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Research Article

Lycium barbarum and *Delphinium denudatum* for Hastened Lipid Oxidation and Microbial Stability of Heat-Desiccated Milk Model System

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Abstract

Heat desiccation of milk viz., khoa is one of the common processing methods of various dairy products. Khoa is a traditional dairy product used as the base for many sweets. Being nutrient dense, they are vulnerable to lipolysis and proteolysis. Due to excessive pro-oxidant formation in the human body, oxidative stress is produced and the immunity of individuals is lowered, leading to several lifestyle diseases and making people prone to COVID-19 in the current scenario. The present study aimed to prepare functional khoa exploring antioxidants of two locally available herbs *Lycium barbarum* and *Delphinium denudatum* to make them functional dairy products. The value-added khoa was optimized for its preparation with the fortification of 4% *Lycium barbarum* and 0.50% *Delphinium denudatum*. The fortified khoa was optimized and standardized for its preparation and subjected to an invitro antioxidant profile viz. total phenols, total flavonoids content, DPPH, ABTS and FRAP assays and product storage profile. The value-added khoa prepared with 4% *Lycium barbarum* and 0.50% *Delphinium denudatum* had a shelf life of more than 28 days at refrigeration storage. Thus, functional khoa can be prepared with the fortification of 4% *Lycium barbarum* and 0.50% *Delphinium denudatum*.

Keywords: *Delphinium Denudatum*; Khoa; Milk Model System; *Lycium Barbarum*

Introduction

Khoa is a heat dried, desiccated, evaporated milk solids commonly used in sweet Indian deserts. Khoa is an important intermediate product that can be used to make a wide range of sweets. It is a heat-concentrated milk product made after the milk has been partially dried**.** It is called as kava, khoya, or mawa. Its exact origin isn't known but it has been prepared as the base material for milk-based confections by milk traders and halwa is in Indian sub-continent for centuries. It is having a uniform whitish colour with brown tinge, a slightly granular texture, and a rich nutty flavour. Due to the high concentration of lactose, it possesses a mildly cooked and sweet taste. The standard recovery of khoa should be more than 20 to 25%. There is documentation of sweets made from khoa such as sihakesara and morandeka in the scriptures from the early Buddhist and the Jain period. This sihakesara and morandeka have been used as desserts at the end of meals. Khoa has been using as a base material in numerous sweets such as burfi, kalakand, gulabjamun, kalajamun, rabri, khurchan,milk cake, peda, basundi, pantua, and lalmohan. About 600,000 metric tons of khoa is produced annually in the Indian sub-continent, utilizing 7% of total milk production just in India [24].

Lycium barbarum (Goji berry) is a traditional Chinese herb of family Solanaceae. It has been used as a functional food product for more than 2500 years for medicinal and nutritional purposes. Popular name "wolfberry" is derived from the Chinese character

"gou," which is related to the word "wolf [1]. Goji berries contain abundant bioactive molecules suchas polysaccharides, scopoletin (6-methoxy-7-hydroxycoumarin), the glucosylated precursor, a stable vitamin C analog 2-O-β-d-glucose-adenosyl-l-ascorbic acid, carotenoids, betaine, cerebroside, β-sitosterol, flavonoids, amino acids, minerals, and vitamins(7;22).The polysaccharide constitute 5-8% of dried fruit, carotenoids (zeaxanthin) about 0.03-0.5% and phenols (caffeic acid, caffeoylquinic acid, chlorogenic acid, p-coumaric acid). *Lyciumbarbarum* polysaccharide (LBP) is the primary bioactive component which possesses a wide range of biological properties, including antioxidant and immuno-modulatory effects (7;8). This fruit is rich source of vitamin C at higher levels than those found in oranges, vitamin E-which is very rarely found in fruits but found only in grains and seeds, beta-sitosterol, which is an anti-inflammatory agent, as well as a large number of essential fatty acids. These essential fatty acids are essential for production of hormones in our body and for the smooth functioning of the brain and nervous system, chaperone - a sesquiterpene that provides benefits for blood pressure and heart, reduces menstrual discomfort, and used in cervical cancer treatment, solavetivone is a powerful anti-fungal and anti-bacterial compound, physalin is a natural compound that is active against all major types of leukaemia, betaine - used by the liver to produce choline, which calms nervousness, boosts memory, enhances muscle growth, and protects against fatty liver disease.

