in today's world as urbanization continues unchecked.

Prior to the current situation, a large-scale outbreak of Ebola in several African nations and further abroad in 2014 was met with a rapid response from international public health organizations including the World Health Organization (WHO, 2015; Walker et al., 2021). Here we can see a response from the sort of public health apparatus which arose in the wake of the Spanish flu epidemic, specifically in food safety during an epidemic. WHO provided descriptions of the symptoms and methods of transmission for Ebola to an international audience, along with instructions on how to properly prepare food to avoid transmission via food vectors (Charlebois, 2011; WHO, 2015). The use of the internet to rapidly distribute this information to affected people around the world is another advantage that modern efforts to maintain food safety and the efficacy of supply lines have over the post World War One world (Reid, Perez, and Schenker 2021).

Resources for food safety information have been even more plentiful during the current outbreak, with not only the World Health Organization, but also national entities like the US Food and Drug Administration providing detailed guidelines for maintaining safe practices when handling and preparing food. The FDA's information resources on food during the COVID-19 outbreak do not only include detailed safety guidelines for individuals and organizations, but also a detailed account of the strategies in place to manage the crisis and what should be expected regarding food availability and delivery during the ongoing situation (FDA, 2020).

3. Pandemic Origins

Concern regarding food safety during the pandemic first arose as news of the virus' likely origins in a Wuhan, China "wet market" began to spread widely during early 2020. It was reported that the infection originated in the Huanan seafood market in Wuhan or at another live animal market in the region, from which it spread throughout China's Hubei province in the early days of the outbreak (Readfeam, 2020).

This is not the first time a widespread outbreak of a coronavirus type infection has likely originated in a Chinese "wet market". Both COVID-19 and SARS, which was at the centre of a large-scale public health crisis in 2003, likely originated in bats, which transmitted the infection to other animals which then transmitted it to humans via these wet markets (Woodward, 2020). Here we see the first and most crucial intersection between the COVID-19

crisis and food safety.

The structure of a wet market sees numerous different live and dead animals kept in close quarters along with large numbers of human shoppers interacting with and consuming the animals. This provides a perfect environment for viruses like COVID-19 and SARS, which can be transferred from animals to humans, to make the leap and rapidly spread among a large population. Animals are killed, butchered, and skinned in front of prospective customers, a process which can send particles carrying infectious material into the air where they can be breathed in and spread (Krause and Gruber, 2020). This perfect storm of tightly packed people and animals in unsanitary conditions mirrors the deadly combination of close quarters and lack of proper sanitation that allowed Spanish flu to spread so effectively in the trenches of WWI.

On January 1 2020, the Huanan seafood market was shut down and wet markets across China followed suit as a permanent ban on the loosely regulated markets was put into place by the Chinese government. Further, the government made a commitment to crack down on illegal markets and the illegal farming of many of the species (Slaoui and Hepburn, 2020).

Despite the measures directly targeting the wet markets and associated industries, it is still unknown exactly how the virus was transmitted to humans. Understanding how this virus jumped species is crucial to mitigating the risk of future outbreaks, but scientists are still unsure precisely which animal in which location transmitted it to humans (Guo et al. 2020). Passage through food is possible, but several interactions with several different species (pangolins being one suggestion) are possible origins to the jump to humans as COVID-19 travels easily from one species to another. This is evidenced by a tiger in a New York Zoo becoming infected within the early weeks of the outbreak. SARS was transmitted from bats to a cat-like species of civets during the 2003 epidemic (Readfeam, 2020).

4. Globalization, Food, and COVID-19

Beyond China, steps are being taken on an international scale to prevent the spread of the virus. On March 16th 2020, following the example of other nations, Canada closed its borders to non-essential traffic with an exemption for Americans. Canada was the first North American nation to enact a closure on this scale during the outbreak. The US, meanwhile, heavily restricted travel from affected regions (Levenson, 2020).

This response, the closure of borders, is a recognition of the primary factor which has enabled COVID-19 to reach pandemic proportions in its spread across the globe. That factor is, of course, globalization. In 1918, the First World War was an exceptional situation. It was this unusual circumstance which caused people to travel across the surface of the Earth and communicate the Spanish flu to far flung territories. In 2020, international travel is constant, normal, and global. Every condition that allowed for the Spanish flu to flourish has been multiplied. To understand how, we must examine the global supply chain and the broader effects of globalization within the context of COVID-19 (Hailu, 2020).

It is not constructive to be alarmist about the challenge's globalization poses for food safety, nor to leave the suggestion that globalization is a force which should be resisted to prevent pandemics of this nature (McCabe and Erdem, 2021). The World Health Organization recognizes several significant benefits which are afforded to nations that are integrated into the global food trade. First and foremost, the diversity of foods which can be rapidly delivered anywhere in a globalized world allows for greater nutrition and more robust public health by introducing a broad variety of food and higher quality food to consumers. Secondly, food trade provides a valuable economic boon to participating nations, allowing developing states to monetize agriculture in a way that can accelerate their development and ensure a higher standard of living for their citizens (WHO, 2014).

There are, however, significant challenges in maintaining standards of quality for food on the international market. The 21st century has seen a sharp increase in recalls of food, despite domestic measures for detecting disease becoming steadily more advanced (Maras, 2015). This increase also comes despite agreements like the World Trade Organization (WTO, 2014) Agreement on the Application of Sanitary and Phytosanitary Measures, which created rules for member states regarding food safety in the global market.

A survey by Swiss Reinsurance Company Ltd. found that by 2015, the number of annual food recalls in the US had doubled when compared to the number of recalls in 2002. The survey ultimately concluded that the globalization of the food supply chain made risk management for food recalls significantly more difficult. Multi-million-dollar hits were taken by affected firms, reflecting the significant financial risk which comes with the economic benefits of global food trade (Maras, 2015).

The economic component of the global food supply chain is as much a centre of vulnerability as issues such as food quality and sanitation. Not only are the prices of foods which have become a standard part of diets

worldwide tied to international supply and demand in such a way that fluctuations in the economy of even a few developing nations can have significant consequences for food prices worldwide, but global finance makes shocks not directly related to food strong factors in food prices and the viability of food production and processing around the world. As an example, a banking crisis in Iceland had devastating effects on seafood producers internationally due to their financing being heavily sourced from Icelandic banks (Sowinski, 2019). With small shocks having far-reaching effects in a global system, a crisis of this pandemic's nature, which has negative impacts on food production and finance around the globe, could be absolutely devastating.

5. Supply Chain Impacts

On a smaller scale, it is valuable to understand the effects the pandemic has had on the food supply chain through examination of the impacts on those industries that are most directly related to food. Farms, food processing facilities, restaurants, grocery stores, and food banks are the most direct points of contact between people and food and thus in this situation they become the most direct indicators of the challenges which COVID-19 creates in the realm of food.

Farms, the immediate source of food commodities, are facing several challenges during the pandemic. While the early days of the outbreak did not see Canada facing shortages of key foods, the closure of the Canadian border means that migrant workers will at best be delayed in arriving in Canada and at worst be unable to make the harvest season at all. The potential impact of this is huge, with millions of pounds of crops being harvested by migrant workers on a yearly basis (MacLeod, 2020).

The Canadian government has made an exception for temporary foreign workers, which will allow them to enter the country despite the border closure, but this exception comes with an onerous set of conditions including a 14-day quarantine period, housing which can accommodate self-isolation (including only 3 workers being allowed in housing that usually houses 40 in some areas), rigorous sanitation procedures for worker facilities, daily communication with isolated workers to ascertain whether or not they are symptomatic, and physical distancing during work (MacLeod, 2020). These measures, while probably essential to prevent the spread of the infection, create significant additional costs for farmers; costs which may be insurmountable for some. The workers will need to be paid for a full two-week period in which they will not be working, the social distancing measures will require changes to standard procedures for harvest, and the restrictions on housing will mean the cost of sheltering workers will be multiplied.

Canadian government officials reiterate that there is no shortage of food, but there are challenges regarding allocation of food from farms and food processing facilities which, until recently, served grocery stores and restaurants in consistent, but distinct ways (Martin, 2020). Demand for dairy products has plummeted due to the closure of many businesses, creating an excess of supply which farmers are reacting to by dumping large quantities of milk. Given the unique quota system Canada has, discarding milk was not expected, unlike in other countries like the United States (Charlebois et al., 2021). This is sixth time in 55 years that Dairy Farmers of Ontario has called upon dairy farmers to dispose of raw milk. This process is a part of the supply management system which governs Canadian dairy farms and ensures consistency of prices via controls on supply (BBC News, 2020). Given that the breakdown in demand is the result of restaurants and stores closing, not a lack of public desire to purchase dairy products, this event reveals an inefficiency in the way the dairy market operates under the conditions of an outbreak. The wastage of this milk could be made unnecessary with alternative modes of delivery.

Food processing facilities, the second step in the movement of food from earth to table, are also heavily affected by COVID-19 and the resulting government response. The CFIA has laid out an extensive series of guidelines for food processing facilities which aim to protect the health of employees at these facilities. As food and food packaging have not yet been documented to function as vectors for infection, employee health is the primary concern in responding to COVID-19 in food processing facilities (CFIA, 2020)

These guidelines include expanded sanitation procedures and a requirement for the social distancing measures (2 metres distance) recommended to the public to be followed by employees working at these facilities. These are just the most significant of a long list of requirements and recommendations issued by the CFIA (CFIA, 2020).

As with the guidelines for farms, there are challenges in maintaining this new standard and completing normal work procedures. Many facilities are not equipped for staff to operate at a regular distance of two metres, with machinery and floor layouts requiring workers to stay in much closer proximity despite the guidelines (CFIA, 2020). The CFIA rules allow for exceptions in these cases, but punitive action from the CFIA is not the sole motivating factor for wanting to avoid violation of social distancing. Losing workers to infection has its own economic consequences, both for continuing productivity and the ability of a facility that has been the site of an

infection to remain in operation.

Like farms, food processing facilities have been impacted by the closure of restaurants and the need for people to eat at home. Preparing and packaging food for sale to a restaurant is a different process than preparing and packaging food for sale to a grocery store. Restaurants require bulk packages of food in large containers while individual consumers require smaller packaging. Shifting production to meet this altered demand is a challenge for processing facilities (Demetrakakes, 2020).

The aforementioned grocery stores are also naturally affected. While demand for some grocery store products rises by as much as 500%, supplies from south of the border may not be consistent and the stores themselves pose a risk of infection not only to their own employees, but also to their customers (Hollingsworth, 2020).

Government guidelines have also been issued to grocery stores. For example, in British Columbia an extensive series of guidelines recommends expanded sanitation procedures, limits on the number of customers allowed within a store at one time, floor markings indicating appropriate routes through the store and positions to maintain social distancing, the removal of customer packaging such as reusable bags from store surfaces, turning away customers who are visibly symptomatic, and encouraging effective hygiene practices for staff. Similar measures have been taken nationwide (BC Ministry of Health, 2020). The same concerns regarding employee health that necessitate extra care at food processing facilities are also at play in grocery stores, with the additional complicating factor that grocery stores have a steady flow of new people entering interacting with staff and facilities during business hours. Unsanitary conditions within a store could spread infection to outside communities, while vice versa infection in the general populace could be transmitted to store employees.

The risks inherent in this sort of public gathering and interaction with the public spurred the closure of restaurants across Canada and the world, but business continues through avenues such as takeout and delivery which do not pose as high a risk for the public as the traditional sit-in meal.

The aforementioned risks of grocery shopping for the public have allowed restaurants to stay afloat by selling products such as bags of flour which may be sold out at grocery stores or may simply not be easily accessible to customers unwilling to risk a trip to a crowded store. As previously noted, supplies for restaurants are not the same as food supplies sold in grocery stores, so the specific nature of these products may vary when sold through these avenues. For example, a bag of flour will likely be a bulk 50-pound bag which may be divided at retail rather than the traditional 5 pound bag. This process not only allows for restaurants to remain financially solvent during a long period of closure, but also prevents wastage as food which had already been ordered before restaurants were forced to close can be sold in its raw form (Domonoske, 2020).

Despite these strategies for continuing under adverse conditions, the restaurant industry is still suffering financially, and restaurant owners are seeking financial aid from the federal government to prevent mass bankruptcies and permanent closures as they face rent and mortgage payments after a month or more without regular business. Over 90% of restaurant owners expressed grave concern regarding the situation in a poll by Restaurants Canada (Sagan, 2020).

This may truly be the COVID-19 epidemic exposing weaknesses in the food service industry that already existed, such as landlords charging unsustainably high rent and restaurants operating with immense overhead that leaves them extremely vulnerable to even small market shocks, much less a global pandemic resulting in multiple months of ongoing closures (Nunn, 2020). Radical change may be necessary for food delivery of all kinds if restaurants, which are filling the role of grocery stores for many people, are unable to operate or even survive in times of crisis.

6. Conclusion

Overall, the key conclusion to draw from this information is that any response must consider the global food supply chain as it relates to travel, urbanization, international business, and all other related factors in a holistic way.

It is the interconnectedness of people, nations, and institutions that allowed this virus to spread in the way it has, and food is a key part of that interconnectedness. In a world where food is shared across borders via a global system of trade, all people are eating at the same table and public health ceases to be an issue that can be confined by national borders. The food safety risk here is twofold: Food can be contaminated at several points in the supply chain and directly affect the health of consumers, and secondly, unsanitary food practices in one nation can trigger chains of infection that spread internationally. In this situation, as always, food is central to public health.

Nor can these issues be addressed without an up-to-date and holistic understanding of the food supply chain

within national borders. The difficulties in maintaining supply and keeping people fed faced by restaurants, grocery stores, and other purveyors of food during this situation reveal how fragile a system that cannot immediately adjust to a temporary change in the consumption habits of Canadians and people the world over.

China responded to the issue of illegal farming and wet markets only after a pandemic caused by factors that had already been recognized by academics as increasing the risk of a human infection like COVID-19 had already occurred. This failure to act in advance of negative circumstances had consequences worldwide, but it cannot truly be said that this is entirely the fault of the Chinese government. The responses of other nations, while ultimately responsible, were scattered and inconsistent. Standards need to be set and enforced internationally for the safe sale, export, and consumption of food and the response measures for international public health events of this nature.

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Effect of Scopoletin and Carotenoids on Postharvest Physiological Deterioration (PPD) of Transgenic High Beta Carotene Cassava

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Abstract

Cassava tubers suffer from postharvest physiological deterioration (PPD) which normally sets in within 72 hours of harvest. This study examines the role of scopoletin and carotenoids in the onset or delay in PPD in two transgenic varieties EC20-7 and EC20-8 compared to a wild variety TME-7. Scopoletin and carotenoids were quantified by liquid chromatography-mass spectrometry. The scopoletin content (0.10 – 0.20 nmol/g) in the fresh varieties was not significantly ($P>0.05$) different from the amount in stored cassava roots (12.58 – 14.90 nmol/g). The carotenoid content values in EC20-7 variety were 6.66 $\mu\text{g/g}$ (α -carotene), 80.45 $\mu\text{g/g}$ (β -carotene) and 5.98 $\mu\text{g/g}$ (lutein). As for EC20-8, α -carotene, β -carotene and lutein values were 6.19 $\mu\text{g/g}$, 69.11 $\mu\text{g/g}$ and 3.12 $\mu\text{g/g}$, respectively. There was no significant ($P>0.05$) difference between the varieties in α -carotene content but in their lutein content. The results indicate that carotenoids are more relevant in the delay of PPD and scopoletin content is not a major factor in PPD vascular streaking or discoloration. Hence scopoletin content of cassava varieties may not be considered as a chemical marker for determining the potential of PPD in cassava tubers.

Keywords: postharvest physiological deterioration (PPD), metabolites, vascular discoloration, cassava, carotenoid

1. Introduction

Cassava (*Manihot esculenta* Crantz) is one of the major staple crops in Africa. The plant is highly cultivated because of its resistance to drought and pests as well as being a rich source of starch and energy (Edoh *et al.*, 2014). However, the tubers undergo post-harvest physiological deterioration (PPD), an active physiological response triggered by harvesting, and this poses a greatest hurdle in the utilization of cassava. PPD is characterized by a rapid discoloration of the cassava tubers and occurs within 24-48 hours after harvest. It is triggered by wounds on the tubers which occur during harvesting and handling. The discoloration first appears in the vascular system around the wounds (Reilly *et al.*, 2007) from where it spreads to the rest of the tuber. In just a few days after harvest, a blue-black coloration known as vascular streaking will accumulate in the vascular bundles in the parenchyma (Reilly *et al.*, 2003). The deteriorated cassava roots have an unfavourable appearance and taste, which makes them commercially unacceptable. Some conventionally bred high beta-carotene varieties known as yellow cassava which were envisaged to assist in combating both vitamin A deficiency (VAD) and postharvest physiological deterioration have been introduced (Sanchez *et al.*, 2006). Another strategy is a transgenic modification of cassava varieties to increase their carotenoid content through genetic engineering as β -carotene content is associated with a reduction in post-harvest physiological deterioration and is thought to be due to oxidative nature of carotenoids (Rudi *et al.*, 2010; Sanchez *et al.*, 2006). Therefore, it is important to have information on the chemical and biochemical properties of cassava varieties with pronounced delay in postharvest physiological deterioration. The accumulation of stress response metabolites is an important indicator in PPD development as the process involves a wide range of compounds including coumarins and phenolic compounds and also other compound classes such as phytosterols and fatty acids such as palmitic, linoleic, and oleic acids and their derivatives (Sakai *et al.*, 1986). The accumulation of 22 diterpenes was also confirmed in wounded cassava roots (Sakai and Nakagawa, 1988), and this is an unusual plant stress response. However, it is the hydroxycoumarins that accumulate most ‘dramatically’ during PPD (Bayoumi *et al.*, 2010).

The most significant one of these hydroxycoumarins is scopoletin and its glucoside scopolin, while esculetin and its glucoside esculin are also accumulated but in less significant quantities. These secondary metabolites may act as anti-oxidants or antimicrobial agents (Buschmann *et al.*, 2000, Sakai and Nakagawa, 1988). Scopoletin (7-hydroxy-6-methoxychromen-2-one) is synthesized as part of the phenylpropanoid metabolism. There is evidence that scopoletin is involved in plant defensive response in cassava, tobacco, tomato, and other plants (Sudha and Ravishankar, 2002, Sun *et al.*, 2014). At the onset of PPD, the level of scopoletin in cassava roots rises rapidly, increasing from less than 1 ng/mg to 60-80 ng/mg in fresh roots in 48 hours, and remains at a high concentration during further development of PPD (Buschmann *et al.*, 2000). The dramatic accumulation of scopoletin in PPD indicates that it plays an important role in the deterioration process. Supporting this is a pruning treatment that delays PPD and also lowers scopoletin synthesis in cassava roots (Van Oirschot *et al.*, 2000). Scopoletin may scavenge ROS with its hydroxyl group; to support this, scopolin, the glycoside of scopoletin whose hydroxyl group is bound to a glucose residue, does not show ROS scavenging activity (Reilly *et al.*, 2003). Wheatley *et al.* (1985) applied a range of phenolic compounds to fresh cassava root samples, and found that scopoletin was the only one that induced a rapid PPD discoloration, implying that scopoletin might act as a signaling molecule to trigger PPD (Wheatley and Schwabe, 1985). However, there is no evidence that scopoletin might have a signaling function in PPD, while there is considerable evidence for its anti-oxidant and anti-microbial activities. This study examines the role of scopoletin and carotenoids in the onset or delay in PPD in two transgenic varieties EC20-7 and EC20-8 compared to a wild variety TME-7.

2. Materials and Methods

All reagents were of analytical grade unless otherwise stated. HPLC grade solvents were obtained from J.T. Baker and Sigma-Aldrich USA. LC-MS grade water was obtained from Honeywell part of Thermo Fisher Scientific, USA. Scopoletin was quantified by liquid chromatography-mass spectrometry using a Sciex 6500 QTRAP while an Agilent HPLC coupled to Thermo Finnigan LCQ Advantage ion trap mass spectrometer was used to quantify the carotenoids. All experiments were carried out at Donald Danforth Plant Science Center (DDPSC) St. Louis, Missouri USA in collaboration with the National Root Crops Research Institute (NRCRI), Umudike, Nigeria.

2.1 Sample Collection

Identification and selection of two conventionally bred (UMUCASS) high beta-carotene cassava or yellow cassava stakes were with the assistance of the Genetic Resource Unit and Cassava Programme of NRCRI. Collection of two transgenic bred (EC-20) high beta carotene cassava stakes and wild type (TME-7) were carried out with the assistance of the International Institute for Crop Improvement (IICI) Department of Donald Danforth Plant Science Center. The cassava stakes were planted in the greenhouse at the Center, and harvested four months after planting (4MAP).

2.2 Postharvest Physiological Deterioration (PPD) Set-up for Experimental Cassava Varieties

At 12 weeks the experimental cassava roots were carefully harvested for PPD experiment. The unbroken cassava roots from each of the experimental genotype (TME 7 (wild type), EC20-8 and EC20-7 (transgenic type)) were stored in a growth chamber at Donald Danforth Plant Science Center for five days. The oxidative vascular streaking and discoloration associated with postharvest physiological deterioration (PPD) was observed at 48 hours after harvesting of the roots. The ambient room conditions during the storage period was 27 °C and 60 % relative humidity (Ukpabi *et al.*, 2014). The deterioration was imaged through a digital camera and the results were analyzed by visual inspection of the images.

2.3 Sample Preparation

This was carried out as described by Gamez-Meza *et al.* (1999). Fifty (50) mg of dried and ground roots was weighed. 100 µL of 0.1% formic acid was added to the powder and vortexed for one minute. The samples were homogenized in a TissueLyser II at 15 Hz for 10 minutes, and then allowed to extract overnight at a concentration of 100 mg/mL with 100 % LC-MS grade methanol in a 4 °C cold room. The samples were centrifuged at 13.2 rpm for 10 minutes at 4 °C, and the supernatant collected. Samples were extracted two more times for 30 minutes and the supernatant was collected as previously described; the pooled supernatants were dried using a speed-vac. Samples were clarified using 0.8 µm PES spin-filters prior to separation.

2.4 Carotenoid Profiling and Quantification

Separation of the carotenoids was achieved by injecting 40 µL of sample onto the Agilent HPLC using a 250 x 2.0 mm YMC RP-C30 column. Ninety six (96) % methanol with 4 % water (aq) was used as mobile phase (A) and 90 % methyl-t-butyl ether, 7 % methanol with 3 % water (aq) as mobile phase (B). The gradient initiated at

100 % A with a hold for four minutes, then increased linearly to 85% B over 30 minutes, then a ramp back to 100 % A. The column was re-equilibrated for ten minutes before the next injection. Carotenoid quantification was accomplished by a combination of HPLC retention times and absorption spectra on an Agilent HPLC coupled to a Thermo Finnigan LCQ Advantage ion trap mass spectrometer by comparing peak areas of carotenoid standards of an external calibration curve prepared using analytical grade standards (PMSF-DDPSC protocol, 2016).

2.5 Determination of Scopoletin

Separation of scopoletin was achieved by injecting 2 μ L of sample onto the Eksigent micro LC 200 using a 100 x 0.5 mm Targa C18 column. 0.1% formic acid (aq) was used as mobile phase (A) and acetonitrile with 0.1% formic acid as mobile phase (B). The gradient initiated at 95% A for three minutes, then increased linearly to 95% B over 5 minutes with a hold for 5 minutes, then a ramp back to 95% A. The column was re-equilibrated for ten minutes before starting the next injection. Quantitation was accomplished on a Sciex 6500 QTRAP by comparing peak areas of endogenous target analytes to that of an external calibration curve prepared using analytical grade standards (Gamez Meza *et al.*, 1999).

2.6 Statistical Analysis

All experiments were carried out in triplicates. Statistical significance was established using one-way analysis of variance (ANOVA), and data were reported as mean \pm standard deviation. Mean comparison and separation was established using Duncan Multiple Range Test ($P < 0.05$).

3. Results and Discussions

3.1 Postharvest Physiological Deterioration (PPD)

The results of the PPD set-up for 120 h are presented Figure 1 and 2.

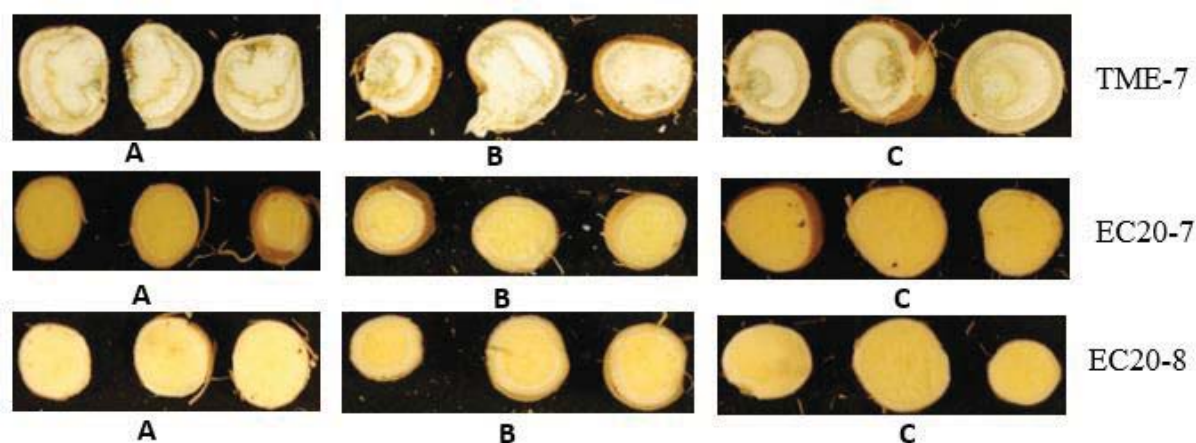


Figure 1. Photographs of postharvest physiological deterioration (PPD) in replicates of the cut experimental cassava lines after 48 hours

EC20-7 = Transgenic type 1

EC20-8 = Transgenic type 2

TME-7 = Wild Type

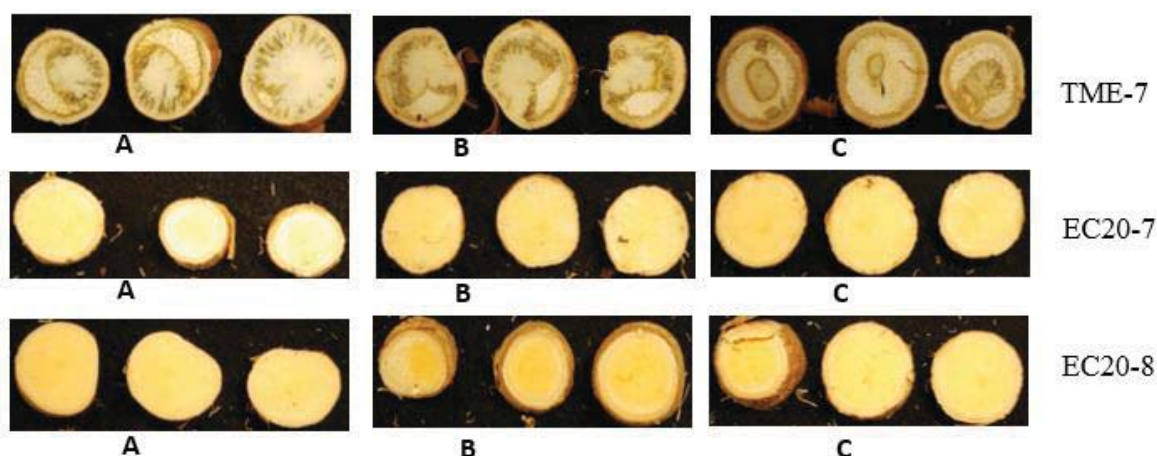


Figure 2. Photographs of postharvest physiological deterioration (PPD) in replicates of the cut experimental cassava lines after 120 hours

EC20-7 = Transgenic type 1

EC20-8 = Transgenic type 2

TME-7 = Wild Type

Figure 1 and 2 show the colour changes in cassava at 48 h and 120 h of storage. While the wild variety TME-7 (white) showed symptomatic appearance of PPD from the onset of harvest, the transgenic EC20-7 and EC20-8 varieties exhibited non-symptomatic appearance or delayed PPD even after 120 hours of harvest.

The result indicates that only EC20 varieties which had delayed PPD have appreciable quantities of carotenoids. Therefore, it is possible that the presence of carotenoids in the cassava roots contributed significantly to the delay of PPD vascular discolouration in these varieties as earlier suggested by Sanchez *et al.* (200) and Morante *et al.* (2010). Postharvest physiological deterioration in cassava is known to be linked to oxidative bursts (Sayer, *et al.* (2012) and carotenoids are also known to effectively scavenge reactive oxygen species (ROS) and other free radicals from different origins. This scavenging property has the potential to deliver protection against oxidative damage to plants and other organisms that undergo photosynthesis at all levels of complexity (Edge, *et al.* (2010).

3.2 Scopoletin Levels

Table 1 shows the result of scopoletin levels in fresh and stored un-deteriorated experimental cassava varieties.

Table 1: Scopoletin levels (nmol/g) of experimental cassava roots on dry matter basis

| Lines | Fresh Roots | Stored Roots |
|--------|------------------------|--------------------------|
| TME-7 | 0.15±0.08 ^a | 14.66±11.18 ^a |
| EC20-7 | 0.10±0.04 ^a | 12.58±3.52 ^a |
| EC20-8 | 0.20±0.61 ^a | 14.90±8.27 ^a |

Values are mean of triplicate determination, values with the same letter are not significantly different ($P>0.05$) using Duncan Multiple Range Test

EC20-7 = Transgenic type 1

EC20-8 = Transgenic type 2

TME-7 = Wild Type

Previous studies indicated that scopoletin content of cassava roots increased with PPD up to 72 hours after harvest (Okeke *et al.*, 2017). Hence, PPD vascular discolouration was linked to scopoletin content of the roots. In the fresh cassava roots, the scopoletin content in the varieties ranged from 0.10 – 0.20 nmol/g. Though there were observed differences in PPD rates among the cassava roots, no significant ($P>0.05$) differences were observed in the scopoletin content of the fresh cassava varieties used in this study which agreed with previous study by Aristizabal *et al.* (2007) which reported that scopoletin content in freshly harvested cassava roots are usually low but increased within hours of harvest. The scopoletin content of the stored cassava roots ranged from

12.58 – 14.90 nmol/g which indicated a remarkable increase. From this study, there is an indication that the scopoletin content of both the TME-7 (white) and EC20 (yellow) varieties increased during storage. Nevertheless, the transgenic EC20-7 and EC20-8 varieties with scopoletin content of 12.58 nmol/g and 14.90 nmol/g exhibited non-symptomatic appearance or delayed PPD (Plate 2) after 120 hours of harvest. These results suggest that scopoletin is not the major cause of PPD as previously considered (Wheatley, *et al.* 1985). Thus, scopoletin content of cassava varieties may not be used as a chemical marker for determining the PPD potential of cassava varieties.

3.3 Carotenoids

The results in Table 2 show the carotenoids identified in the study.

Table 2. Quantity of identified carotenoids of the experimental cassava roots ($\mu\text{g/g}$) on dry matter basis

| Carotenoids | Alpha carotene | Beta carotene | Lutein |
|-------------|------------------------------|--------------------------------|------------------------------|
| TME-7 | ND | ND | ND |
| EC20-7 | 6.66 \pm 2.05 ^b | 80.45 \pm 10.86 ^b | 5.98 \pm 1.60 ^c |
| EC20-8 | 6.19 \pm 4.34 ^b | 69.11 \pm 45.80 ^b | 3.12 \pm 2.17 ^b |

Values are mean of triplicate determination, values with the same letter are not significantly different ($P=0.05$) using Duncan Multiple Range Test

EC20-7 = Transgenic type 1; EC20-8 = Transgenic type 2; TME-7 = Wild Type

The detected and quantified carotenoids were alpha (α)-carotene, beta (β)-carotene and lutein. The variety EC20-7 had the highest α -carotene (6.66 $\mu\text{g/g}$), β -carotene (80.45 $\mu\text{g/g}$) and lutein (5.98 $\mu\text{g/g}$) content followed by EC20-8 with α -carotene (6.19 $\mu\text{g/g}$), β -carotene (69.11 $\mu\text{g/g}$) and lutein (3.12 $\mu\text{g/g}$). There were no significant ($P>0.05$) differences between EC20-7 α -carotene (6.66 $\mu\text{g/g}$); β -carotene (80.45 $\mu\text{g/g}$) and EC20-8 α -carotene (6.19 $\mu\text{g/g}$); β -carotene (69.11 $\mu\text{g/g}$) but a significant ($P<0.05$) difference was observed in the lutein content of EC20-7 (5.98 $\mu\text{g/g}$) and EC20-8 (3.12 $\mu\text{g/g}$). The three carotenoids -alpha (α)-carotene, beta (β)-carotene and lutein detected and quantified in this study were not found or detected in TME-7 variety. It is evident that the lutein content of EC20-7 was comparatively higher than the wild variety. The experimental result show that only EC20 varieties have appreciable amount of α -carotene therefore it is possible that this type of carotenoid is what is relevant in postharvest physiological deterioration (PPD) not necessarily beta-carotene or lutein. Alpha-carotene is known to impart yellow, orange or red colouration to many fresh foods (Cazzonelli, 2011) and Rodriguez-Amaya, 2001).

5. Conclusion

The transgenic high β -carotene varieties (EC20) which had delayed PPD had appreciable quantities of α -carotene, therefore, it is possible that this carotenoid is more relevant in the delay of PPD discolouration. Also, scopoletin content of the roots tremendously increased in the first three days of storage, and microscopic images showed that enhanced scopoletin content is not a major cause of PPD vascular streaking or discolouration in the roots. Hence scopoletin content of cassava varieties may not be considered as a chemical marker for determining the PPD potential of cassava varieties as previously suggested.

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Ethnobotanical Uses of Non-cultivated Edible Fruit Species in the Department of Oussouye (South Senegal)

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Abstract

Forests are an immense reservoir of biological resources and provide the local population with subsistence needs, especially for edible fruits. This study contributes to a better knowledge of the use modes of non-cultivate edible plant species in the area of *Kasa*, traditional name for the department of Oussouye. Ethnobotanical surveys, based on an interview guide, oral discussions and direct observations were conducted among 178 people from the department of Oussouye, stronghold of the *Diola* ethnic group. A factorial correspondence analysis highlighted the relationship between species and categories of use. The frequency of citation, informant consensus factor and use value showed the socio-cultural importance of the species. The data collected identified 62 edible species divided into 31 families and 54 genera. The fruit species inventoried are used for different purposes. They are a food source with 62% of citations, energetic 19%, technological 14%, medicinal 13%, cultural 6% and agronomic 2% for the populations. Two species stand out for their high use value factor (UVt). These were *Elaeis guineensis* (12.24) and *Borassus aethiopum* (7.56). In addition to their use value, species such as *Mangifera indica*, *Neocarya macrophylla*, *Parkia biglobosa*, *Anacardium occidentale*, *Ceiba pentandra*, *Parinari excelsa*, stood out for their categories and organs used. These results inform us about the level of use of fruit species for different needs and open up avenues for research in sustainable management of this resource with the aim of reducing poverty.

Keywords: plant species, Diola, added value, potential uses, *Kasa*

1. Introduction

Tropical forests constitute an immense reservoir of biological resources for pharmacopoeia, food, construction, timber industry and handicrafts among local populations. Thus, they provide local populations with subsistence needs. In rural areas, people's lives depend on non-timber and timber forest products (Bikoué & Essomba, 2007).

For a long time, the place occupied by non-timber forest products (NTFPs) and timber products in development policies has been minimal and often limited to indigenous uses. However, the contribution of these products to food security and primary health care has been amply demonstrated, as nearly 80% of the population in developing countries use them for health care or food (Allabi *et al.*, 2011). In this sense, traditional medicine relieves more than 70% of the populations of the Third World (Malaise, 1992) and 80% of the African peoples (Jiofack *et al.*, 2009). Thus, traditional medicine has become part of the culture of African populations although it still remains informal (Sofowara, 1982). In Senegal, 550 plants are considered medicinal and toxic in the pharmacopoeia (Kerharo & Adam, 1974). However, the use of plants requires a wider and deeper knowledge in order to be able to integrate them into the socio-economic development processes of the populations.

In Africa, non-cultivate plants are used daily by the great majority of peasant peoples (Grivetti *et al.*, 1987). These plants essentially contribute to the food and survival of rural people by providing scarce nutrients in their diet (Ayessou *et al.*, 2011). In Senegal, the species most used by the populations have been determined by

ethnobotanical methods based on inventories and uses of species in domains such as food and medicine (Diop, 2011; Dieng, 2017; Djihounouck *et al.*, 2018). However, this assessment does not cover the department of Oussouye even though fragmentary data on the uses of a limited number of species in this locality happened to be collected. Data collection on the uses of plant species is an important step in supporting decentralized programs for the conservation and development of forest resources (Ayessou *et al.*, 2009; 2011; Diop *et al.*, 2010), hence the need to conduct this study among the populations in the department of Oussouye in order to contribute to better management of these natural resources. This study contributes to the knowledge of the uses of edible fruit species in the *Kasa* area. To this end, the specific objectives of the study are to:

- ~ identify the different categories of uses of the inventoried wild edible species;
- ~ describe their uses to optimize the contribution of forest genetic resources to sustainable socio-economic development;

2. Methodology

The department of Oussouye is located in southwestern Senegal and covers 891 km² i. e. 12.14% of the area of the Ziguinchor region. It is bordered to the east by the Kamobeul marigot, the north by the Casamance River, the west by the Atlantic Ocean and Cape Roxo, and the south by Guinea Bissau. It includes five communes that total seventy-five (75) villages, with a population of 48.331 inhabitants in 2013 (ANSD, 2015). (**Figure 1**)

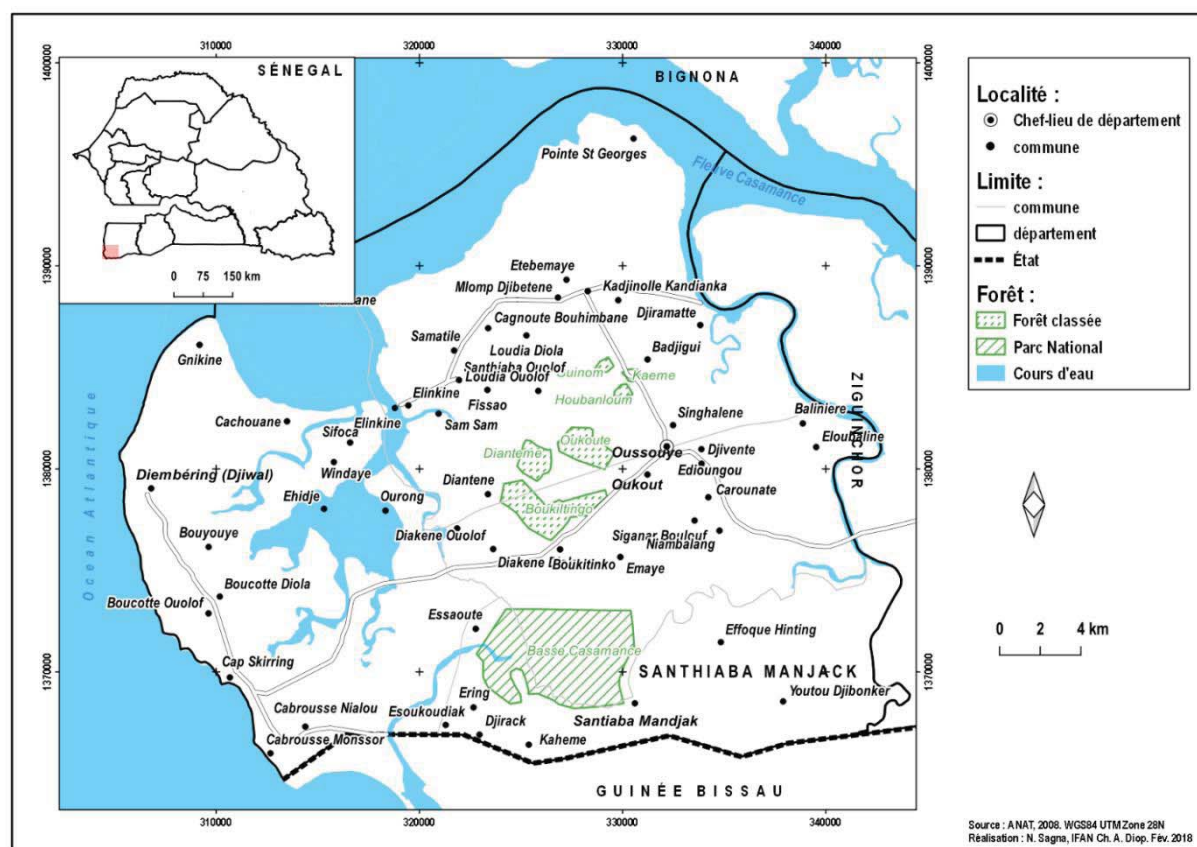


Figure 1. Map of the administrative division, hydrographic network and forest areas of the Oussouye department (Source: ANAT, 2008. WGS84 UTM Zone 28N. Production: N. Sagna Ifan Ch. A. Diop Feb. 2018).