Delphinium denudatum (Jadwar), the roots are used since ancient times in Ayurveda. Beta-sitosterol is the key bioactive element, along with flavonoids, carotenoids, and amino acids, which have antioxidant, antibacterial, anticancer, anticonvulsive, and anti-ageing properties. It is used to reduce depression, and anxiety and for treating insomnia. The chemical constituents include presence of alkaloids like delpho-curarine, staphisagrine,delphinine, condelphine, isotalatizidine, denudatine, panicutine, 3-hydroxy-2-methyle-4H-pyran-4-one, diterpinoid alkaloid 8, and acetyl-hetero-phyllisine [25].

Food can be a major contribution to reducing oxidative load *in vivo* as it can be a potent vehicle to deliver antioxidants at the cellular level. So, it is within this situation that functional food has developed and the value addition in our traditional products with natural antioxidants is of utmost required. We're talking about individual immunity levels in the current circumstance when the entire planet is dealing with an unprecedented COVID-19 epidemic and its mutants subsequently. What better method for our confectioneries and sweets to become immunogenic than this? We chose *Lyciumbarbarum* and *Delphinium denudatum* to be integrated as an antioxidant and immunogenic sources in this manner forward. Therefore, the study aimed to study total antioxidant properties, total phenolic content and total flavonoid content of *Lycium barbarum* and *Delphinium denudatum* in heat coagulated milk model system.

In the present research work, we utilized fruit shrubs and wild flowers viz. *Lycium barbarum* and *Delphinium denudatum* respectively for the development of value-added khoa to make it a functional dairy product with a better shelf-life. The development of value-added khoa with these natural sources of antioxidant not only increase the shelf-life of the products but also made it functional products for consumers. Therefore, this value addition is having twin benefits.

Materials and Methods

Sources of materials

Raw milk

Hygienically milked full fat buffalo milk was used that was presterilised, standardized at 6% fat and 9% solid not fat buffalo milk.

Chemicals and media

The chemicals and media used in the quality analysis of product were of analytical grade and chemicals used in the product preparation were of food grade. They were procured from standard firms like Qualigens, Hi-Media, etc.

Preparation of *Lycium barbarum* and *Delphinium denudatum* extracts as a source of antioxidants

Dried fruits of *Lycium barbarum* and dried roots of *Delphinium denudatum* were blended into fine powder with a blender and used for the preparation of aqueous ethanolic extracts. The *Lycium barbarum* and *Delphinium denudatum* extract were prepared by process of cold maceration for 72 hours with occasional stirring. The mixture was filtered and then the filtrate was collected. The filtrate was lyophilized (Innova, USA) 120 hours cycle and 96 hours cycle respectively for *Lycium barbarum and Delphinium denudatum* to obtain a lyophilized powder form. They were used as a source of antioxidants in the form of finely blended extract powder [27].

Three concentrations of each antioxidant viz. T_1 (2%), T_2 (4%) and T_3 (6%) of *Lycium barbarum* and T_1 (0.25%), T_2 (0.50%) and T_3 (0.75%) of *Delphinium denudatum* in khoa as heat desiccated milk model system.

In vitro Antioxidant Potential Profile of *Lycium barbarum* and *Delphinium denudatum* extracts

Ferric Reducing Antioxidant property (FRAP)

Sample (100 µl) is mixed with 3 ml of working FRAP reagent (a) Acetate buffer 300 mM pH 3.6. A weighing of 3.1grams of sodium acetate trihydrate was done and then add 16 ml of glacial acetic acid and then make the volume to 1 L with distilled water. b) TPTZ (2, 4, 6-tripyridyl-s-triazine) (M.W. 312.34) 10Mm in 40Mm HCl (M.W. 36.46) c) FeCl3. $6H_2O$ (M.W. 270.30) 20 mM. a b and c in the ratio of 10:1:1 were mixed to prepare the working FRAP reagent at the time of use and absorbance (593 nm) is measured at 0 minutes after vortexing. Thereafter, samples are placed at 37^0C in the water bath. Then, absorption is measured again after 4 minutes. Ascorbic acid standards (100µM-1000µM) were processed in the same way [27].