The study population is made up of the Diola who are the majority ethnic group in the department of Oussouye. The language spoken by this ethnic group bears the same name (*Diola*) and is made up of several dialects. When the dialect is not mastered during the ethnobotanical survey, we call in an interpreter to ensure good communication. A first interview was conducted with randomly selected people in villages. This contact visit allowed us to get an idea of how the species studied contribute to the life of the population and select the areas of activity. The choice of the villages where the surveys should be conducted was made taking into account the local forest resources that allow harvesting activities. The newly created villages called batogat were not chosen because they do not have cultural originality due to the loss of ancestral values. Villages affected by the

Casamance conflict were excluded from the survey. Based on those criteria, 34 villages were selected for data collection. These villages are located in five (5) traditional communities (*An Alufay, Esulalu, Ejamat, Dyiwat and Her or Haer*). Each community is distinguished from the others in its dialect, organization and social practices. The choice of interviewees was non-probabilistic and their identification was done for convenience. Their selection by study area and village depended on their availability. They were chosen with the help of the village Chiefs, targeting individuals whose activity, experience or status was related to the topic. They were herders, gatherers, traditional family caterers, and farmers. The interviews were carried out between 2012 and 2014 mainly with indigenous adults, with a preference for men who have a better knowledge of non-cultivate species and who have experience in their mode of use. The choice of these people was made in order to reduce the risk of uncertain or vague answers. Data were collected through semi-structured interviews and casual conversations.

Semi-structured interviews were conducted using an interview guide which included the following headings: interviewee identification, form of use, socio-cultural importance of the species, and description of use modes. Casual conversations were used to estimate responses and gather new information (Martin, 1995).

The local name of each plant studied was transcribed into local language based on specialized documents (Adam, 1970; Berhaut, 1967) or by using the Diola alphabet codified with reference to the local language of the *An Alufaye* community.

The identification of fruit plants was done either on site or in the botany laboratory of IFAN and the botany and biodiversity laboratory of the Department of Plant Biology (BV) using the various flora of Senegal by Berhaut (1979; 1976; 1975a; 1975b; 1974; 1971; 1967) and Van Der Berghen (1988), and the works of Hawthorne and Jongkind (2006), Arbonnier, (2002) and Hutchinson and Dalziel (1954), Wieschus (2000) and the herbarium collections of Dakar and IFAN. The nomenclature adopted is that of the database of the Conservatoire et Jardin Botanique (C.J.B) of the city of Geneva, which is regularly updated (Lebrun & Stork, 1997; 1995; 1992; 1991).

Qualitative data were processed using the content analysis method (Faucault, 1966; Négura, 2006). This process consists of a systematic and methodical examination of textual and / or visual documents. Applied to qualitative data, it captures the contextual dimension of these documents and highlight the categorical determinants.

Quantitative data were processed according to descriptive statistics using response rate (Van den Eyden *et al.*, 1994; Cotton, 1996).

The data obtained were analyzed with Epi Info software and the results were processed by Excel spreadsheet which allowed us to establish cross-tabulations [species /organs], [species/use], [species/method of collection], [species/stage of development] and the R software (Core Team, 2017) with the factoextra package (Version: 1.0.5), for Factorial Correspondence Analysis (FCA) allowing to study the relationships between different species and categories of use. A test of independence of Chi² with a threshold of significance of 5% was previously carried out in order to verify the independence between the different species and the categories of use. The information collected was analyzed on the basis of ethnobotanical indicators.

➤ **Frequency of Citation (FC)** is used to determine the level of use of different species in a category and ranges from 0 to 100. The value 0 indicates that the species is not used in this category and 100 when the species is used in this category by all interviewees. It is expressed by the following formula:

FC = (S/N) 100% where:

- **S** : number of citations of a species in a category;

- **N** : total number of informants.

➤ **Informant Consensus Factor (ICF)** measures the variability of species use forms (Trotter & Logan, 1986). Always between 0 and 1, the value of this ICF is high when only one or a small number of species are cited by a large proportion of informants for a specific use category. Conversely, the greater the diversity of species cited for the same use, the closer the value will be to 0. The ICF is calculated from the following formula:

ICF = Nur - Nt / (Nur - 1) where,

- **Nur** (user-reports number) is the number of uses indicated in a given category;

- **Nt** (number of taxa) the number of species involved in this same use category.

➤ **Use Value (UV_(K))** is a way of expressing the importance of each family or species for the population interviewed. It significantly identifies the species with a high use value in a given environment (Dossou *et al.* ,

2012). It was calculated according to the method used by Philips & Gentry (1993) and Camou-Guerrero and his team (2008) using the following formula:

$$UV_{(k)} = \frac{\sum_i^n si}{n} \text{ where,}$$

- $UV_{(k)}$ is the ethnobotanical use value of species k within a given use category,
- si is the number of uses assigned by respondent i within this category,
- n is the number of respondents for a given category of use.

The total use value (UV_t) of species k is then calculated by summing the use values of that species within the different use categories by the formula:

$$UV_t = \sum_1^p UV \text{ where,}$$

- UV_t represents the total use value of the species,
- UV is the use value of the species for a given use category, p is the number of use categories.

3. Results

Thirty-four (34) villages were visited as part of this survey. The number of interviewees varied from 38 to 4 per area and 2 to 6 in each village. In total, eighty-two (82) informants were interviewed individually or in groups. This amounts to identifying one hundred and seventy-eight (178) interviewees in the five traditional areas.

3.1 Use Values

Data collected made it possible to inventory 62 non-cultivate edible fruit plant species distributed in 31 families. *Apocynaceae* and *Annonaceae* are the most represented with 6 species each. They are followed by *Anacardiaceae* (5 species) and *Chrysobalanaceae*, *Arecaceae* and *Rubiaceae* (4 species each). The other families are made up of *Sapindaceae*, *Caesalpiniaceae*, *Moraceae*, *Celastraceae* (3 species each), *Bombacaceae* and *Zingiberaceae* (2 species). The remaining 19 families are each represented by 1 species. (**Table 1**)

Generic diversity is also important with 54 genera in total. The family *Anacardiaceae* is the most diverse with 5 genera, followed by *Annonaceae*, *Arecaceae* and *Rubiaceae* with 4 genera each, *Apocynaceae*, *Chrysobalanaceae*, *Sapindaceae*, *Celastraceae*, *Caesalpiniaceae* with 3 genera and *Moraceae*, *Bombacaceae* with 2 genera each. The remaining families are represented by only one genus each. (**Table 1**)

These species are grouped in 6 use categories which are food, medicinal, technological, agronomic, energetic and cultural. The organs used are 7 in total, namely fruit, wood, leaf, root, bark, exudate and flower. (**Table 1**)

The analysis of the results shows:

- 6 use categories concerning 3 species: *Elaeis guineensis*, *Neocarya macrophylla* and *Parinari excelsa*;
- 5 use categories represented by 8 species;
- 4 use categories for 17 species;
- 3 use categories for 9 species;
- 2 use categories for 15 species;
- and 1 use category for 10 species.

Regarding the organs harvested, the results indicate:

- 6 organs used in *Elaeis guineensis*, *Mangifera indica* and *Cola cordifolia*;
- 5 organs exploited in 15 species;
- 4 organs in 15 species;
- 3 organs in 6 species;
- 2 organs in 13 species;
- and 1 organ in 10 species.

The use values are:

- high in two palm trees: *Elaeis guineensis* with 12.24 and *Borassus aethiopum* with 7.56;

- medium [3.87-2.35] in *Neocarya macrophylla*, *Mangifera indica*, *Parkia biglobosa*, *Anacardium occidentale*, *Ceiba pentandra*, *Parinari excelsa*, *Dialium guineense* and *Adansonia digitata*.
- low to very low for the rest of the species.

These species are useful to the population in several fields: food, medicine, technology, agronomy, energy and culture. The organs used are fruits, wood, leaves, bark, roots, flowers and exudate.

The food use of the species is the most frequent, followed by the medicinal use. Our results show that there is not a close proportionality between the use value of the species and its organs used. A species can be highly prized for one organ in a given environment, while another species with multiple uses is poorly known (**Table 1**). For example, *Borassus aethiopicum* (UVt = 7.56) with five use categories and four organs used, is highly used while *Parinari excelsa* (UVt = 2.59) with six use categories and five organs exploited, is very little sought after.

Table 1. Categories, organs used and use value of species

| Families | Scientific name | Local Name | Use categories | Organs used | Use value (UV _t) |
|-------------------------|---|--|----------------------------|------------------------|------------------------------|
| <i>Arecaceae</i> | <i>Elaeis guineensis</i> Jacq. | ka bekel, ka hiit | Fo, Med, Tech, Ag, En, Cul | Fr, Wo, Le, Ro, Ex, Fl | 12.24 |
| <i>Arecaceae</i> | <i>Borassus aethiopicum</i> (L.) Mart. | ka lahaay | Al, Med, Tech, En, Cul | Fr, Wo, Le, Ro, Fl | 7.56 |
| <i>Chrysobalanaceae</i> | <i>Neocarya macrophylla</i> (Sabine) Prance | bu bita, bu ñafay, beel | Fo, Med, Tech, Ag, En, Cul | Fr, Wo, Le, Ba, Ro | 3.87 |
| <i>Anacardiaceae</i> | <i>Mangifera indica</i> L. "Perse" | bu mangali bu jóoluay, bu mangu bu jóoluay | Fo, Med, Tech, Ag, En | Fr, Wo, Le, Ba, Ro, Fl | 3.76 |
| <i>Mimosaceae</i> | <i>Parkia biglobosa</i> (Jaq.) Benth. | bu nalay, bu níók | Fo, Med, Tech, Ag, En | Fr, Wo, Le, Ba, Ro | 3.7 |
| <i>Anacardiaceae</i> | <i>Anacardium occidentale</i> L. | bu talakasa, bu bisa | Fo, Med, Tech, Ag, En | Fr, Wo, Le, Ba, Ro | 3.5 |
| <i>Bombacaceae</i> | <i>Ceiba pentandra</i> (L.) Gaertn | bu sana | Fo, Med, Tech, Ag, En | Fr, Wo, Le, Ba, Ro | 3.23 |
| <i>Chrysobalanaceae</i> | <i>Parinari excelsa</i> Sabine | bu wel, bu fujay, e liik | Fo, Med, Tech, Ag, En, Cul | Fr, Wo, Le, Ba, Ro | 2.59 |
| <i>Caesalpiniaceae</i> | <i>Dialium guineense</i> Willd. | bu fulan, bu foyitay | Fo, Med, Tech, En | Fr, Wo, Le, Ro | 2.43 |
| <i>Bombacaceae</i> | <i>Adansonia digitata</i> L. | bu koŋa, bu baak | Fo, Med, Tech, Cul | Fr, Le, Ba | 2.35 |
| <i>Nymphaeaceae</i> | <i>Nymphaea spp</i> | bu kikif, e bahál | Fo, Tech | Fr, Le, Ro | 1.99 |
| <i>Apocynaceae</i> | <i>Landolphia dulcis</i> (Sabine) Pichon | bu bot, bu ñohol, bu ñohon | Fo, Med, Tech | Fr, Wo, Le, Ba, Ro | 1.98 |
| <i>Annonaceae</i> | <i>Xylopia aethiopica</i> (Dunal) A. Rich. | bu ñew ba finjoe | Fo, Med, Tech, En | Fr, Wo, Le, Ro | 1.85 |
| <i>Annonaceae</i> | <i>Uvaria chamae</i> P. Beauv. | bu ñew | Fo, Med, Tech, En | Fr, Wo, Le, Ba, Ro | 1.74 |
| <i>Sapindaceae</i> | <i>Aphania senegalensis</i> (Juss. Ex Poir.) Radlk. | bu uł | Fo, Med, Tech, En | Fr, Wo, Le, Ro | 1.74 |
| <i>Sapindaceae</i> | <i>Allophyllus africanus</i> P. Beauv. | bu singilit, bu huł a mata, bu fankahen | Fo, Med, Tech, En | Fr, Wo, Le, Ro | 1.66 |
| <i>Apocynaceae</i> | <i>Saba senegalensis</i> (A. DC.) Pichon | bu híndik, bundok | Fo, Med, Tech, En | Fr, Wo, Le, Ex | 1.63 |
| <i>Arecaceae</i> | <i>Phoenix reclinata</i> Jacq. | bu faba faba, bu sanjab, bu juka, bu jak | Al, Med, Tech, Ass | Fr, Wo, Le, Ro | 1.62 |
| <i>Rubiaceae</i> | <i>Sarcocephalus latifolius</i> (Sm.) | bundufáy, | Al, Med, Tech, | Fr, Wo, Le, | 1.6 |

| | | | | | |
|-----------------------|--|---|--------------------------|---------------------------|------|
| | E.A.Bruce | buntunfáy, bu kundufáy | En | Ro | |
| <i>Anacardiaceae</i> | <i>Spondias mombin</i> L. | bu ɽeɽu, bu ɽiɽu | Fo, Med, Tech, En | Fr, Wo, Le, Ba, Ro | 1.54 |
| <i>Rubiaceae</i> | <i>Gardenia erubescens</i> Stapf. & Hutch. | bu kookay ba nale | Fo, Med, Tech | Fr, Wo, Le, Ba, Ro | 1.51 |
| <i>Apocynaceae</i> | <i>Landolphia heudelotii</i> A. DC. | bu hemb | Fo, Med, Tech | Fr, Wo, Le, Ro, Ex | 1.48 |
| <i>Verbenaceae</i> | <i>Vitex doniana</i> Sw. | bu jink, bu kuf | Fo, Med, Tech, En | Fr, Wo, Le, Ba, Ro | 1.48 |
| <i>Anacardiaceae</i> | <i>Sorindeia juglandifolia</i> (A. Rich.) Planch. | bu totol, bu totol e kaw, bu lalalen, bu foot, bu singilit | Fo, Med, Tech, En | Fr, Wo, Le, Ro | 1.41 |
| <i>Avicenniaceae</i> | <i>Avicennia germinans</i> (L.) L. | bu bej | Fo, Med, Tech, Ag, En | Fr, Wo, Le, Ro | 1.4 |
| <i>Sterculiaceae</i> | <i>Cola cordifolia</i> (Cav.) R. Br. | bu gítin, bu jíkin | Fo, Med, Tech, Ag, En | Fr, Wo, Le, Ro, Ba, Ex | 1.38 |
| <i>Celastraceae</i> | <i>Salacia senegalensis</i> (Lam.) D.C. | bu fumb, bu lál | Fo, Med | Fr, Wo, Le, Ba, Ro | 1.27 |
| <i>Apocynaceae</i> | <i>Voacanga africana</i> Stapf. | bu ɽefukál, bu ɽenf e jaamen, bu hi fan hi f, buntiñ, bungól | Fo, Med, Tech, Cul | Fr, Wo, Le, Ex | 1.26 |
| <i>Annonaceae</i> | <i>Annona senegalensis</i> Pers. | bu lálóf | Fo, Med, Tech, En | Fr, Wo, Le, Ba, Ro | 1.22 |
| <i>Rutaceae</i> | <i>Zanthoxylum zanthoxyloides</i> (Lam.) Zepern. & Timler | hu ɽuna, bu joginam, bu kosindal | Fo, Med, Tech, En | Fr, Wo, Le, Ba, Ro | 1.2 |
| <i>Icacinaceae</i> | <i>Icacina oliviformis</i> (Poir.) J. Raynal var. <i>oliviformis</i> | bu tima, buntima | Fo, Med, Tech | Fr, Le, Ro | 1.18 |
| <i>Apocynaceae</i> | <i>Landolphia hirsuta</i> (Hua) Pichon | bu gisay | Fo, Tech | Fr, Ex | 1.12 |
| <i>Zingiberaceae</i> | <i>Aframomum cereum</i> (Hook. f.) K. Schum. | bu humay, bu hefay | Fo, Med, Tech | Fr, Ro | 1.09 |
| <i>Rubiaceae</i> | <i>Psychotria peduncularis</i> (Salisb.) Styerm. | bu gílen, bu lel e haf | Fo, Med, Tech | Fr, Wo, Le, Ro | 1.07 |
| <i>Rubiaceae</i> | <i>Macrosphyra longistyla</i> (DC.) Hiern. | bu juɽ e jaamen, bu kokol, bu giɽ e jaamen | Fo, Med | Fr, Wo, Le, Ro | 1.02 |
| <i>Moraceae</i> | <i>Ficus lutea</i> Vahl | bu fok (bu kunful), bu kunfun, bu fok (bu ganful) | Fo, Med, Tech, Ag, En | Fr, Wo, Le, Ex | 0.99 |
| <i>Polygalaceae</i> | <i>Aroxima afzeliana</i> (Oliv.) Stapf. | bu miiton | Fo, Med | Fr, Le | 0.91 |
| <i>Passifloraceae</i> | <i>Passiflora foetida</i> L. | mu teñay, bu kuma kuma, bu fobek, bu ting, bu sikín | Fo, Med | Fr, Wo, Fe | 0.89 |
| <i>Meliaceae</i> | <i>Azadirachta Indica</i> A. Juss. | bu niwakin | Fo, Med, Tech, En | Fr, Wo, Le, Ro | 0.78 |
| <i>Capparidaceae</i> | <i>Ritchiea capparoides</i> (Andr.) Britt. | bu naanaa a mata, bu lulumay a mata, bu saɽ a mata, e toj e heeji | Fo, Med | Fr, Wo, Le, Ro | 0.72 |
| <i>Moraceae</i> | <i>Treulia africana</i> Decne. | buitók | Fo, Med, Tech | Fr, Wo, Ex | 0.71 |

| | | | | | |
|-------------------------|---|--|-------------------|------------|------|
| <i>Chrysobalanaceae</i> | <i>Chrysobalanus ellipticus</i> Sol. ex Sabine | bu ɲoñ, bu sima, bu uɭ e jakaɭ | Fo, Med, Tech, En | Fr, Wo | 0.61 |
| <i>Cucurbitaceae</i> | <i>Cucumis metuliferus</i> E. Mey. ex Naudin | bu konkombra e firika, bu konkombura, e giɭ e jaamen | Fo, Med | Fr, Le | 0.61 |
| <i>Ebenaceae</i> | <i>Diospyros ferrea</i> (Willd.) Bakh. | bu wing a ligen, lalalen | Fo, Med, En | Fr, Wo | 0.35 |
| <i>Clusiaceae</i> | <i>Mammea africana</i> Sabine | báál | Fo, Med | Fr, Le | 0.33 |
| <i>Caesalpiniaceae</i> | <i>Detarium senegalense</i> J.F. Gmel. | bu gawuj, bu bunkut ba teñey | Fo, Med | Fr, Le | 0.29 |
| <i>Sapindaceae</i> | <i>Pancovia bijuga</i> Willd. | buntañ, bu luñay | Fo, Med, En | Fr, Le, Wo | 0.28 |
| <i>Caesalpiniaceae</i> | <i>Tamarindus indica</i> L. | bu dahar | Fo, Med | Fr, Le | 0.22 |
| <i>Apocynaceae</i> | <i>Landolphia owariensis</i> P. Beauv. | bu tiyok, bu tik | Fo, Med | Fr, Ro | 0.17 |
| <i>Zingiberaceae</i> | <i>Aframomum elliotii</i> (Bak.) K. Schum. | bu humay e kobol, bu hefay e kobol | Fo | Fr | 0.13 |
| <i>Euphorbiaceae</i> | <i>Drypetes floribunda</i> (Müll. Arg.) Hutch. | bu huuta, bunkóɭ | Fo | Fr | 0.12 |
| <i>Arecaceae</i> | <i>Calamus deerratus</i> G. Mann & H. Wendl. | ka hihá, ka liyá | Fo, Tech | Fr, Wo | 0.12 |
| <i>Annonaceae</i> | <i>Monanthes barteri</i> (Baill.) Verdc. | bu ɭew ba yine | Fo | Fr | 0.11 |
| <i>Sapotaceae</i> | <i>Synsepalum brevipes</i> (Baker) T. D. Penn. | bundukul | Fo | Fr | 0.09 |
| <i>Rhamnaceae</i> | <i>Ziziphus mauritiana</i> Lam. | sidem | Fo | Fr | 0.09 |
| <i>Annonaceae</i> | <i>Uvaria thomasi</i> Sprag. Et Hutch. | bu ɭew bu jal | Fo, Med | Fr, Ro | 0.09 |
| <i>Annonaceae</i> | <i>Annona glauca</i> Schumach. & Thonn. | bu lala | Fo, Med | Fr, Ro | 0.07 |
| <i>Ulmaceae</i> | <i>Celtis toka</i> (Forssk.) Hepper & J.R.I. Wood | buwintol | Fo | Fr | 0.06 |
| <i>Moraceae</i> | <i>Ficus capensis</i> Thunb. | bu fok | Fo | Fr | 0.06 |
| <i>Chrysobalanaceae</i> | <i>Chrysobalanus orbicularis</i> Schumach. | nu loña | Fo | Fr | 0.05 |
| <i>Zygophyllaceae</i> | <i>Balanites aegyptiaca</i> (L.) Del. | | Fo | Fr | 0.02 |
| <i>Anacardiaceae</i> | <i>Pseudospondias microcarpa</i> (A. Rich.) Engl. | bu nunu | Fo | Fr | 0.02 |

Fo: food; **Med**: medicinal; **Tech**: technological; **Ag**: agronomic; **En**: energetic; **Cu**: cultural.