Total phenolic content (TPC)

The extract solution (0.1 ml) having 1000 µg of extract was then mixed with 46 ml of distilled water in a volumetric flask. To this, 1 ml Folin-Ciocalteu reagent was added. Then, the flask was thoroughly shaken. The mixture was then allowed to react for 3 minutes. After that, 3 ml aqueous solution of 2% Na_2CO_3 was added. After 2 h incubation at room temperature, absorbance of each mixture was measured at 760 nm. Similar procedure was applied to the standard solutions of gallic acid, and then a standard curve was obtained. Total phenolic contents were expressed as µg gallic acid equivalents per mg of the extract. Then, all tests were carried out in triplicate. After this, Gallic acid equivalent values were reported as X ± SD of triplicates [27].

Determination of total flavanoids

0.5 ml of sample was added to 0.5 ml of 2% $AlCl_3$ ethanol solution. After one hour at room temperature, the absorbance was measured at 420 nm. A yellow color indicated the presence of flavonoids. Extract samples were evaluated at a final concentration of 0.1 mg/ml. Total flavonoid content was calculated as quercetin (mg/g) using the following equation based on the calibration curve: y = 0.0255x, R2 = 0.9812, where x was the absorbance and was the quercetin equivalent (mg/g) [27].

DPPH radical scavenging activity

Various concentrations (80 and 90µg/mL) of antioxidant extracts and samples were mixed with 3.0 mL of a methanolic solution containing DPPH radical (6×10^{-5} mol/L). The mixture was shaken vigorously and left to stand for 60 min in the dark. Its value is measured at 517 nm using UV-Vis spectrophotometer. DPPH radical-scavenging activity was calculated with the following equation

DPPH radical scavenging activity (%) = $((A_{DPPH} - A_S)/A_{DPPH}) \times 100$

where A_{DPPH} is the absorbance without samples. A_s is the absorbance in the presence of the samples [27].

ABTS radical scavenging activity

The stock solutions included 7 mM ABTS solution and 2.4 mM potassium persulphate solution. The working solution was then prepared by mixing the two stock solutions in equal quantities and allowing them to react for overnight at room temperature in the dark. Dilution of the sample was done by mixing ABTS solution (1 mL) with ethanol (60 ml) for obtaining an absorbance of 0.706 ± 0.001 units at 734 nm. Preparation of fresh ABTS solution was done for each assay. Different concentrations (20-100 µg) of the antioxidant plant extracts and 1ml each of the Khoa extracts was allowed to react with ABTS solution (1 mL). The absorbance was measured at 734 nm after 7 min using a Perkin Elmer Lambda 35 UV-Visible Spectrophotometer. ABTS radical scavenging activity was calculated according to the following equation:

ABTS radical scavenging activity (%) = $((A_C - A_S)/A_C) \times 100$

where A_c is the absorbance without samples. A_s is the absorbance in the presence of the samples [27].

Preparation of khoa

Full fat raw buffalo milk was procured from the market and boiled in a karahi (of different sizes and shapes) over a brisk non – smoky fire. The milk was stirred vigorously and constantly with a circular motion by a khunti. During this operation, all parts of the pan with which the milk comes into contact were lightly scraped to prevent the milk from scrotching. Constant evaporation of moisture took place and milk was thickening progressively. At a certain concentration, heat coagulation of milk proteins begins and the concentrate becomes progressively insoluble in water. This stage was marked by an abrubt change in colour. The heating was continued with greater control thereafter and the speed of stirring cum scraping was increased. The viscous mass reached a semi-solid consistency and beginning to dry up. The final product was ready when it showed signs of leaving the bottom and sides of the karahi and sticking together. The khoa-pat was invariably made after re-

moving the pan from fire and working the contents up and down into a single compact mass.

Sensory evaluation

A semi-trained experienced sensory evaluation panel consisting of scientists evaluated for various sensory attributes using 9 points descriptive scale [27]. In this, the extremely desirable scale is 9 and the extremely poor scale is 1. Coded samples were prepared by cooking khoa. Samples were then served to the sensory panellists. The water was provided between the two samples evaluation for rinsing of mouth. Sensory performa was used for evaluating value-added Khoa.

Physico-chemical properties

Thiobarbituric Acid Reacting Substances (TBARS) Value

10 grams of sample containing the antioxidant was finely blended with 50 ml of 20% TCA in homogeniser/blender for 2 minutes followed by allowing the resultant extract to stand for 10 minutes. The extract was filtered in a test tube through the Whatman filter paper no. 42. 3 ml of this extract was mixed with equal volume of 0.1% (w/v) TBA reagent. Simultaneously, 3 ml of 20% TCA was mixed with an equal volume of 0.1% TBA reagent for blank preparation. In a boiling water bath, the contents of each test tube were mixed thoroughly and boiled for 35 minutes. At 532 nm, the absorbance value of the samples was then measured by a spectrophotometer and calculation of TBA value was done by comparing the test sample absorbance values with that of the standard graph prepared by using known concentrations of malonaldehyde. For standard graph preparation, 0.3055 grams of 1,1,3,3, Tetraethoxy Propane (TEP) was dissolved in 100ml of 95% absolute alcohol and a final concentration of 1 mg malondialdehyde/ml which was used for further preparation of solution. Preparation of the working standard solution of TEP was done by diluting 0.3 ml of the stock solution to a volume of 100 ml by distilled water. The diluted solution contained 3 grams/ml of malondialdehyde. The standard graph was prepared from this solution by using different concentration of malondialdehyde [29].

Free Fatty Acid (% Oleic Acid)

25 grams of khoa sample containing the antioxidant was blended with 137 ml of chloroform for two minutes in presence of 0.5 to 1 teaspoon of anhydrous sodium sulphate. The extract was filtered through the Whatmann filter paper no.12. An aliquot of 25ml of the extract was transferred to 125 ml conical flask. Then, 10 drops of 0.2% phenolpthalien indicator was added to it. The sample was titrated with 0.1 n 90% alcoholic potassium hydroxide to the end point (pink colour). Another 25 ml of the extract was placed in a pre-weighed beaker for the estimation of the fat weight after the complete vapourization and removal of chloroform at 80^0C in a drying over (28). FFA could be calculated as:

$$\text{FFA (\% Oleic acid)} = \frac{(0.1 \text{ ml } 0.1 \text{ N alcoholic KOH} \times 0.282 \times 100)}{\text{Weight of fat}}$$

Microbiological Profile

The microbiological profile analysis was done which included the Total plate count, Psychrophilic count, Coliform count and Yeast and Mould count in the sample were determined by the method given by APHA (1993).

Sample preparation

10grams of the khoa samples were taken aseptically and blended with 90 ml of 0.1 percent sterile peptone with a pre-sterlized blend. Serial ten-fold dilution of this sample was made in the pre-sterlized tubes containing 9 ml of 0.1 percent of peptone water. The sample preparation was done near flame under Laminar air flow (Thermo Electron Corporation D-63505 Langenselbold, Robert Boschstr. 1, Germany).

Total plate count

23.5 grams of plate count agar media which was procured from Hi Media Laboratories Pvt. Ltd., Mumbai (Code No. M091) was suspended in 1000ml distilled water. The media was then boiled to dissolve the suspension. The final pH was adjusted to 7.0 ± 0.2. The media was then sterilized by autoclaving at 15lb pressure (121^0C) for 15 minutes and then cooled to 45 ± 2^0C.For plating of the sample, the pour plate technique was followed.1ml of the inoculum was taken in duplicate and media was poured upto 2/3rd level of the pre-sterilized petriplates. These plates were then incubated at 35 ± 2^0C for 24 hours. Following incubation plates showing 30-300 colonies were counted and expressed as $\log_{10}$cfu/g of sample.

Pschyrophilic count

The sample was prepared in the same manner as for the total plate count. The plates were then incubated at 4 ± 1^0C for 10-14 days and the colonies were counted and expressed as $\log_{10}$cfu/g of sample.

Coliform Count

41.5g of Voilet Red Bile Agar procured from Hi Media Laboratories Pvt. Ltd., Mumbai (Code No. 049) was suspended in 1000ml of distilled water. It was then boiled to dissolve the medium completely and cooled to 45^0C. The final pH was adjusted to 7.4 ± 0.2^0C. Pour plate with overlay technique was used for the inoculation of

suitable sample dilution. The plates were later incubated at 35 ± 2^0C for a period of 24 hours. The colonies were then counted and the results were expressed as $\log_{10}$cfu/g of sample.