Fr : fruit; **Le** : leaf ; **Ba** : bark; **Wo** : wood ; **Ro** : root; **Ex** : exudate; **Fl** : flower.

3.2 Use Categories

Non-cultivate fruit species can be useful in several ways. The results on their use modes show 6 categories of use (**Table 2**). These are:

- food where all species are mentioned;
- medicinal with 52 useful species;
- technological with 39 species;
- energetic with 27 species;
- agronomic with 10 species;

- cultural with 9 species.

The most used species in these different categories are: *Elaeis guineensis* with 378 citations, *Borassus aethiopum* with 343 citations, *Mangifera indica* with 266 citations, *Parkia biglobosa* with 227 citations, *Ceiba pentandra* with 226 citations, *Neocarya macrophylla* with 212 citations and *Parinari excelsa* with 210 citations (**Table 2**).

The frequency of citation (**Table 2**) is greater than 50% in:

- 38 species in the food sector;
- 9 species in the energy sector;
- 6 species in the technological sector;
- 4 species in the cultural sector;
- 1 species in the medicinal f sector and less than 50% in agronomy.

This frequency of citation of species by populations can be high, medium or low depending on the category of use. For example, *Borassus aethiopum*, *Phoenix reclinata* are highly used in cultural field whereas they are not in medicine. A low use value may be due to a lack of knowledge about the plant or the disappearance of certain species due to forest erosion.

Table 2. Frequency of citation of the different use categories of non-cultivate edible fruit species

| Scientific name | Number of citation | Frequency of Citation (FC) in % | | | | | |
|---|--------------------|---------------------------------|------|------|-----|-----|-----|
| | | Fo | Med. | Tech | Ag | En | Cul |
| <i>Mangifera indica</i> | 266 | 100 | 57 | 28 | 43 | 96 | |
| <i>Elaeis guineensis</i> | 378 | 100 | 49 | 100 | 65% | 100 | 99 |
| <i>Landolphia dulcis</i> | 127 | 100 | 49 | 6 | | | |
| <i>Parkia biglobosa</i> | 227 | 100 | 44 | 24 | 18 | 90 | |
| <i>Nauclea latifolia</i> | 126 | 100 | 39 | 4 | | 55% | |
| <i>Adansonia digitata</i> | 163 | 100 | 38 | 57 | | | 3 |
| <i>Neocarya macrophylla</i> | 221 | 100 | 38 | 16 | 5 | 96 | 4 |
| <i>Anacardium occidentale</i> | 197 | 100 | 34 | 1 | 7 | 98 | |
| <i>Aphania senegalensis</i> | 141 | 100 | 34 | 1 | | 37 | |
| <i>Uvaria chamae</i> | 134 | 100 | 28 | 24 | | 55 | |
| <i>Saba senegalensis</i> | 112 | 100 | 26 | 22 | | 1 | |
| <i>Salacia senegalensis</i> | 101 | 100 | 23 | | | | |
| <i>Borassus aethiopum plant</i> | 143 | 100 | 21 | 100 | | 98 | 100 |
| <i>Ceiba pentandra</i> | 126 | 100 | 21 | 80 | 60 | 9 | 54 |
| <i>Landolphia heudelotii</i> | 114 | 100 | 21 | 18 | | | |
| <i>Annona senegalensis</i> | 97 | 100 | 15 | 4 | | | |
| <i>Parinari excelsa</i> | 210 | 100 | 60 | 32 | 1 | 78 | 33 |
| <i>Vitex doniana Sw</i> | 115 | 100 | 10 | 60 | | 18 | |
| <i>Aframomum cereum</i> | 90 | 100 | 9 | 1 | | | |
| <i>Dialium guineense</i> | 189 | 100 | 7 | 34 | | 89 | |
| <i>Nymphaea spp</i> | 84 | 100 | 1 | 1 | | | |
| <i>Allophylus africanus</i> | 137 | 98 | 6 | 5 | | 59 | |
| <i>Phoenix reclinata</i> | 134 | 96 | 1 | 5 | | | 61 |
| <i>Xylopia aethiopica</i> | 133 | 94 | 44 | 21 | | 4 | |
| <i>Macrosphyra longistyla</i> | 80 | 93 | 5 | | | | |
| <i>Sorindeia juglandifolia</i> | 113 | 91 | 7 | 2 | | 37 | |
| <i>Atroxima afzeliana</i> | 74 | 89 | 1 | | | | |
| <i>Mombin spondias</i> | 121 | 88 | 4 | 52 | | 4 | |
| <i>Icacina oliviformis var. oliviformis</i> | 93 | 87 | 23 | 4 | | | |
| <i>Cola cordifolia</i> | 104 | 82 | 22 | 16 | 1 | 6 | |
| <i>Passiflora foetida</i> | 70 | 80 | 5 | | | | |
| <i>Gardenia erubescens</i> | 115 | 78 | 29 | 33 | | | |
| <i>Avicennia germinans</i> | 108 | 73 | 4 | 7 | | 48 | |
| <i>Landolphia hirsuta</i> | 91 | 73 | 1 | 37 | | | |
| <i>Treculia africana</i> | 58 | 63 | 6 | 1 | | | |
| <i>Cucumis metuliferus</i> | 51 | 61 | 1 | | | | |

| | | | | | | |
|--------------------------------------|-----|----|----|----|---|-----|
| <i>Ficus lutea</i> | 78 | 57 | 6 | 5 | 1 | 26 |
| <i>Voacanga africana</i> | 100 | 52 | 2 | 59 | | 1 7 |
| <i>Psychotria peduncularis</i> | 84 | 48 | 17 | 2 | | 35 |
| <i>Chrysobalanus ellipticus</i> | 44 | 46 | 1 | 2 | | 4 |
| <i>Ritchiea capparoides</i> | 51 | 37 | 26 | | | |
| <i>Mammea africana</i> | 26 | 30 | 1 | | | |
| <i>Detarium senegalense</i> | 26 | 29 | 2 | | | |
| <i>Pancovia bijuga</i> | 23 | 23 | 4 | | | 1 |
| <i>Tamarindus indica</i> | 18 | 21 | 1 | | | |
| <i>Diospyros ferrea</i> | 28 | 65 | 5 | | | 16 |
| <i>Landolphia owariensis</i> | 15 | 65 | 5 | | | |
| <i>Monanthes barteri</i> | 55 | 65 | | | | |
| <i>Drypetes floribunda</i> | 60 | 60 | 1 | 1 | | |
| <i>Aframomum elliotii</i> | 10 | 60 | | | | |
| <i>Uvaria thomasi</i> | 7 | 7 | 1 | | | |
| <i>Synsepalum brevipes</i> | 6 | 7 | | | | |
| <i>Ziziphus mauritiana</i> | 6 | 7 | | | | |
| <i>Zanthoxylum zanthoxyloides</i> | 92 | 6 | 65 | 38 | 1 | 54 |
| <i>Azadirachta indica, neem tree</i> | 63 | 6 | 4 | 6 | 0 | 61 |
| <i>Celtis toka</i> | 5 | 6 | | | | |
| <i>Ficus capensis</i> | 5 | 6 | | | | |
| <i>Annona glauca</i> | 5 | 4 | 2 | | | |
| <i>Calamus deerratus</i> | 10 | 2 | | 10 | | |
| <i>Balanites aegyptiaca</i> | 2 | 2 | | | | |
| <i>Chrysobalanus orbicularis</i> | 2 | 2 | | | | |
| <i>Pseudospondias microcarpa</i> | 2 | 2 | | | | |

Fo : food; **Med** : medicinal; **Tech** : technological; **Ag** : agronomic; **En** : energetic; **Cul** : cultural

Table 3 shows the fidelity index of the 62 species used in the six use categories. Thus, the fidelity index is greater than 50% for 47 species in the food sector and for only 1 species, namely *Calamus deerratus*, in the technological field. The rest of the species have fidelity indices below 50%.

The results of the Factorial Correspondence Analysis (**FCA**) between the use categories and the 62 harvested species are presented in **Figure 2**. The prior Chi2 independence test showed a statistically significant relationship between the use categories and the 62 species ($X^2 = 4975.9$, $df = 305$, $p\text{-value} < 2.2e-16$). These results show that the frequency of use of these categories varies according to the species exploited. **Table 3** presents the inertia of each of the factorial axes obtained after transformation of the original variables by the FCA. The percentage of inertia of each of the first 2 dimensions (axes) is higher than the average threshold $(1/5) \times 100 = 20\%$. The first two dimensions account for 69% of the total inertia of the original variables. Thus, all the analyses will be done in the factorial plane composed by these first two dimensions.

Table 3. Distribution of the inertia according to the dimensions (Dim)

| Axes | Dim1 | Dim 2 | Dim 3 | Dim 4 | Dim 5 |
|----------------------------------|-------|-------|-------|-------|-------|
| Proper value | 0.27 | 0.21 | 0.10 | 0.07 | 0.07 |
| Inertia | 39.12 | 29.89 | 14.65 | 52.5% | 5.83 |
| Cumulative percentage of inertia | 39.12 | 69 | 83.66 | 94.16 | 100 |

Figure 2 of the FCA allows us to isolate three groups (G1, G2, G3). Dimension 1, which accounts for 39.12%, opposes group 3 in the negative abscissa to groups 1 and 2 in the positive abscissa. Dimension 2 (29.89%) also opposes group 2 in the negative ordinates to group 1 in the positive ordinates.

Group 1 characterizes the species used in the technological and cultural categories. The species *Elaeis guineensis* (3.3), *Borassus aethiopum* (5.5), *Ceiba pentandra* (8.1), which have a contribution above the threshold (average of the absolute contributions = 1.6), represent this group; group 2 includes species for agronomic and energy use characterized by *Parkia biglobosa* (7.2), *Zanthoxylum zanthoxyloides* (3.9) and group 3 of species for food and medicinal use characterized by *Salacia senegalensis* (3.1), *Sarcocephalus latifolius* (1.8). This study shows that the species:

Parinari excelsa, *Elaeis guineensis*, *Borassus aethiopum*, *Calamus deerratus*, *Ceiba pentandra*, *Voacanga africana*, *Psychotria peduncularis*, *Phoenix reclinata* (8 species) are more related to technological and cultural uses;

Azadirachta indica, *Zanthoxylum zanthoxyloides*, *Diospyros ferrea*, *Mangifera indica*, *Anacardium occidentale*, *Parkia biglobosa*, *Neocarya macrophylla*, *Dialium guineense*, *Avicennia germinans*, *Allophylus africanus* (9 species) are related to agronomic and energy uses;

Aroxima afzeliana, *Aframomum cereum*, *Adansonia digitata*, *Aframomum elliotii*, *Annona glauca*, *Annona senegalensis*, *Aphania senegalensis*, *Balanites aegyptiaca*, *Cola cordifolia*, *Chrysobalanus ellipticus*, *Cucumis metuliferus*, *Chrysobalanus orbicularis*, *Dryicus topetibicularis*, *Feticus topetibicularisunda*, *Deticus topetibicularisea*, *Feticus topetibicularis*, *Fevericus senegalensi*, *Gardenia erubescens*, *Icacina oliviformis*, *Landolphia dulcis*, *Landolphia heudelotii*, *Landolphia hirsuta*, *Landolphia owariensis*, *Mammea africana*, *Monanthes barteri*, *Macrosphyra longistyla*, *Nymphaea -spp*, *Pancovia bijuga*, *Passiflora foetida*, *Pseudospondias microcarpa*, *Ritchiea capparoides*, *Synsepalum brevipes*, *Sorindeia juglandifolia*, *Sarcocephalus latifolius*, *Spondias mombin*, *Saba senegalensis*, *Salacia senegalensis*, *Treulia africana*, *Tamarindus indica*, *Uvaria chamae*, *Uvaria thomasi*, *Vitex doniana*, *Xylopiya aethiopica*, *Ziziphus mauritiana* (45 species) are correlated with food and medicine.

Use categories with an above-average absolute contribution (16.6) have helped build dimensions 1 and 2. Thus:

- The food use category (31.9) has contributed significantly to building dimension 1;
- The energy use category (24.1) has contributed negatively to dimension 1.

Dimension 1 opposes food and medicinal categories to energy and agronomic categories and therefore distinguishes the species used in these different categories.

- The cultural use category (37) has contributed positively to building dimension 2.

Analysis of the results (**Figure 2**) shows that food and medicinal species in group 3 outnumber the species in groups 1 and 2. These results also show that the nature of the subsistence needs of the *Kasa* populations is mainly food and medicinal. These results also reveal categories that are highly correlated with each other and with the species.

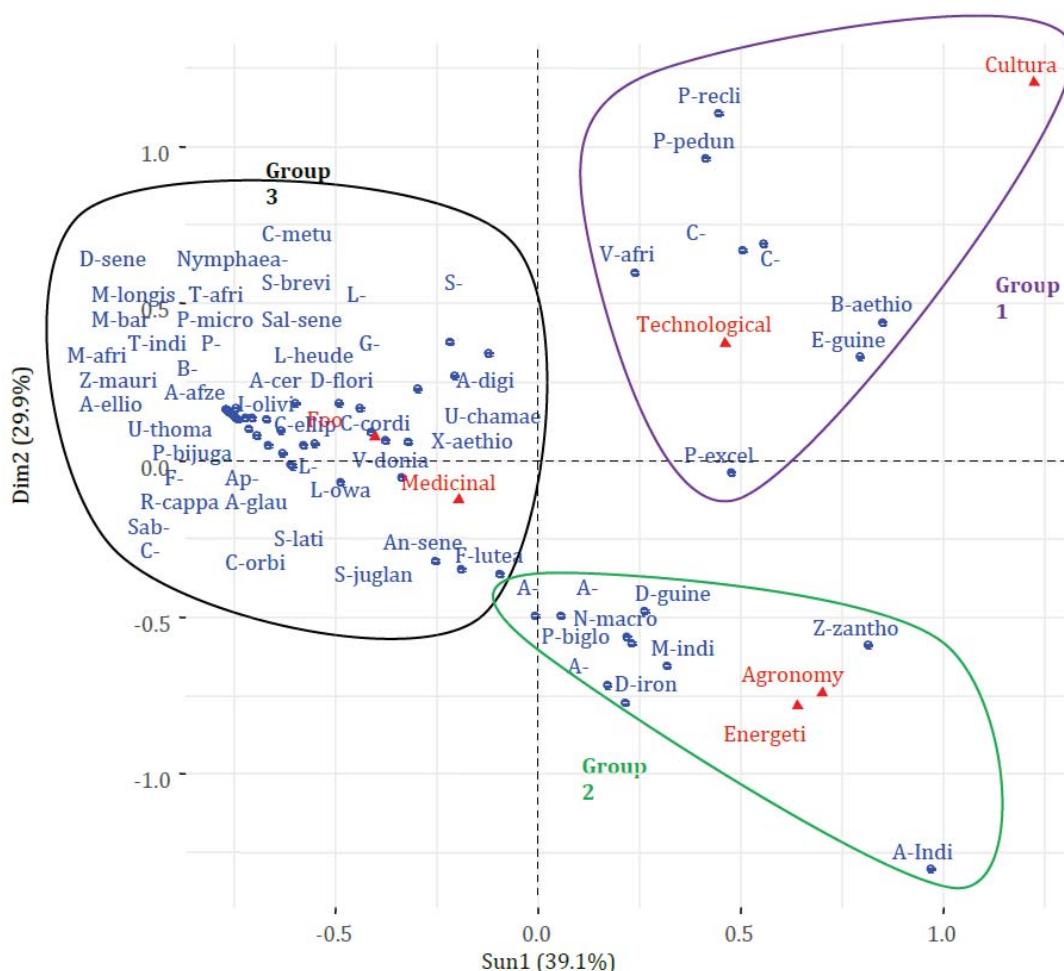


Figure 2. Factorial Correspondence Analysis (FCA) of the matrix of 62 species X 6 use categories

The consensus factors are very high for the six defined use categories. They are between 0.9 and 0.8 (Table 4). This shows that the plant species used and their use modes in the different Diola communities vary very little. Species use is most harmonious in food, cultural, and energy categories, where the consensus factor is 0.98-0.97. The agronomic category, which is strongly marked by female activity, has the lowest consensus factor at 0.89.

Table 4. Use and their category consensus factor (ICF)

| Use category | Number of citation | Number of Species | ICF |
|---------------|--------------------|-------------------|------|
| Food | 3128 | 62 | 0.98 |
| Energy | 943 | 28 | 0.97 |
| Technological | 716 | 40 | 0.94 |
| medicinal | 678 | 53 | 0.92 |
| Cultural | 325 | 8 | 0.98 |
| Agronomic | 85 | 10 | 0.89 |

In total, with regard to species use in the context of use value, use categories, organs used, our results show that the local population harvests different organs for multiple uses. *Elaeis guineensis*, *Borassus aethiopum*, *Mangifera indica*, *Neocarya macrophylla*, *Parkia biglobosa*, *Anacardium occidentale*, *Ceiba pentandra*, *Parinari excelsa*, are the most used species for the daily needs of the local population.

4. Discussion

Our results indicate 62 edible fruit species distributed in 31 families and 54 genera. They present a good generic and specific diversity compared to that of the 75 fruit species belonging to 35 families in the Séguéla region (Ambé, 2001). The results show a dominance of *Apocynaceae* and *Annonaceae*, and confirm those obtained by

(Ambé, 2001) and (Ouattara *et al.*, 2016).

Among the species studied, *Elaeis guineensis* (12.24) and *Borassus aethiopum* (7.56) have the highest use values with a relatively high number of use categories and organs used. *Elaeis guineensis* has 6 categories and 6 organs, *Borassus aethiopum* 5 categories and 5 organs. These two species are therefore highly sought after by the populations due to their high socio-economic and cultural values. They are used in the cultural field as well as in profane and sacred rites. Moreover, these species are an essential link in technology (construction of houses, production of means, etc.) and their products are abundantly consumed throughout the sector.

The *Anacardiaceae* which come in second position, when referring to use value, are essentially represented by *Mangifera indica* (3.74) with 5 categories and 6 organs and *Anacardium occidentale* (3.5) with 5 categories and 5 organs. These two species are of great economic importance, in particular *Anacardium occidentale*, the seeds of which have a high value in national and international markets as well as its alcoholic juice which is highly prized during public ceremonies and events (Djihounouck, 2018). This alcoholic juice is a substitute for palm wine in winter periods when it is scarce.

The results do not show a close proportionality between use categories, organs used and use value. The use of a species depends on its socio-economic importance, availability and accessibility. Therefore, the use value of a family is not proportional to its specific diversity as pointed out by Guèye (2012), Djihounouck *et al.* (2019). In addition, the species *Elaeis guineensis*, *Borassus aethiopum*, *Neocarya macrophylla*, *Parkia biglobosa*, *Anacardium occidentale*, *Ceiba pentandra* have significant use values between 12.24 and 3.23. The level of exploitation of these species by the local population has been highlighted by Dossou (2010), Lougbegnon *et al.* (2011), Diatta (2016) who have shown that the importance given to a species depends on its capacity to meet the needs of the populations in the different use categories. *Borassus aethiopum* has a high use value and paradoxically, it is not abundant in our study area. When a species is not abundant and has a high use value, this means that it is under strong pressure as pointed out by Camou-Guerrero *et al.* (2008), Dossou (2010).

Our results show a certain homogeneity of uses of plant species in the *Kasa* area with a high consensus factor (ICF) for all use categories. They show six categories of use among the Diola populations in the *Kasa* area. These are: food, medicinal, technological, agronomic, energetic and cultural.

Food, energy, technological and medicinal categories are the most important in terms of citation with 3128; 943; 716 and 678 respectively. These results are comparable to those obtained by Galeano (2000) among the forest communities in Colombia, where he noted a high citation index for the “food” and “medicinal” categories. These results show that subsistence needs are the primary concern of the populations that exploit plant species. The medicinal category complete the medical centers and districts, which can explain its position.