Yeast and Mould Count

39 g of Potato Dextrose Agar obtained from Hi-Media Laboratories Pvt. Ltd. Mumbai (code no. M096) was suspended in one litre of distilled water, boiled to dissolve the medium completely and sterilized by autoclaving at 25 lb pressure (121 C) for 15 minutes. The final ph was adjusted to 3.5 at 25 C. Pour plate with overlay technique was followed for inoculation of suitable sample dilution and the plates were incubated at 37 C for 5 days. The colonies were counted and results were expressed as $\log_{10}$cfu/g of sample.

Technical programme

Our study included a series of experimental steps which were characterised for verification by the application of various analytical tests. Initially, extracts of *Lyciumbarbarum* and *Delphinium denudatum* were prepared. On preparation, they were subjected to analysis for their *in vitro* antioxidant potential through estimation of their Total Phenols and total Flavonoids. Also, their radical scavenging activity was estimated through DPPH, ABTS and FRAP assays. Followed to this preparation and standardisation of khoa were done. Optimization and standardization of the process of incorporation of different levels of extracts i.e. 2%, 4%,6% of *Lycium barbarum* and 0.25%, 0.50%, 0.75% *Delphinium denudatum* to khoa was done to make it functional. Optimization and standardization of process of incorporation of different levels of extracts i.e. 2%, 3%,4% of *Lyciumbarbarum* and 0.15%, 0.25%, 0.35% *Delphinium denudatum* to chhana was done to make it functional. Product quality profile was analysed which consisted of sensory evaluation of the product, and the *in vitro* antioxidant profile of antioxidant extracts incorporated khoa in terms of Total Phenols, Total Flavonoids and radical scavenging activity through DPPH, ABTS and FRAP assays. Based on the sensory evaluation, final levels of incorporation i.e. 4% of *Lycium barbarum* and 0.50% *Delphinium denudatum* to khoa, to make it functional were selected for further experimental proceedings. The prepared khoa was subjected to refrigerated storage at 4±1°C and was analyzed on weekly intervals for 28 days. The prepared khoa was analyzed for product storage profile which included physicochemical tests viz. estimation of FFA and TBARS and microbiological profile on week intervals.

Results and Discussion

The present study is to unravel the effects of herbs and nuts, rich in bioactive compounds on the quality and shelf life of milk and milk products. In order to expedite the characteristic properties of *Lycium barbarum* and *Delphinium denudatum*, nuts and herbs are amalgamated in khoa to improve the functional property and extend the shelf life of khoa.

In vitro antioxidant profile of *Lycium barbarum* in heat-coagulated milk model system.

Total phenolic assay is based Folin Ciocalteau method. Folin Ciocalteau reagent contains phosphotungstic/phosphomolybdic acid complexes. There is transfer of electrons from phenolic compounds to form a blue chromophore (phosphotungstic/phosphormolybdenum complex) and the maximum absorption depends on the concentration of phenolic compounds. The molecule 1, 1-diphenyl-2-picrylhydrazyl (a,a-diphenyl-b_picrylhydrazyl; DPPH is a stable free radical in which delocalisation of the spare electron occurs over the molecule so that the molecule does not dimerize. The delocalization of electrons is responsible for deep violet color. When DPPH solution is mixed with a substrate (AH) that can donate an atom of hydrogen, this gives rise to the reduced form with the loss of this violet color. ABTS measures the loss of color when an antioxidant is added to the blue-green chromophore ABTSÆ + (2,2-azino-bis (3-ethylbenz_thiazoline-6-sulfonic acid)). The antioxidant reduces ABTSÆ+ to ABTS and decolorize it. ABTSÆ+ is a stable radical not found in the human body. Trolox (6-hydroxy-2,5,7,8- tetramethylchroman-2-carboxylic acid), can be used as an antioxidant standard which is a water-soluble analog of vitamin E. FRAP method is used to measure the ability of the antioxidant to reduce ferric iron and TPTZ (2,4,6-tripyridyl-s-triazine) to ferrous form at low pH.