The agronomic category has the lowest ICF (0.89) as information in this area is mainly held by women, who are not the priority target of the survey. Furthermore, the ICF for the medicinal category is the second lowest (0.92) since only traditional healers and a few initiated people have knowledge of the use of plants.

These results confirm the high value of the ICF for the different use categories of plant species in arid and semi-arid zones of Africa (Gning *et al.* 2013; Ayantunde *et al.* 2009; Cheikhyoussef *et al.* 2011). The ICF provides information on the good knowledge of species exploited by local populations on the one hand, and on natural resources important for the survival and well-being of rural populations on the other hand (Gueye, 2012). The rural population retains a fairly homogeneous knowledge of the uses of plant species transmitted from generation to generation through oral tradition. This knowledge could be a basis for local development policy.

The food category covers the activities of gathering and consumption of plants. All of the species inventoried are food, but their importance varies according to their frequency of citation by informants. Of the 574 citations for the 7 organs used in this category, *Elaeis guineensis* and *Nymphaea* spp. are the most frequently cited with 29% and 27% respectively, followed by *Borassus aethiopum* 21%, *Landolphia dulcis* 20% and *Adansonia digitata* 17%.

The energy studied is that which comes from firewood of woody species, in general, or from charcoal. It covers 42% of the species inventoried. This high frequency of citation reveals a significant energy demand by the populations and the diversity of species mobilized to meet this need. This category includes the priority activities of rural women, most of whom are housewives. Carrière *et al.* (2005) showed that among the Betsileo of Madagascar, firewood is the main category of useful plants for domestic use harvested in post-agricultural recruits and ranks first ahead of other uses such as pharmaceuticals, craft and construction.

The technological category concerns the making of means and includes 63% of species. The species *Elaeis guineensis* and *Borassus aethiopum* have the highest citation frequencies with 49% and 40% respectively and are

therefore the most used in the technological field. Their leaves are used for construction (house roof, hut, fence, etc.) and for crafting service products (brooms, baskets, mats, fish traps, basketwork, furniture, fibers, ropes, benches, belts to climb palm trees, ...); their trunks or stipes are used to construct bridges, beehives, dikes and habitats (pillar, frame, ceiling, roof, hut). These results confirm those of Sambou *et al.* (1992) and Blinck (2002). In addition, palm leaves and stipes give these species a high market value (Johnson, 2010).

The medicinal category represents 82% of the species inventoried. This study has enabled us to identify 96 pathologies among the *kasa* populations. The species frequently cited by the populations for the treatment of pathologies are: *Mangifera indica*, *Elaeis guineensis*, *Landolphia dulcis*, *Parkia biglobosa* and *Xylopia aethiopica*. The frequency of use of a species for disease treatment is an index of reliability of its effectiveness. Some authors even argue that plant species used repeatedly by the population in the medical field are generally effective and interesting in the search for bioactive molecules (Trotter & Logan, 1986; King *et al.*, 1996; Guèye, 2012). The fruit species used in the *kasa* have multiple medicinal uses except *Drypetes floribunda*, *Mammea africana*, *Atoxima afzeliana*, *Nymphaea spp*, *Cucumis metuliferus*, *Tamarindus indica*, *Annona glauca*, *Diospyros ferrea* which are only used to treat a single pathology.

The cultural category represents 12% of the species. It covers sacred or profane rites. The leaves of *Elaeis guineensis* and *Phoenix reclinata* are used in dancing initiation ceremonies. *Elaeis guineensis* provides brooms that certain fetish leaders always hold as a sign of identification at the sacred throne and its traditional wine commonly called “bunuk” is used for worshipping the fetishes traditionally called *bàkin* in order to enter into communion or set a contract with the ancestors and the gods (Loubelo, 2012). *Borassus aethiopum* is used as a symbol of prohibition to non-initiated people in a festivity or of recognition to a heritage or in a traditional dance or to create coffins. *Psychotria peduncularis* is used for blessings. *Ceiba pentandra* is used to create coffins often with the stem of *Carapa* Aubl. spp. also reported by Djihounouck (2010).

The agronomic category concerns 15% of the species. The leaves of *Mangifera indica* and *Ceiba pentandra*, and the fruits of *Parkia biglobosa* are often used as fertilizers for cultivated land. Evaluation of the impact of fertilization by the *néré* (*Parkia biglobosa*) and shea (*Vitellaria paradoxa*) on sorghum seed yields has shown an increase of 50 - 70% in Burkina Faso (Kessler, 1992).

5. Conclusion

This study identified the needs of the populations in relation to the 62 fruit species exploited in the *Kasa* area and highlighted the relationship between these species and the needs of the populations. The various products derived from these species have different uses grouped into six categories. These uses have made it possible to understand the importance of these resources for the local population in all areas of life. The uses of fruit species are relatively homogeneous for the different categories; however, they are more marked for food needs, followed by those of energy, technology, medicine, agronomy and culture. The use values, categories and organs used obtained allowed the identification of the most important species for the populations. In the *kasa* area, these are *Elaeis guineensis*, *Borassus aethiopum*, *Mangifera indica*, *Neocarya macrophylla*, *Parkia biglobosa*, *Anacardium occidentale*, *Ceiba pentandra*, *Parinari excelsa*. Thus, the estimation of the threat to these species by the use value assessed through harvesting has defined these species with the exception of *Elaeis guineensis*. This species has been little affected by human activities because of its strong predominance in the forest. The study reveals 7 parts of the plants exploited (fruit, wood, leaf, bark, root, flower and exudate) with a preference for fruits with food use. For a sustainable management of these non-cultivate edible and multiple-use fruit species, it is necessary to observe the population dynamics of the exploited species and involve local users in the resource, especially in the search for income.

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Annex: List of plant species used (abbreviated) for AFC

| <i>Allophyllus africanus</i> (A-afri) | <i>Drypetes floribunda</i> (D-flori) | <i>Passiflora foetida</i> (P-foeti) | <i>Allophyllus africanus</i> (A-afri) | <i>Drypetes floribunda</i> (D-flori) | <i>Passiflora foetida</i> (P-feti) |
|--|--|--|---------------------------------------|--|--|
| <i>Aroxima afzeliana</i> (A-afze) | <i>Dialium guineense</i> (D-guine) | <i>Pseudospondias microcarpa</i> (P-micro) | <i>Aroxima afzeliana</i> (A-afze) | <i>Dialium guineense</i> (D-guine) | <i>Pseudospondias microcarpa</i> (P-micro) |
| <i>Aframomum cereum</i> (A-cer) | <i>Detarium senegalense</i> (D-sene) | <i>Psychotria peduncularis</i> (P-pedun) | <i>Aframomum cereum</i> (A-cer) | <i>Detarium senegalense</i> (D-sene) | <i>Psychotria peduncularis</i> (P-pedun) |
| <i>Adansonia digitata</i> (A-digi) | <i>Elaeis guineensis</i> (E-guine) | <i>Phoenix reclinata</i> (P-recli) | <i>Adansonia digitata</i> (A-digi) | <i>Elaeis guineensis</i> (E-guine) | <i>Phoenix reclinata</i> (P-recli) |
| <i>Aframomum elliotii</i> (A-ellio) | <i>Ficus capensis</i> (F-cap) | <i>Ritchiea capparoides</i> (R-cappa) | <i>Aframomum elliotii</i> (A-ellio) | <i>Ficus capensis</i> (F-cap) | <i>Ritchiea capparoides</i> (R-cappa) |
| <i>Avicennia germinans</i> (A-ger) | <i>Ficus lutea</i> (F-lutea) | <i>Synsepalum brevipes</i> (S-brevi) | <i>Avicennia germinans</i> (A-ger) | <i>Ficus lutea</i> (F-lutea) | <i>Synsepalum brevipes</i> (S-brevi) |
| <i>Annona glauca</i> (A-glau) | <i>Gardenia erubescens</i> (G-eru) | <i>Sorindeia juglandifolia</i> (S-juglan) | <i>Annona glauca</i> (A-glau) | <i>Gardenia erubescens</i> (G-eru) | <i>Sorindeia juglandifolia</i> (S-juglan) |
| <i>Azadirachta indica</i> (A-Indi) | <i>Icacina oliviformis</i> (I-olivi) | <i>Sarcocephalus latifolius</i> (S-lati) | <i>Azadirachta indica</i> (A-Indi) | <i>Icacina oliviformis</i> (I-olivi) | <i>Sarcocephalus latifolius</i> (S-lati) |
| <i>Anacardium occidentale</i> (A-occi) | <i>Landolphia dulcis</i> (L-dul) | <i>Spondias mombin</i> (S-mom) | <i>Western anacardium</i> (A-occi) | <i>Landolphia dulcis</i> (L-dul) | <i>Mombin spondias</i> (S-mom) |
| <i>Annona senegalensis</i> (An-sene) | <i>Landolphia heudelotii</i> (L-heude) | <i>Saba senegalensis</i> (Sab-sene) | <i>Annona senegalensis</i> (An-sene) | <i>Landolphia heudelotii</i> (L-heude) | <i>Saba senegalensis</i> (Sab-sene) |
| <i>Aphania senegalensis</i> (Ap-sene) | <i>Landolphia hirsuta</i> (L-hirsu) | <i>Salacia senegalensis</i> (Sal-sene) | <i>Aphania senegalensis</i> (Ap-sene) | <i>Landolphia hirsuta</i> (L-hirsu) | <i>Salacia senegalensis</i> (Sal-sene) |
| <i>Balanites aegyptiaca</i> (B-aegyp) | <i>Landolphia owariensis</i> (L-owa) | <i>Treculia africana</i> (T-afri) | <i>Balanites aegyptiaca</i> (B-aegyp) | <i>Landolphia owariensis</i> (L-owa) | <i>Treculia africana</i> (T-afri) |

| | | | | | | | |
|---|--|--|---|--|--|---|--|
| <i>Borassus aethiopum</i> (B-aethio) | <i>Mammea africana</i> (M-afri) | <i>Tamarindus indica</i> (T-indi) | (B-aegypt) | (L-owa) | <i>Borassus aethiopum</i> (B-aethio) | <i>Mammea africana</i> (M-afri) | <i>Tamarindus indica</i> (T-indi) |
| <i>Cola cordifolia</i> (C-cordi) | <i>Monanthes barteri</i> (M-bar) | <i>Uvaria chamae</i> (U-chamae) | <i>Cola cordifolia</i> (C-cordi) | <i>Monanthes barteri</i> (M-bar) | <i>Uvaria chamae</i> (U-chamae) | <i>Cola cordifolia</i> (C-cordi) | <i>Monanthes barteri</i> (M-bar) |
| <i>Calamus deerratus</i> (C-deer) | <i>Mangifera indica</i> (M-indi) | <i>Uvaria thomasi</i> (U-thoma) | <i>Calamus deerratus</i> (C-deer) | <i>Mangifera indica</i> (M-indi) | <i>Uvaria thomasi</i> (U-thoma) | <i>Calamus deerratus</i> (C-deer) | <i>Mangifera indica</i> (M-indi) |
| <i>Chrysobalanus ellipticus</i> (C-ellip) | <i>Macrosphyra longistyla</i> (M-longis) | <i>Voacanga africana</i> (V-afri) | <i>Chrysobalanus ellipticus</i> (C-ellip) | <i>Macrosphyra longistyla</i> (M-longis) | <i>Voacanga africana</i> (V-afri) | <i>Chrysobalanus ellipticus</i> (C-ellip) | <i>Macrosphyra longistyla</i> (M-longis) |
| <i>Cucumis metuliferus</i> (C-metu) | <i>Neocarya macrophylla</i> (N-macro) | <i>Vitex doniana</i> (V-donia) | <i>Cucumis metuliferus</i> (C-metu) | <i>Neocarya macrophylla</i> (N-macro) | <i>Vitex doniana</i> (V-donia) | <i>Cucumis metuliferus</i> (C-metu) | <i>Neocarya macrophylla</i> (N-macro) |
| <i>Chrysobalanus orbicularis</i> (C-orbi) | <i>Nymphaea</i> -spp | <i>Xylopiya aethiopica</i> (X-aethio) | <i>Chrysobalanus orbicularis</i> (C-orbi) | <i>Nymphaea</i> -spp | <i>Xylopiya aethiopica</i> (X-aethio) | <i>Chrysobalanus orbicularis</i> (C-orbi) | <i>Nymphaea</i> -spp |
| <i>Ceiba pentandra</i> (C-pentan) | <i>Parkia biglobosa</i> (P-biglo) | <i>Ziziphus mauritiana</i> (Z-mauri) | <i>Ceiba pentandra</i> (C-pentan) | <i>Parkia biglobosa</i> (P-biglo) | <i>Ziziphus mauritiana</i> (Z-mauri) | <i>Ceiba pentandra</i> (C-pentan) | <i>Parkia biglobosa</i> (P-biglo) |
| <i>Celtis toka</i> (C-toka) | <i>Pancovia bijuga</i> (P-bijuga) | <i>Zanthoxylum zanthoxyloides</i> (Z-zantho) | <i>Celtis toka</i> (C-toka) | <i>Pancovia bijuga</i> (P-bijuga) | <i>Zanthoxylum zanthoxyloides</i> (Z-zantho) | <i>Celtis toka</i> (C-toka) | <i>Pancovia bijuga</i> (P-bijuga) |
| <i>Diospyros ferrea</i> (D-fer) | <i>Parinari excelsa</i> (P-excel) | | <i>Diospyros ferrea</i> (D-fer) | <i>Parinari excelsa</i> (P-excel) | | <i>Diospyros ferrea</i> (D-fer) | <i>Parinari excelsa</i> (P-excel) |

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The Feasibility of Controlled Environment in Horticulturally Poor Region: The Case of New Brunswick in Canada

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Abstract

More than 90% of the money spent on food in the Canadian province of New Brunswick was spent on food that was imported to the province from either other provinces or out of the country. The feasibility of controlled environment agriculture in the Canadian province of New Brunswick depends on a large variety of factors, some of which have no available data. Few studies have looked at this issue, including consumers' willingness to pay for locally grown produce in that region. The study aims at understanding how agriculture can serve the region differently to increase its food autonomy and how consumers would be receptive to more locally grown produce. From the information in the survey conducted, unless CEA (Controlled Environment Agriculture) crops can compete with conventionally grown and imported alternatives pricewise, it could face many issues in New Brunswick and Canada considering the economic uncertainties surrounding COVID-19. Canadians were also surveyed specifically about paying a premium for food that they considered local, not necessarily Canada as a whole, and many of the larger regions in Canada, such as Ontario and Quebec, consider food grown within their region as local – a definition which would not include New Brunswick.

Keywords: food security, controlled-environment agriculture, horticulture, greenhouses, vertical farms

1. Introduction

About 90% of the money spent on food in the Canadian province of New Brunswick was spent on food that was imported to the province from either other provinces or out of the country (Haghiri et al., 2009). In a situation such as COVID-19, this can post potential problems if there are any disruptions to cross-border shipping. CEA (Controlled Environment Agriculture) could allow for New Brunswick to grow fresh produce outside of the regular growing season. However, greenhouses have upfront costs involved, and may potentially be more expensive than regular farming operations. Many CEA operations are built in cities, necessitating a larger cost for land, and many CEA operations also require heating and electricity costs (Ceres, 2020; Laate, 2013). These additional costs would need to be recouped somehow, and may increase the cost of produce for consumers, impacting whether consumers would be willing to pay for them (Dieterle, 2016; Giraud, 2021). Consumer attitudes towards CEA crops will also impact whether customers are willing to buy the products and must also be considered (Walters et al., 2021).

Many studies have been conducted on consumer willingness to pay (WTP) for food, but only one has been conducted in New Brunswick, which was done in 2009, and focused on customer WTP for integrated pest management (Haghiri et al., 2009). In the study by Haghiri et. al. (2009), almost all respondents would not pay more than 10% extra for the produce using integrated pest management techniques. There was no available data on New Brunswicker's WTP for local food, nor was there any recent data on their WTP in general, which had likely changed as a result of the pandemic and a variety of other factors since the original study in 2009.

This study aims at understanding the role of CEA and how it supports a broader food autonomy agenda for governments. It also aims to comprehend how CEA-grown produce is perceived by consumers. Consumer attitudes towards CEA agriculture are also important to consider when assessing feasibility. If consumers believe that CEA crops are worse than conventionally grown crops, they will not purchase CEA grown crops when at the grocery store, especially if CEA crops are more expensive than their conventionally grown counterparts.

This report will present the findings from a survey regarding consumer attitudes towards CEA grown crops and consumer WTP and their implications for the feasibility of CEA agriculture in New Brunswick. It also includes additional considerations with regards to supplier fees, which may impact the cost of all food in certain grocery chains and could have future implications of consumer WTP if their grocery bills become even more expensive.

1.1 Food Autonomy and Consumer Perception

Food security is a priority for all countries around the world. In the Western world, food security is often taken for granted (Fieldhouse and Thompson, 2012; Charlebois and Vandertuin, 2021). Becoming more food autonomous is known to be a continuing pursuit for most countries around the world (Benke and Tomkins, 2017; Kolinjivadi, Mendez and Dupras, 2019). Food autonomy is founded on four fundamental concepts: Access for all people to enough quality food and to food resources, at a reasonable cost (Wakefield et al., 2015). Unlike food sovereignty, food autonomy is about producing more food in an open economy which embraces trade. Canada's economy, like many other countries, is trade reliant (Modongo, Oteng and Kulshreshtha, 2018). With efficient distribution systems, underserved markets, or region where the primary production of food is a challenge, can remain food secure (Charlebois and Christensen-Hughes, 2015). Nonetheless, disruption within the supply chain can make that very region more vulnerable, almost instantly (Watts et al., 2015; Power, 2017).

The COVID pandemic had brought an heightened sense of food insecurity in many Canadians (Brady and Brady, 2020). Canada's climate does not allow for domestic agriculture to fully participate in making Canada food anonymous all year round (Zheng, Dixon and Ferguson, 2011; Gomez et al., 2019; Chen et al., 2020). Producing more domestically all year around went from being an after-thought to a political and socio-economical priority during the pandemic for most provinces in Canada, including New Brunswick. One way to achieve such a goal is to build more CEA capacity (Ineck et al., 2020; Miliauskienė et al., 2021). The will to buy locally CEA-grown produce has largely been understudied in a Canadian environment, especially in the context of a pandemic when food security concerns have been acute.

Consumers have often desired locally produced vegetable and fruits, but sales have not always given the same story (Abdullah et al., 2016). Many factors have pushed consumers away from locally grown produce, such as price, availability, and the perception of growing in a greenhouse, or other CEA-type facility (Cavaliere et al., 2014). Some consumers have had concerns about CEA as it may not reconstruct the exact natural environment food has conventional been grown in (Cholette et al., 2013; Shamshiri et al., 2018). Reaction to locally grown produce using new technologies is largely misunderstood in Canada, and the Atlantic region where New Brunswick is located.

1.2 Methodology

The participants of this study were randomly recruited across Canada. Even if the study focuses on one province, have data from other provinces would bring perspective on how New Brunswick consumers compare with other Canadians. The selected participants agreed to participate voluntarily only on the criteria of being a consumer in Canada and had to be an adult. The data used in this paper was obtained from a survey on consumer awareness and purchase behavior from an accredited third-party field house which was selected to collect the data for this project. The survey was conducted online, and the sample design allowed it to be representative of the Canadian population, based on age, gender and regional representation.

The survey was composed of questions to assess consumer WTP and attitudes towards CEA grown crops across the country to assess the viability of CEA produce. The survey was sent out in two parts in September 2020. The first part of the survey went out to 7,950 Canadians and the second part surveying 8,124 Canadians, with a total of 10,266 unique users responding to the survey. There was a total of 272 New Brunswickers who answered the survey, with 220 answered the first part and 222 answering the second. For instrument validity, we conducted a pilot-test and made very minor adjustments as a result.

The survey assessed what consumers considered to be local to them in order to better understand what produce people would be more willing to pay a premium on, their attitudes towards CEA crops in order to assess whether or not consumers viewed them positively to determine whether or not consumers would be willing to buy CEA crops, what they considered important when buying produce in order to determine whether or not they are even looking for local food and if there are any factors that could prove prohibitive for CEA crop feasibility, how much extra they would be willing to pay for locally grown produce to determine whether or not it would be feasible with any price discrepancies, their grocery shopping habits in order to see where CEA crops would need to get on the shelves in order to be feasible, and dietary habits as that may impact people's WTP. A copy of the survey questions can be found in Appendix A.

Caution must be taken with WTP surveys, as consumer habits do not always reflect their responses when they reach the grocery store, and the reality of the price differences is visible. Nevertheless, they can provide valuable indicators and trends particularly when comparing regional differences.

2. Attitudes towards CEA Crops and WTP

In order to assess the feasibility of CEA, consumer attitudes towards CEA crops and WTP with regards to these crops must be considered. The survey questioned respondents on the perceived quality and value of CEA crops in relation to their conventionally grown counterparts, what they considered to be important when choosing what fruits and vegetables to buy and asked about their WTP for locally grown fruits and vegetables. This data can help assess whether or not consumers are interested in buying CEA crops, even in the case that they are more expensive than their conventionally grown counterparts. What consumers consider important is also important to consider when assessing feasibility, because while consumers may value locally grown food, they may not be looking for it at the grocery store or consider it important when choosing what produce to purchase. The premium consumers are willing to pay is important because if CEA crops are more expensive than their conventionally grown counterparts, consumers will likely stick to produce within their price range.

2.1 Attitudes: Canada as a Whole

Overall, Canadians value produce that they consider local, the definition of which varied by province in the survey. Some respondents considered that anything grown in their region was local while others considered local to be anything grown within their own province. Of survey respondents, 51.6% either agreed or strongly agreed with the statement “I would pay a premium for off-season fresh produce grown locally in greenhouses (or using other technologies) versus imported alternatives”, and 27.4% of respondents considered CEA grown produce to be better than conventionally grown field crops as opposed to only 9.2% who considered CEA grown produce to be worse than the conventionally grown alternatives.

How is the quality and value of fruits and vegetables grown in greenhouses or rooftop farms, as compared to conventional land-grown foods? (n=7950)

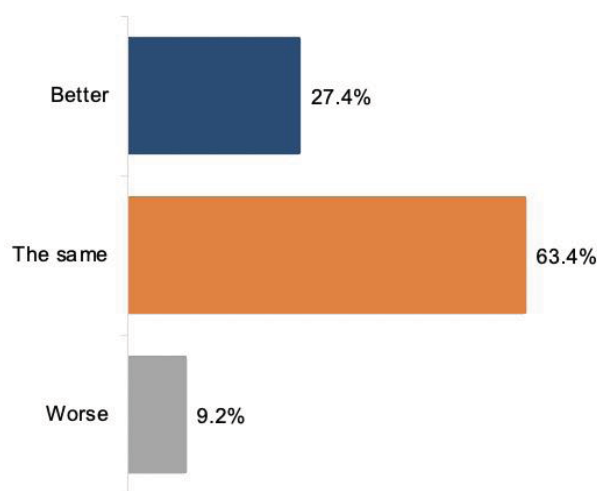


Figure 1. Graph showing Canadian perceptions of CEA grown crops in comparison to conventionally grown crops

When asked about what kind of premium respondents would be willing to pay for locally grown produce, only 20.5% of respondents said that they would be unwilling to pay any kind of premium at all while 43.3% of respondents said they would be willing to pay a premium greater than 10%. Overall, Canadians value locally grown produce very highly, and say that they would be willing to pay a premium. However, when asked what they consider to be important when choosing produce at the grocery store, 61.7% of respondents said that price was an important factor, compared to only 32.3% saying that where it was produced was important, trailing behind taste at 37.6%.

Which of the following options are important to you when choosing fresh produce? (Please select all that apply) (n=7950)

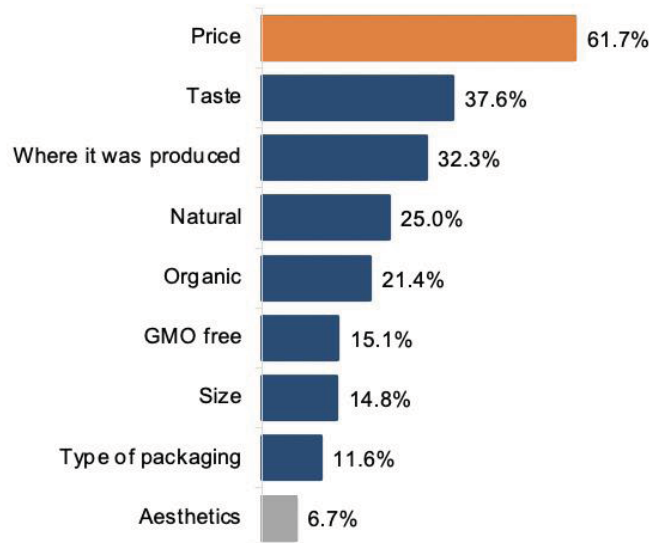


Figure 2. Graph showing what factors are important to Canadians when choosing fresh produce

2.2 Attitudes: New Brunswick

Compared to Canada as a whole, respondents from New Brunswick were more likely to be willing to pay a premium for locally grown (meaning grown within the province, to New Brunswick respondents) CEA produce in the off-season. Over half (56.8%) either agreed or strongly agreed with the statement “I would pay a premium for off-season fresh produce grown locally in greenhouses (or using other technologies) versus imported alternatives”. Only 17.3% said that they would be unwilling to pay any kind of premium for locally grown produce, although 39.6% were willing to pay a premium greater than 10%.

How much of a premium are you willing to pay for locally grown fresh produce? (n=220)

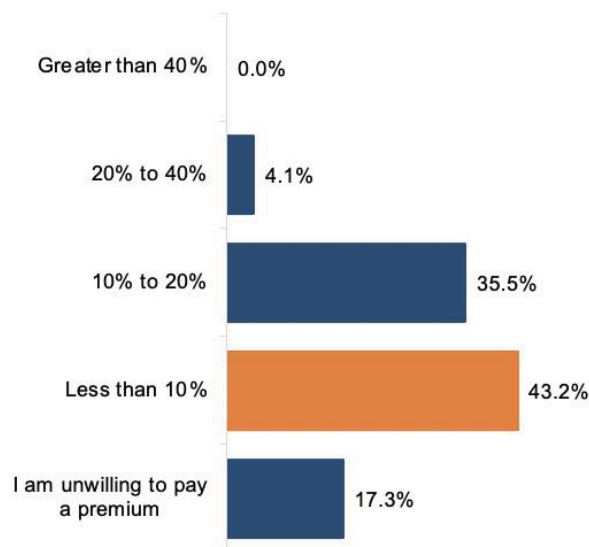


Figure 3. Graph showing how much of a premium New Brunswickers are willing to pay for fresh local produce

Of the respondents, 27.7% said that CEA grown crops were better compared to 9.1% who said they were worse, which is slightly more positive than the rest of Canada. However, 73.2% of respondents said that price was important when choosing fresh produce, and while 37.3% said that where it was produced was important, it still trailed behind taste to which 40.9% it was important.

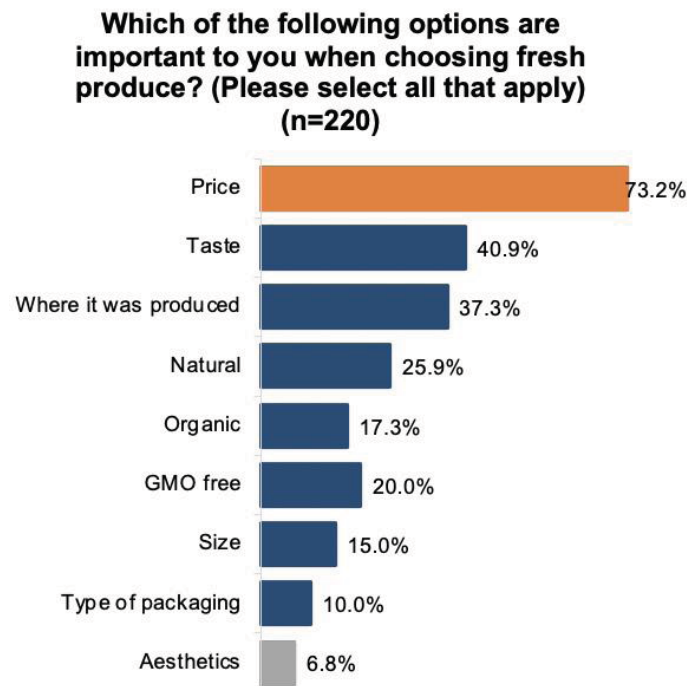


Figure 4. Graph showing what factors New Brunswickers consider important when choosing fresh produce

Compared to Canada as a whole, respondents from New Brunswick were even more likely to consider price important, though were also more likely to consider where something was grown.

2.3 Implications of Attitudes

Both Canadians as a whole and New Brunswickers in particular would be willing to pay extra money for locally grown produce over imported alternatives, and the premiums that they claim to be willing to pay are not insignificant. However, combined with the fact that the majority of respondents, both from New Brunswick and the rest of Canada, consider price to be an important factor when choosing groceries, this may not translate to people actually purchasing local produce. While many still consider where food was being grown to be important, it is only around half the amount that consider price important. This creates the paradox of people considering locally grown food to be more valuable but are neither looking for it nor necessarily willing to pay more for it. Though a significant percentage of respondents claim to be willing to pay more for greenhouse-grown produce in the off-season, this may not translate upon arriving at the grocery store with a strict budget. However, just because people consider price to be important that does not necessarily preclude them from ever paying more, they may still be willing to pay slightly more for certain things. Nevertheless, as most people are not considering where produce is grown as important, CEA grown crops do not have a major advantage when it comes to being grown locally. Instead, they are at an immediate disadvantage if they have a higher price tag, and consumers are not likely to be searching for food that is specifically locally grown.

3. Grocery Shopping Habits

In order to be viable, CEA grown crops need to reach consumers. This means being on the shelves where consumers are looking for them and overall availability to consumers. However, this also means dealing with regulations surrounding the food supply chain, such as additional fees charged to suppliers in an industry where margins are razor thin. In light of the pandemic, grocery store chains began building online infrastructure to serve their customers, and Loblaws, one of the major grocery store chains in Canada, has begun to pass the costs for this development onto their suppliers (see Appendix B). The fees charged to all of Loblaws suppliers are

increasing by 1.36% as of January 3, 2021. Walmart is reportedly imposing similar fees on suppliers as well. This will not just affect the prices of any CEA grown crops, but all food. Consumers will absorb any increases in cost, increasing how much they pay for groceries on a regular basis, which may decrease their WTP. Any CEA start-ups will also need to consider where Canadians are buying their produce and the surrounding fees to guarantee that they can afford the extra costs associated with specific stores while also making sure that they can get their produce to consumers. CEA may not be able to be sold in certain places, like farmer's markets, who may have rules about who can set up a stall.

3.1 Grocery Shopping Habits: Canada as a Whole

When surveyed about where they get the majority of their fresh produce, 74.9% of respondents said that they got their produce at major grocery store chains. This would include chains such as Loblaws, Walmart, and Sobeys. Of the respondents who shop at major grocery stores for their fruits and vegetables, 88.3% either agreed or strongly agreed with the statement "Fruits and veggies are an important part of my household's diet" and 68.7% of respondents who shop at major grocery stores considered price to be an important factor when choosing what produce to buy. The next most common place for Canadians to buy produce was at farmer's markets, where 10.8% did their shopping, followed by small independent stores (9.2%), people who grow their own produce (2.8%), and people who shop at convenience stores (2.3%).

3.2 Grocery Shopping Habits: New Brunswick

Compared to the rest of Canada, respondents from New Brunswick were less likely to shop at major grocery stores for their produce, with only 68.9% saying that they bought most of their fruits and vegetables there. Of those who shopped at major grocery stores, 88.3% either agreed or strongly agreed with the statement "Fruits and veggies are an important part of my household's diet" and 74.4% of those shopping at major grocery stores considered price to be important. The next most common place to buy produce was at small independent grocery stores, where 17.1% of respondents bought their produce, then farmer's markets (9.0%), people growing their own produce (3.2%), and then convenience stores (1.8%).

3.3 Implications of Grocery Shopping Habits

The vast majority of respondents said that they buy most of their produce at major grocery chains, meaning that any CEA suppliers will be subjected to any and all supplier fees charged by major grocery chains if they plan on reaching the majority of consumers. This potentially includes the 1.36% increase in supplier fees being charged by Loblaws and whatever fee increases that Walmart is imposing, which can impact profitability. While some smaller suppliers may be exempted from these fees, this is not a guarantee and the fact that suppliers need to play on major grocery chains' terms is incredibly important because most Canadians go to these stores and if CEA crops cannot be profitable in these stores because of the increased fees, then the majority of Canadians will not have access to the products, which would be detrimental to CEA's feasibility. Likewise, customers who go to major grocery chains are more likely on average to consider price to be an important factor when choosing what produce to buy. If CEA cannot compete on price with the rest of the products in the store and therefore is unprofitable for the store, the major grocery chains may stop working with the CEA suppliers.

New Brunswick has a larger percentage of respondents who go to independent grocery stores than Canadians as a whole, with 17.1% buying their produce there. While CEA suppliers may have better luck with smaller independent stores for negotiating fees, it is still less than one fifth of New Brunswickers, and not a very large market. They may be able to be profitable on those shelves, but the major grocery chains still have most of the consumers that CEA suppliers need to reach.

4. Produce and Premiums

Consumers who are willing to pay a premium for certain commodities may not be willing to pay more for other commodities. Certain crops are better suited to CEA than others, so it is important to assess what consumers do not want to pay a premium for, in case of a difference in price between CEA and conventionally grown crops. Consumers were asked what produce they were unwilling to pay a premium for and were able to respond with what they were unwilling to pay a premium for and write in their answer. As it was a respondent generated list, many of the produce types are not relevant to Canadian CEA concerns. Commonly grown CEA crops in Canada are bell peppers, cucumbers, tomatoes, and lettuce. Should consumers be unwilling to pay a potential premium on these commodities, CEA is immediately less viable because people will be more likely to flock to the cheaper options when presented with them.

4.1 Produce and Premiums: Canada as a Whole

Overall, 15.1% of respondents were least willing to pay a premium on strawberries, which was the highest result

for all the produce provided, followed by 14.2% who were least likely to pay a premium on bananas. As for CEA crops, 11.1% were least likely to pay a premium on lettuce while 4.5% were least likely to pay a premium on peppers. Numbers were not provided for cucumbers and tomatoes, likely because there were an extremely small number of respondents who answered with those vegetables.

4.2 Produce and Premiums: New Brunswick

Of respondents from New Brunswick, 20.3% were the least willing to pay a premium for strawberries, followed once again by bananas at 13.5%. Of the CEA crops we collected data on, 10.8% were least willing to pay extra for lettuce and 4.5% were least willing to pay a premium for peppers.

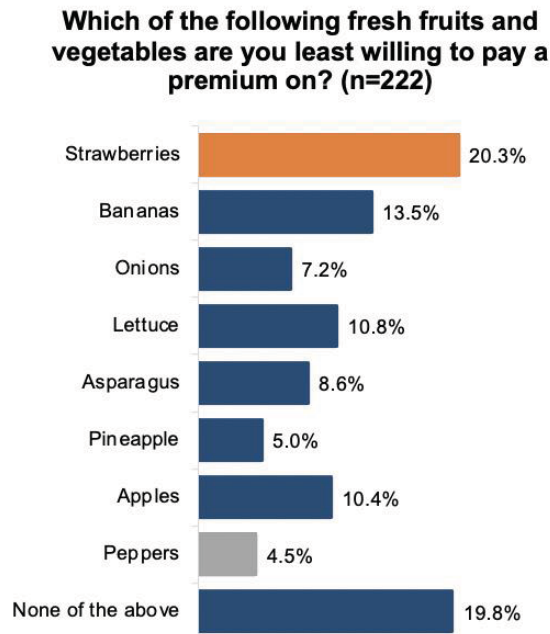


Figure 5. Graph showing what produce New Brunswickers are least willing to pay a premium for from a respondent generated list

4.3 Implications of Produce and Premiums

Fortunately for CEA suppliers, the majority of respondents were still willing to pay a premium on peppers. Respondents were less likely to be willing to pay a premium for lettuce, which is one of the more common greenhouse crops, which may pose a slight issue depending on what crops CEA producers choose to grow. Further market scans on how much of these products that consumers are buying would be valuable, to assess how much demand exists, but unfortunately there is little consumer data available for the grocery purchasing habits of Canadians.

5. Conclusion

The feasibility of CEA in New Brunswick will depend on a large variety of factors, some of which have no available data. However, from the information in the survey conducted, unless CEA crops are able to compete with conventionally grown and imported alternatives price-wise, it could face many issues in New Brunswick and Canada as a whole in light of the economic uncertainties surrounding COVID-19. Canadians were also surveyed specifically about paying a premium for food that they considered local, not necessarily Canada as a whole, and many of the larger regions in Canada, such as Ontario and Quebec, consider food grown within their region as local – a definition which would not include New Brunswick. The food security that CEA could provide would be beneficial not only in the province of New Brunswick but in other regions where similar market conditions can be found. It would be useful in preventing any uncertainties that come from wildfires, closing borders, droughts, and floods, but these solutions cannot only be made for short-term protections, they must be economically viable even in times when they are not needed, of which the biggest concern is price. Before making a decision on feasibility, the overall premium that would be charged to consumers for CEA crops and whether or not CEA suppliers can afford to make it into major grocery stores must be determined in light of consumer perceptions, WTP, and supplier profit margins.

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Enhancing the Nutritional Quality of Vegetable Amaranth through Specific Food Preparation Methods

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Abstract

Food preparation methods applied to African traditional vegetables vary greatly depending on preferences of various consumers. Vegetable amaranth is one of the most preferred vegetable, with high nutritional quality. The bioaccessibility of some minerals such as iron is, however, low since it is non-heme, and is also bound by anti-nutrients such as oxalates. This study aimed at evaluating the nutrient retention of amaranth vegetable dishes prepared using selected Kenyan traditional recipes, and to enhance the iron bioavailability of amaranth dishes using food preparation methods. Nutrient retentions of amaranth prepared by three common food methods were analyzed. In-vitro iron bioavailability of amaranth dishes with or without bioavailability enhancers as well as an amaranth meal incorporating a common maize meal staple food was also studied. The nutrient retentions of the various dishes used in this study was fairly high with at least 85% retention of minerals and an increase of up to 45% in three carotenoids. It can be concluded that incorporating vitamin C, adding an iron rich vegetable and boiling of the vegetable significantly improves the iron bioavailability and hence improves the iron uptake by the body. Incorporating lemon juice enhanced dialysable iron of the selected recipe by up to 66%. There was no significant ($P \leq 0.05$) effect by the amaranth components on the iron bioavailability of ugali. These methods could therefore be incorporated into household recipes to increase micronutrient intake.

Keywords: micronutrient malnutrition, bioaccessibility, anti-nutrients, ingredients

1. Introduction

The genus *Amaranthus* consists of many species, which are often considered as pseudo-cereals in Europe and America, but are mostly grown as vegetables in Africa (Achigan-Dako et al., 2014). It is considered as one of the African indigenous vegetables. The edible parts of the plants range from seeds, leaves and tender shoots. It is one of the most commonly consumed African indigenous vegetables in Kenya, East Africa and other parts of Africa (Kansiime et al., 2018). It is a cheap source of micronutrients that can contribute to reduced cases of micronutrient malnutrition. Apart from being a rich source of most micronutrients, amaranth is also a source of phytochemicals that are useful to the human body. In recent years, its production has risen from that of a subsistence crop, to a commercial crop, finding itself on the shelves of most supermarkets in urban areas. In some case, supply cannot match demand (Cernansky, 2015). Its tender leaves and stem are used in many countries in Africa in the form of infusions, salads, sauces, soups; singly or mixed with other vegetables (Achigan-Dako et al., 2014).

This vegetable is, however, not consumed raw. It goes through food preparation methods which vary among consumers based on convenience and taste preferences rather than nutrient retention (Hossain et al., 2017). Preparation of these vegetables is done in different ways, according to the traditional recipes and culinary traditions of different communities (Musotsi, 2017). The general preparation process for African Indigenous

vegetables involve sorting, destalking, washing and sometimes cutting (Musotsi et al., 2017), followed by varied cooking durations with unclear effects on the nutritional quality. These cooking methods induce a series of changes in physical properties, chemical properties and enzymatic modifications in various foods (Rothwell et al., 2015), affecting concentration and bioavailability of nutrients. Findings by different researchers on phytochemical and biological changes during cooking have been inconsistent and sometimes contradictory (Zhao et al., 2019). In order to maximize the nutritional benefits from amaranth vegetables, it is important to subject them to a cooking method that results in optimal nutrient retention and bioavailability (Habwe, 2012).

Despite the notable increase in consumption of indigenous vegetables including amaranth, micronutrient malnutrition still remains a public health problem in several parts of Africa, the most affected being children and women of child bearing age. In Kenya, 27.2% of women in reproductive age have anemia (Global Nutrition Report, 2019). In some areas of Kenya 76% of children have been reported to have been anemic at least at one point since birth (Kao et al., 2019). This hidden hunger could be linked to the high rates of morbidity and mortality among children and women especially in rural areas.

Though the common approach for combating iron deficiency anemia comprise supplementation and food fortification, diet modification and proper food preparation methods would be a cheaper and more sustainable approach. Modification of the diet may be done to improve nutritional value of common dishes and iron bioavailability. These may be through providing dietary information to include iron rich foods and improve cooking skills (Lion et al., 2018). Amaranth vegetable as a good source of dietary iron, can contribute to addressing the challenge of iron deficiency anemia. However, the bioavailability of the iron in amaranth is low due to its non-heme nature, as well as the presence of anti-nutrients such as oxalates which bind iron. This would result decreased absorption of most of the iron contained in the vegetable in the upper gut. Bioavailability of iron from a food source in the body is usually influenced by enhancers and inhibitors (Kapil, 2017). It has been noted that iron absorption in the upper gut can range from 1% to 40%, and this can be doubled with certain cooking practices or by changing the composition of the meal such as by addition of other vegetables and fruits containing ascorbic acid (Kapil, 2017).

The aim of this study was to evaluate the nutrient retention of amaranth vegetable dishes prepared using some traditional recipes, and also to enhance the iron bioavailability of amaranth dishes using food preparation methods.

2. Materials and Methods

2.1 Experimental Design and Treatments

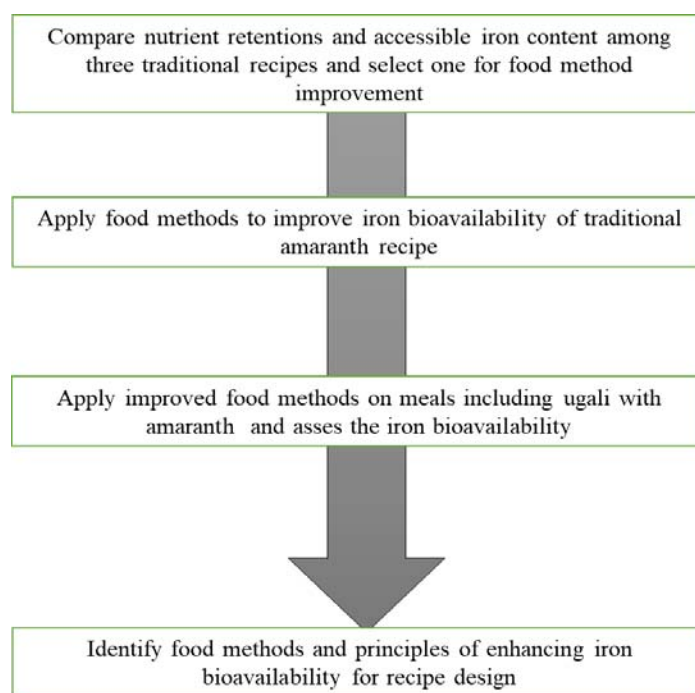


Figure 1. Summary of the study flow

The study was carried out sequentially in three stages, involving different treatments at each stage. In the first stage, three traditional cooking methods were selected based on literature (Faber et al., 2010; Musotsi et al., 2005; Musotsi et al., 2017; Oluoch et al., 2012). Freshly harvested leaves were prepared using three (3) traditional recipes and fresh leaves (uncooked) as well as a mixture of the common ingredients used as control. The effect of cooking on retention of vitamins, minerals and oxalates, as well as the effect of cooking on iron bioavailability was determined. Thereafter, one of the selected recipes was chosen for improvement through use of iron bioavailability enhancers including high ascorbic acid ingredients (Blanco-Rojo & Vaquero, 2019). The effect of boiling as well as addition of iron rich vegetables to amaranth iron bioavailability was also studied. At the third stage, the iron bioavailability of the traditional and improved cooking methods was analyzed in combination with a common staple, maize meal “*ugali*” (thick porridge).

2.2 Experimental Material

This study was carried out using three varieties of vegetable amaranth; Madiira 1 (*A. cruentus*), Madiira 2 (*A. cruentus*) and AH-TL-Sel (*A. hypochondriacus*). These are improved varieties that were developed by World Vegetable Centre and released in Kenya and Tanzania. The vegetables were grown in the open field in Shanhua, Taiwan. Planting was done uniformly in trays and later transplanted into the open field after three weeks. Later the leaves were harvested uniformly at six weeks after planting, just before flowering. The edible parts (leaves and tender stems) were then separated and the vegetables from the three varieties mixed in the ratio 1:1:1. Other ingredients used in recipe preparation including onions, tomatoes, lemons and soybean oil were obtained from local supermarket in Shanhua, Taiwan. Moringa leaves were obtained from the World Vegetable Center in Taiwan, while maize flower was obtained from Kenya.

2.3 Chemicals

All reagents used were of analytical reagent (AR) grades. They included pepsin (P-7000, from porcine stomach mucosa), hydrochloric acid, pancreatin (P-1750, from porcine pancreas), bile extract (B-8631, porcine), Sodium hydrogen carbonate, sodium hydroxide, trichloroacetic acid (TCA), 4,7-diphenyl-1,10-phenanthroline disulfonic acid, hydroxylammoniumchloride, sodium acetate, H₂SO₄, standard oxalic acid, 2,4-dinitrophenylhydrazine (DNPH), 2,6-dichlorophenolindophenol (DCPIP), metaphosphoric acid thiourea standard L-(+)-ascorbic acid, Acetone, ethyl ether, methanol, potassium hydroxide, hexane, Tetrahydrofuran (THF). The chemicals were purchased from Merck and Sigma–Aldrich Co. (St. Louis, MO, USA).

2.4 Preparation of Samples

The fresh vegetables were destalked to separate edible portions comprising leaves and tender stems. All the vegetables used in the different experiments were washed with water then rinsed with distilled water. Onions and tomatoes were chopped into small pieces which were used for frying the vegetables. All the cooking was done in stainless steel pans over uniform heat on an electric cooker. The samples in the study are shown in Table 1.

Table 1. Samples used in the study

| Sample | Description/ Preparation process |
|--|---|
| Experiment 1: Nutrient retention and iron bioavailability among recipes | |
| Amaranth Raw | These were freshly harvested amaranth leaves which were used as control reference in the study |
| Mixed ingredients | This was the uncooked control consisting of a mixture of amaranth leaves, onions, tomatoes, and oil mixed together but with no cooking. The ratios of ingredients were the same as that used for the cooked samples. The mixture was blended to ensure homogeneity |
| Recipe 1 | Approximately 200 g of fresh whole leaves (not chopped) were placed in 200 g of boiling distilled water in a cooking pan. This was covered and let to boil for 10 minutes, stirring every 3 minutes. The boiled vegetables were then stir-fried without straining in 10 g non-fortified soybean oil, 25 g of chopped red bulb onions and 25 g chopped tomatoes for five minutes. |
| Recipe 2 | Approximately 200 g of fresh vegetables were chopped to medium size (about 1cm wide) and then placed in 200 g of boiling distilled water in a cooking pan. This was covered and let to boil for 10 minutes, stirring every 3 minutes. The boiled vegetables were then stir-fried without straining in 10g non-fortified soybean oil, 25 g of chopped red bulb onions and 25 g chopped tomatoes for five minutes |
| Recipe 3 | Approximately 200 g of fresh vegetables were chopped to medium size (about 1cm wide) and stir-fried in 10 g non-fortified soybean oil, 25 g of chopped red bulb onions and 25 g chopped tomatoes for five minutes. This was without boiling. |
| Experiment 2: Enhancing iron dialysability | |
| Improved Recipe 1 with more tomato | This was prepared as the Recipe 1, with double the quantity of tomatoes (50 g of chopped tomatoes). Components of tomatoes including vitamin C and lycopene have been reported to have positive effects on iron bioaccessibility (Garcia-Casal, 2006; Singh et al., 2016). |
| Improved Recipe 1 with Lemon Juice | This was prepared as Recipe 1, with one tablespoon (10 mL) of freshly squeezed lemon juice added. Lemon juice has been shown to increase iron bioavailability in foods (Singh et al., 2016), and this is attributed to its ascorbic acid as well as other components |
| Samples for boiling effect experiment | Two samples of 100 g fresh amaranth leaves were boiled in 200 mL distilled water for five minutes. After boiling, excess water was discarded in one sample. This was also done with another set of samples, with a boiling time of 15 minutes. Fresh amaranth leaves were used as control. |
| Samples for inclusion of moringa | Three different samples of 100 g of amaranth, 100 g of fresh moringa leaves, and 100 g of a mixture of amaranth leaves and moringa leaves (50:50); were boiled separately in 200 mL distilled water for 5 minutes. The remaining water was not discarded. Dialysable iron was determined in these samples together with fresh amaranth and fresh moringa leaves to determine the effect of adding moringa, a high iron vegetable, on dialysable iron of an amaranth dish. |
| Experiment 3: dializability of iron in amaranth meal combination | |
| Amaranth meals | Recipe 1 (Traditional recipe) and improved Recipe 1 with lemon were prepared. "Ugali" (thick porridge) was prepared using iron fortified maize flour from Kenya (Jogoo Maize Flour). Dialysable iron was determined for the two recipes, ugali, and a combination of ugali with each of the dishes in a ratio of 1:1. |

2.5 Nutrient Retention Analysis

Determination was done for the nutritional components of the amaranth recipes as well as a mixture of all the ingredients (uncooked), and this was compared with the fresh amaranth sample. Components analyzed included oxalates, vitamin C, minerals (Ca, Fe and Zn) and carotenoids (violaxanthin, lutein, α -carotene and β -carotene)

2.5.1 Determination of Oxalates

Determination of oxalates was done by HPLC (Libert, 1981) with modifications suggested by Yu *et al.*, (Yu et al., 2002). A 0.5 g fresh weight of sample was homogenized in 4 mL of 0.5N HCL. The homogenate was heated at

80⁰ C for 10 minutes with intermittent shaking. To the homogenate, distilled water was added up to a volume of 25 mL. About 3 mL of the solution was withdrawn and centrifuged at 12000 rpm for 10 minutes. About 1 ml of supernatant was passed through a micro filter (0.45 μ) before HPLC analysis. Standards were prepared at varying concentrations for quantification. HPLC analysis was done using Shimadzu UV-VIS detector, Hypsil C18 column (5 μ M, 4.6 mm *250 mm) equipped waters 550 was used as the static phase and the mobile phase was a solution 0.01 N H₂SO₄. Flow rate was 0.6 mL min⁻¹, pressure of 62 kgf and detection wavelength of 221 nm.

2.5.2 Determination of Vitamin C

The vitamin C content was determined based on coupling 2,4-dinitrophenylhydrazine (DNPH) with ketonic groups of dehydroascorbic acid through the oxidation of ascorbic acid by 2,6-dichlorophenolindophenol (DCPIP), (Hanson et al., 2004). About 20 grams of blended samples were homogenized with 80 mL of 5% metaphosphoric acid and centrifuged at 7000 rpm for 10 minutes. Two mL of the supernatant was transferred into 20 mL test tube followed by addition of 0.1 mL of 0.2% 2,6-DCPIP sodium salt in water, 2 mL of 2% thiourea in 5% metaphosphoric acid and 1 mL of 4% 2,4-DNPH in 9 N H₂SO₄. The mixtures were kept in water bath at 37°C for 3 h followed by an ice bath for 10 min. to the mixtures, 5 mL of 85% sulphuric acid was added and kept at room temperature for 30 min before reading at OD 520 nm. Commercial L-(+)-ascorbic acid was used for calibration.

2.5.3 Determination of Carotenoids

Determination of carotenoids was done by HPLC (Rodriguez, 2001). About 0.1 g of freeze-dried sample was weighed into a vial. 0.6 mL distilled water and 4.4 mL Acetone was then added, mixed and shaken for 30 min. The mixture was centrifuged and 2.0 mL of supernatant transferred to 10 mL test tube. Nitrogen gas was then used to dry the samples at 36°C. Saponification was done by adding 100 μ L ethyl ether followed by 2.0 mL methanol and 1.0 mL 15% KOH in methanol. The ethyl ether was evaporated using N₂ gas and the samples incubated at 30°C while shaking for 2 h. 3 mL of hexane and water (1.5 mL water + 1.5 mL hexane) was added and mixed by shaking for 1 min. The hexane layer was transferred to 60 mL separating funnel, collected 3 times and washed with water (4 times) then dried under nitrogen gas. To the dried sample, 100 μ L of Tetrahydrofuran (THF) and 1900 μ L Methanol (Merck LC grade) were added and mixed. The solution was then filtered through a 0.22 μ m membrane and 20 μ L injected into HPLC for analysis. Separation and identification of carotenoids was performed using HPLC system (Waters 2695, Milford, MA, USA) equipped with an auto-sampler, a photodiode array detector (Waters 996) monitoring at wavelength between 210 - 700 nm. The static phase was a C 30 Column (YMCTM Carotenoid 3.0 μ m, 4.6 mm \times 150 mm). The running conditions were set at 30°C using a gradient at 1.3 mL/min from 0% to 1% THF in methanol at 0 - 15 min, 1% to 25% THF in methanol at 15 - 25 min, 25% to 70% THF in methanol at 25 - 50 min, and the final 100% THF at 50 - 60 min. Identification of sample carotenoids was performed by comparing retention time and light absorption spectra (350 nm - 700 nm) of known standards. The peak areas were calibrated against known amounts of standards.

2.5.4 Determination of Minerals

Minerals were determined by strong acid digestion method and atomic absorption spectrophotometer (AAS) (AOAC, 2016). The minerals that were determined are calcium, iron and zinc. About 0.3 g of freeze-dried sample was mixed with 5 mL of 36N H₂SO₄ in a digestion flask. The flasks were placed in a digester and heated at 300°C for 2-3 h. The contents were then cooled to about 150°C and 2 mL of 30% hydrogen peroxide (H₂O₂) added. The tubes were placed in the digester at 300°C for further 30 min till the mixture was transparent. The mixture was then cooled to about 40°C and diluted with 50 mL distilled water. The absorbance of the solutions was read by Atomic Absorption Spectrophotometer (AAS) at their respective wavelengths. The various mineral standards were also prepared to make the calibration curve.

2.6 Analysis of Iron Bioaccessibility

2.6.1 In-vitro Iron Dialysability Assay

Dialysability of iron was determined using in vitro dialysability method (Luten et al., 1996) with simulated peptic and pancreatic digestion. A pepsin solution was prepared by dissolving 16 g of pepsin (P-7000, from porcine stomach mucosa) in 100 mL of 0.1 M HCl. The pancreatin solution contained 4 g of pancreatin (P-1750, from porcine pancreas) and 25 g of bile extract (B-8631, porcine) with 1000 mL of 0.1 M NaHCO₃. The sample dry matter content was adjusted to 5 using distilled water, then homogenized. The pH of homogenized sample was adjusted to pH 2.0 with 6 M HCl, then 20 g was weighed into 125 mL conical flasks in three replications. Distilled water was also weighed in the same manner to act as blank.

Peptic digestion- To 20 g of weighed samples, 0.75 mL pepsin solution was added. The mixture was covered well using parafilm and incubated at 37°C for 2 h with shaking.

Titrateable acidity- To one replication of each digested sample, a titration was performed in which 20 mL of gastric digest was mixed with 5 mL of pancreatin-bile suspension and the amount of 0.5 M NaOH needed for this mixture to achieve a pH of 7 ± 0.05 was determined.

Pancreatic digestion- Segment of dialysis tubing (6-8 cm) was soaked in distilled water for about 30 minutes. A solution of 0.5 M NaHCO₃, being equivalent to the volume of 0.5 M NaOH needed for the pancreatic digestion (titrateable acidity), was made up to 25 mL with distilled water. These solutions were transferred into the dialysis tubes, tied on both sides, then placed into the conical flask containing gastric digest and incubated for 30 minutes at 37°C. After 30 minutes, 5 mL of pancreatin-bile mixture was added and the incubation continued for another 2 hours. The dialysis tubes were then removed, washed in distilled water and the contents weighed. To 5 mL of dialysate, 2.5 mL of protein precipitant containing 10% TCA and 10% HCl in distilled water was added, heated in boiling water bath for 10 minutes and centrifuged at 10000 rpm for 5 minutes.

To determine the dialysable iron, 3 mL of the supernatant was reacted with 2 mL of Bathophenanthroline reagent, containing 0.025% of 4,7-diphenyl-1,10-phenanthroline disulfonic acid and 10% hydroxylammoniumchloride in 2 M sodium acetate. The mixture was let to stand for 15 minutes before reading at OD 535 nm. Iron standard was used for calibration.

2.7 Statistical Analysis

The data collected was subjected to Analysis of Variance (ANOVA) using Genstat statistical software. Separation of means for the various treatments was done using Duncan's Multiple Range Test (DMRT).

3. Results

3.1 Nutrient and Oxalate Retention of the Amaranth Dishes

The nutrient contents of the various recipes were calculated on dry weight basis. The change in nutrient content was expressed as percentage of the mixed ingredients in raw form.

Table 2. Amounts of oxalates and Vitamin C in the recipes per 100 g DW

| SAMPLE | OXALATE (mg) | VITAMIN C (mg) |
|--------------------------|-------------------|---------------------|
| Amaranth raw | 2943 ^c | 524.20 ^c |
| Mixed Ingredients | 1720 ^b | 244.93 ^b |
| Recipe 1 | 1592 ^a | 146.45 ^a |
| Recipe 2 | 1605 ^a | 172.98 ^a |
| Recipe 3 | 1677 ^b | 173.99 ^a |
| LSD | 60.68 | 46.11 |

Values are presented as Mean, $n = 3$. Means within the same column with different superscripts were significantly ($P \leq 0.05$) different. LSD= Least Significant difference at 5% level of significance

There were significant ($P \leq 0.05$) differences in the oxalate contents of the various samples. The level of oxalates was lower in the cooked samples compared to the raw samples, with the lowest amounts detected in Recipe 1 (Table 2). Compared to the uncooked mixed ingredient recipe, cooking reduced the oxalate content. Recipe 3, where there was no boiling, retained the highest oxalate content.

There was a reduction in vitamin C content in all the recipes. The amounts in the three recipes were, however, not significantly ($P \leq 0.05$) different. The vitamin C in the mixed ingredients was much lower than in fresh amaranth. This may be due to the loss of the vitamin through oxidation.

Table 3. Carotenoids content in the recipes in 100 g DW

| SAMPLE | VIOLAXANTHIN (mg) | LUTEIN (mg) | α -CAROTENE (mg) | β -CAROTENE (mg) |
|--------------------------|-------------------|--------------------|-------------------------|------------------------|
| Amaranth raw | 8.37 ^c | 47.64 ^c | 2.39 ^a | 22.65 ^c |
| Mixed Ingredients | 4.06 ^b | 23.52 ^a | 1.93 ^a | 12.65 ^a |
| Recipe 1 | 0.00 ^a | 33.51 ^b | 2.35 ^a | 18.20 ^b |
| Recipe 2 | 0.00 ^a | 34.29 ^b | 2.38 ^a | 18.13 ^b |
| Recipe 3 | 3.30 ^b | 31.75 ^b | 2.60 ^a | 16.77 ^b |
| LSD | 0.93 | 3.96 | 0.62 | 2.03 |

Values are presented as Mean, $n = 3$. Means within the same column with different superscripts were significantly ($P \leq 0.05$) different. LSD= Least Significant difference at 5% level of significance

Four carotenoids were detected in the samples including violaxanthin, lutein, alpha carotene and beta carotene. The different carotenoids were affected differently by the different cooking methods. Violaxanthin was completely destroyed in the recipes that involved boiling, while the contents of the other carotenoids increased in the three recipes when compared to the mixed ingredients in raw form.

Table 4. Mineral contents of recipes in 100 g DW

| SAMPLE | CALCIUM (mg) | IRON (mg) | ZINC (mg) |
|--------------------------|-----------------------|--------------------|-------------------|
| Amaranth raw | 2403.09 ^c | 30.15 ^b | 4.42 ^b |
| Mixed Ingredients | 1218.69 ^a | 14.72 ^a | 2.36 ^a |
| Recipe 1 | 1322.81 ^{ab} | 13.82 ^a | 2.01 ^a |
| Recipe 2 | 1418.10 ^b | 12.59 ^a | 2.12 ^a |
| Recipe 3 | 1309.35 ^{ab} | 13.91 ^a | 2.30 ^a |
| LSD | 141.10 | 4.00 | 0.43 |

Values are presented as Mean, $n = 3$. Means within the same column with different superscripts were significantly ($P \leq 0.05$) different. LSD= Least Significant difference at 5% level of significance

There were no significant ($P \leq 0.05$) differences the amounts of the various minerals among the three cooked recipes. The calcium content was slightly higher in all the recipes compared to the uncooked mixed ingredients, while iron and zinc contents were lower. However, these differences were not significant. Recipe 3 showed the lowest losses of iron and zinc.

3.2 Iron Bioaccessibility in the Recipes

The bioaccessible iron was calculated as the dialysable iron percentage of the total iron in each sample.

$$\% \text{ Bioaccessibility} = \frac{\text{Dialyzable iron}}{\text{Total iron}} \times 100\% \quad (1)$$

Table 5. Percentage Iron Bioaccessibility of the dishes

| SAMPLE | % Bioaccessibility |
|--------------------------|---------------------|
| Amaranth raw | 11.02 ^a |
| Mixed Ingredients | 17.53 ^b |
| Recipe 1 | 21.92 ^{bc} |
| Recipe 2 | 24.22 ^c |
| Recipe 3 | 21.59 ^{bc} |
| LSD | 5.89 |

Values are presented as Mean, $n = 3$. Means within the same column with different superscripts were significantly ($P \leq 0.05$) different. LSD= Least Significant difference at 5% level of significance

The three preparation methods/recipes had higher iron dialysability compared to the raw sample. There were however no significant differences in iron dialysability among the dishes (Table 5).

3.3 Enhancement of Dialysable Iron in Amaranth

To improve dialysable iron and improve iron delivery from amaranth meals, further experiments were carried out to identify effects of preparation methods and some ingredients in enhancing iron dialysability in amaranth dishes. The methods that were considered for enhancement in this study were inclusion of high ascorbic acid ingredients as ascorbic acid enhances iron bioavailability; boiling (Blanco-Rojo & Vaquero, 2019; Nomkong et al., 2019; Singh et al., 2016); and cooking the amaranth in combination with high iron vegetables as a way of increasing the total iron content.

The first experiment involved the addition of lemon juice, which is a source of vitamin C into the dish. Recipe 1 from the first experiment was chosen for this improvement. One recipe was made by doubling the amount of tomatoes used in the Recipe 1 (Recipe 1+2X tomato), while another recipe was made by adding 10 mL fresh lemon juice to the Recipe 1 (Recipe 1 + lemon juice), and comparing against the original Recipe 1.

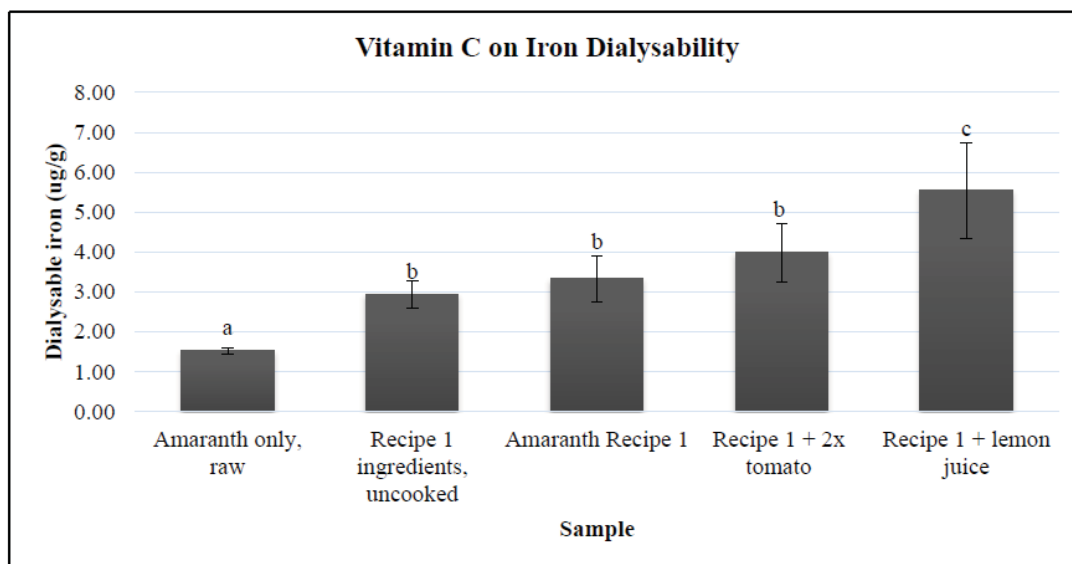


Figure 2. Effect of addition of Vitamin C rich ingredients on dialysable Iron of Amaranth recipes Values are presented as Mean, $n = 3$. Means with different superscripts were significantly ($P \leq 0.05$) different.

The addition of lemon juice, a source of vitamin C had a positive effect on iron dialysability (Figure 2). Increase in the dialysable iron due to doubling the tomato content was, however, not statistically significant ($P \leq 0.05$) compared to the control recipe (Recipe 1). Adding lemon juice significantly ($P \leq 0.05$) enhanced dialysable iron of the traditional recipe (Recipe 1), compared to the raw amaranth. Lemon juice enhanced the dialysable iron by over 200% in comparison to the raw amaranth leaves, while the increase was 66% higher than the traditional recipe.

The effect of boiling on the iron dializability of amaranth was then determined. Studies on other vegetables have shown that boiling can enhance bioavailability of iron (Nomkong et al., 2019). While boiling led to an increase in dialysable iron, discarding excess water reduced the dialysable iron content. This could be attributed to the leaching of iron in the discarded water. Longer boiling (15 minutes) times increased dialysable iron compared to shorter boiling time (5 minutes).

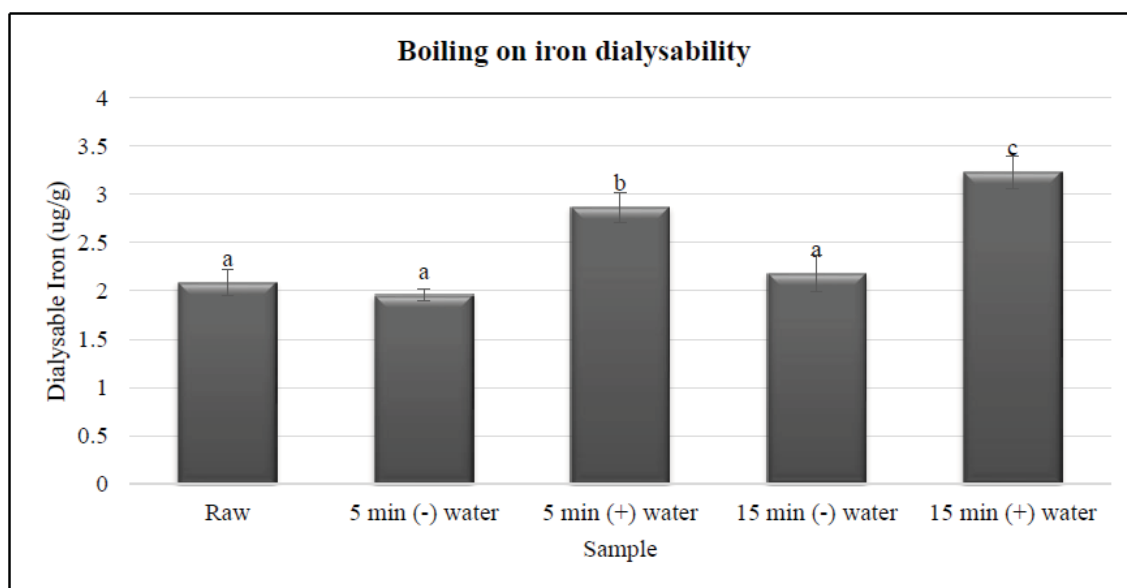


Figure 3. Effect of boiling and removal of excess water on iron dialysability of Amaranth Values are presented as Mean, $n = 3$. Means with different superscripts were significantly ($P \leq 0.05$) different.

Addition of moringa leaves also increased the quantity of dialysable iron of the amaranth. This could be partly because moringa has higher dialysable iron, and also because it contains higher amounts of vitamin C, which is an enhancer (Gopalakrishnan et al., 2016; Shija et al., 2019).

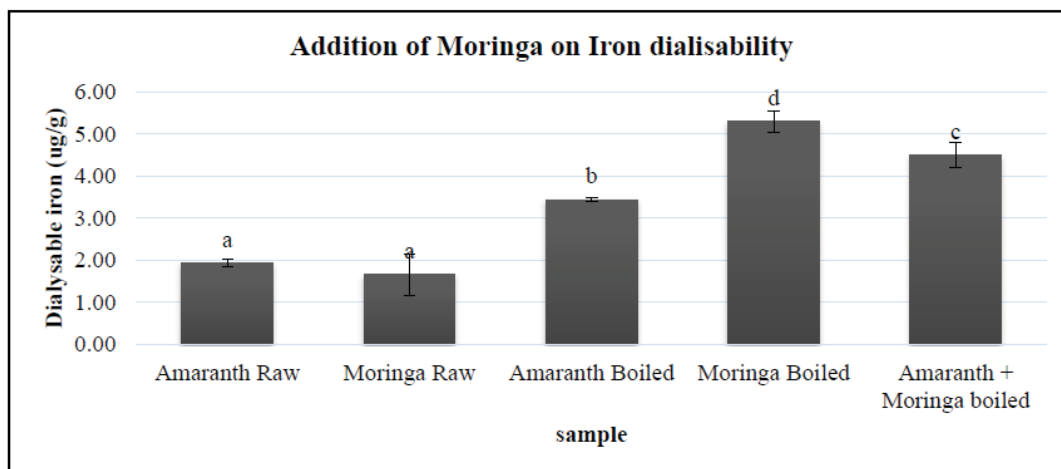


Figure 4. Effect of including Moringa leaves on iron dializability of Amaranth dish

Values are presented as Mean, $n = 3$. Means with different superscripts were significantly ($P \leq 0.05$) different.

The quantity of dialysable iron when the traditional recipe (Recipe 1) and improved recipe (Recipe 1 one with lemon juice) is combined with a common staple, Maize meal/Thick porridge (“ugali”) was then determined to evaluate if the biochemical components of the cooked amaranth had any effect on the dialysable iron of the maize meal, which was iron fortified.

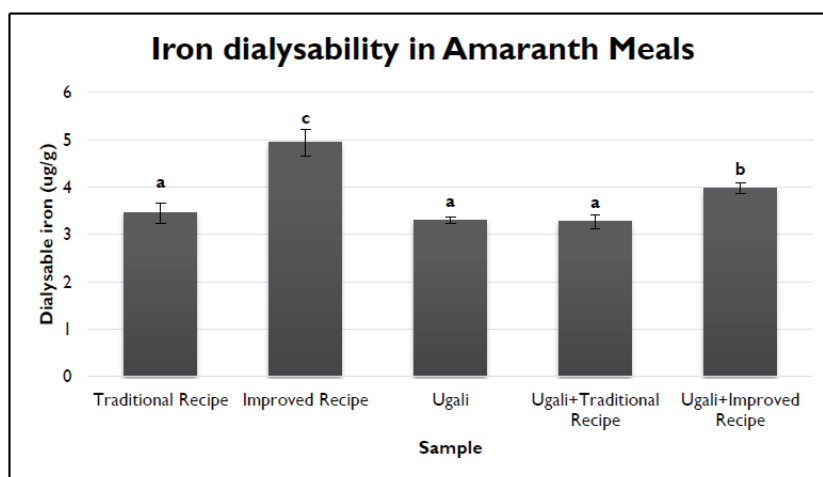


Figure 5. Iron dialyzability in traditional recipe and improved recipe in combination with maize meal

Values are presented as Mean, $n = 3$. Means with different superscripts were significantly ($P \leq 0.05$) different.

The improved recipe with lemon juice was used for this study in comparison with Recipe 1, and each was mixed with “ugali”. Meal combination with improved recipe had significantly higher dialysable iron, at 21% more than the meal combination with the traditional recipe.

4. Discussion

African traditional vegetables form an important part of most diets in rural parts of Africa. Low consumption of micronutrient rich foods including green leafy vegetables has been reported to be associated with the incidence of mineral deficiencies. The minerals whose deficiency is of utmost public health concerns in East Africa are iron (Fe), calcium (Ca) and zinc (Zn) (P. Singh & Prasad, 2018). Despite the content of micronutrients in these

vegetables, the preparation methods can greatly affect their bioavailability in the human body. Some anti-nutrients present in these vegetables such as oxalates may also bind to minerals hence reducing their bioavailability.

Oxalate, a major anti-nutrient in amaranth vegetables is not known to be heat sensitive; and its main reduction strategy could be by boiling and discarding of boiling water (Akhtar et al., 2011). This method can however, be counter-productive as most minerals and vitamins are also lost in the process. Recipe 1 and Recipe 2 in this study, both of which involved boiling, showed statistically significant reduction in the oxalate content (Table 2). The amount of oxalate in these two recipes were not significantly different, showing that there was no effect of chopping on the oxalate contents. The main effect of concern from oxalate is the interference with bioaccessibility of minerals including iron and calcium in the body. Even though many people can induct normal amounts of oxalate rich foods, people with certain conditions such as enteric hyperoxaluria need to lower their oxalate intake (Popova & Mihaylova, 2019). In sensitive people, even small amounts of oxalate can cause burning in the eyes, ears, mouth and throat; while large amounts may cause abdominal pain, muscle weakness, nausea and diarrhea (Natash et al., 2017).

During cooking, some nutritional components are reduced, which are mainly the vitamin components. For instance, a study conducted by Traore *et.al.*, showed that boiling amaranth for 30 minutes completely destroyed the beta carotene in the vegetables (Traoré et al., 2017). All the recipes used in this study led to significant changes in the nutritional attributes of the dishes.

In this study, combination of size reduction or lack of it (chopping) with or without boiling (Table 2 and 3) retained statistically similar content of vitamin C, Iron and Zinc. This would have been due to the effect of the mixed ingredients which are known to contain these nutrients

Vitamin C was significantly reduced in the recipes compared to the raw ingredients by up to 40%. The retention of vitamin C in recipes of this study was, however, much higher than those reported in other indigenous vegetable dishes such as 70% and 93% in African nightshade and spider plant, respectively (Musotsi et al., 2019). Vitamin C for example is easily lost during cooking since it is water soluble and temperature sensitive so it is easily degraded during cooking. Vitamin C cannot be synthesized by the body and therefore must be obtained from the diet (Singh & Prasad, 2018), vegetables forming part of the best sources. The vitamin is a cofactor in numerous physiological reactions such as collagen gene expression, peptide hormone activation, carnitine synthesis, and it is also an effective antioxidant (Lee et al., 2018).

Carotenoids are essential in the human diet owing to their nutrition and health benefits. Apart from being a good source of pro-vitamin A, they also possess antioxidant activity, which helps lower the risks of long-term degenerative diseases. Their bioavailability can however be modulated using dietary factors such as mechanical disintegration, enzymatic maceration of matrix compounds, the addition of lipids, and thermal treatments (Schweiggert & Carle, 2017). In this study, the amounts of carotenoids were much lower in the recipes compared to that in the fresh amaranth sample. However, compared to the mixed ingredients in raw form, three carotenoids, lutein, α -carotene and β -carotene, were found to be enhanced by cooking, while violaxanthin, was not detected in recipes that included boiling. There was no significant ($P \leq 0.05$) difference in the carotenoid contents between the chopped and un-chopped samples. Cooking of vegetables has been reported to promote breakdown of the cellulose structures of the plant cell thereby releasing carotenoids. It also denatures carotenoid-protein complexes hence improving the extractability of the carotenoids in the cooked food (Miglio et al., 2008). It is also assumed that this enhanced extractability is associated with increased bioavailability of these compounds (Lee et al., 2018). Addition of lipids has also been shown to have a positive effect on carotenoid bioavailability (Schweiggert & Carle, 2017). The use of oil in preparing the recipes in this study could have contributed to the release of more carotenoids from the matrix. Cooking was shown to enhance the carotenoid content in amaranth vegetables. There are also studies that show negative effect of cooking on carotenoids. The conflicting results could be due to differences in the starting materials as well as methodologies (Cilla et al., 2018), such as differences in the cooking time.

Minerals in vegetables such as calcium, iron and zinc are quite stable and not affected much by cooking. The iron and zinc contents of the three recipes in this study were not significantly different from that of the uncooked ingredients. There was, however, some increase in calcium content compared to the raw ingredients especially in Recipe 2 (Table 4). This increase can be attributed to release of complexes which are normally formed between calcium and other compounds such as oxalates. Similar results of slight increase in calcium contents on various species of amaranth and other leafy vegetables have also been reported (Amalraj & Pius, 2014).

In-vitro assays have been used to estimate the bioaccessible nutrients from different food products. These assays

mimic the digestive system, with the various enzymes as well as the conditions of the digestive tract controlled. Dialysis tubing is used to determine the amount of iron released from the food matrix, hence recorded as the dialysable iron.

In most foods especially those of plant origin such as amaranth vegetables, the iron concentration may not indicate its bioaccessibility (Amagloh et al., 2017). In this study (Table 4), the iron content of the raw amaranth was much higher than that in the recipes. However, the percent dialysable iron of the recipes was, however, higher than that of the raw amaranth (Table 5). One of the factors that can affect the bioaccessibility of this vegetable iron is their non-heme nature. The non-heme iron mostly exists in complexes, which can be degraded in the gastrointestinal tract during digestion owing to the action of pepsin and hydrochloric acid. Once released from food components, most non-heme iron is present in the ferric form (Fe^{3+}), with low solubility and bioavailability (Han, 2011). Another factor that is known to reduce the bioaccessibility of iron in amaranth leaves is oxalate which binds divalent minerals such as iron hence reducing their bioaccessibility. During cooking, iron is released from the complexes, and some ingredients such as tomatoes used in cooking may also enhanced the bioaccessibility of the iron (Nomkong et al., 2019). In this study, cooking involving boiling and heating during frying may have enhance the bioaccessibility of the iron. On the other hand, cooking itself softens the food matrix, releasing bound components, and also alters inherent mineral absorption inhibitors such as soluble dietary fiber thus improving their bioaccessibility (Platel & Srinivasan, 2015).

This study clearly shows that food preparation methods such as boiling of the vegetables may be used to improve the bioaccessible iron significantly. For instance, inclusion of ingredients that are rich in ascorbic acid results in higher amounts of bioaccessible iron. This is because these vitamin C rich dietary components are capable of reducing the ferric iron to bioaccessible ferrous iron (Blanco-Rojo & Vaquero, 2019). Enhancing effects of vitamin C on mineral bioaccessibility has also been observed in other studies (Singh & Prasad, 2018). In this study, lemon juice was used as a source of vitamin C, and this showed significant improvement in the bioaccessible iron. Other organic acids in the lemon juice such as citric acid could also be responsible for the enhancement of iron dializability. These acids are reported to have the ability of chelating iron to form soluble complexes. They also have the ability to lower pH increases solubilization of iron from the food (Rodriguez-Ramiro et al., 2019)

Inclusion of ingredients which are equally rich in iron also proved to be beneficial in improving the dialysable iron content. As most indigenous vegetables have also been shown to be good sources of iron, including them as ingredients could also result in higher iron content in the dish, hence higher bioaccessible iron. *Moringa oleifera* leaves in this study were used to determine if the anti-nutritional effects of oxalate in amaranth can be masked by including more iron in the dish. Moringa leaves have previously been reported to be rich in iron; Suzana et al reported values of 14.6 mg/100 g leaf extract, this being up to four times higher than spinach (Suzana et al., 2017); while Yang reported values of 9.2 mg/100 g fresh leaves (Yang et al., 2006). Other iron rich vegetables can also be included in amaranth recipes to increase the total iron in the vegetable dishes. Kruger *et al.*, reported that combining of amaranth with spider plant (80:20 ratio) increased iron bioavailability of the dish from 9.7% to 25%, while combination of amaranth with cowpea leaves (80:20 ratio) slightly increased iron bioavailability from 9.7% to 10.1% (Kruger et al., 2015).

Combination of amaranth Recipe1 with “ugali” (50:50) did not result in any significant difference in dialysable iron compared to the Recipe1 alone. In most parts of East Africa, amaranth dishes are eaten in combination with maize meal “ugali”, which is either fortified or unfortified. This study evaluated meal interactions which would affect iron bioavailability and showed that components in amaranth did not have any inhibitory effects on the iron bioavailability of the iron-fortified maize meal.

5. Conclusion

The amaranth dishes prepared using the three different recipes all showed high nutrient retentions. Food based methods such as incorporating high vitamin C foods and high iron foods as ingredients in vegetable preparation significantly enhanced the dialysable iron in the amaranth based recipes, which translated to improvement in the bioavailable iron in the recipes. Use of such methods can be applied in increasing micronutrient intake and reduction of prevalence of micronutrient malnutrition.

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