The total phenolic content and flavonoid content of *Lycium barbarum* extract found in high amounts (Figure 1). This is supported by the findings of [5]. The antioxidant activity with respect to DPPH, ABTS and FRAP of *Lycium barbarum* extract were also found significantly higher as in concordance with observations of [13,20]. The decrease in trend in the total phenolic, flavonoid content and the DPPH, ABTS, and FRAP assays was observed when incorporated into khoa. This decrease in trend of total phenolic content, the total flavonoid content, DPPH, ABTS and FRAP aasay may be attrib-

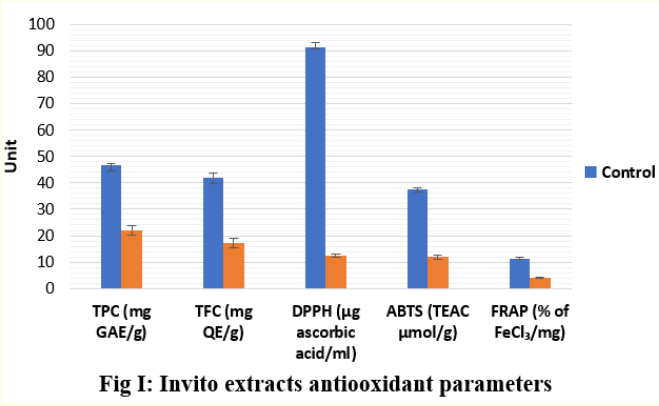

Figure 1: *In vitro* antioxidant profile of *Lycium barbarum* in heat concentrated milk model system (Khoa) (Mean ±S.E.)
*Mean ± SE; n = 6 for each treatment.

uted to resistance offered by khoa food matrices thereby lowering the exhibition of antioxidant activity of *Lycium barbarum* in khoa. [12] stated that all goji berries are rich in phenolics. The results were witnessed to be in non-agreement with the findings of [33] who showed that enriched yogurt with berries have higher phenolic content and higher radical scavenging activity. Similar results were quoted by [6] who found varieties of bioactive elements like flavonoids, carotenoids, polyunsaturated fatty acids having antioxidant properties. [31] concluded the *L. barbarum* was having higher concentrations of carbohydrates and phenolics than *L. chinense Mill*. Fruits. [10] noted the pharmacological activities of *Lycium barbarum* polysaccharides (LBP) and other major components of *Lycium barbarum* and demonstrated significant antioxidant activities. The antioxidant and antimicrobial activities of *Lycium barbarum* flowers, as an alternative resource of naturally-occurring antioxidant compounds, was revealed [17].

In vitro antioxidant profile of *Delphinium denudatum* in heat-concentrated milk model system

The invitro antioxidant profile of *Delphinium denudatum* extract had a remarkably high level of total phenolic content and total flavonoid content (Figure 2). The results found were close to those reported in the studies of [30]. [11] noted that the extract had also been shown to have high radical scavenging activity in terms of DPPH, ABTS, and FRAP assays. However, a decrease in trend was observed in the antioxidant profile of *Delphinium denudatum* after incorporation in khoa. The decline in the exhibition of antioxidants attributes in the heat-concentrated milk model system may be due to heat as well as the stabilizing effect of the khoa matrix (Figure 2).

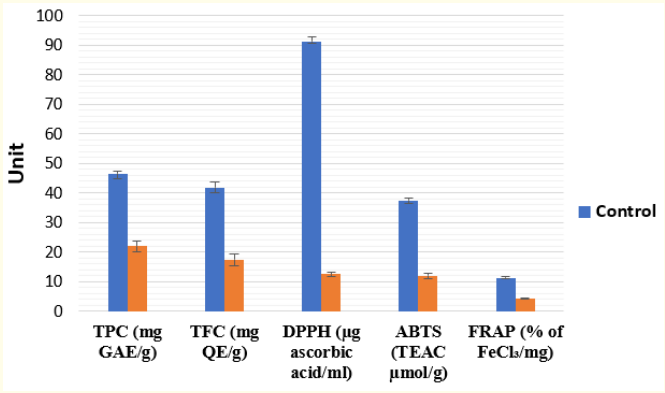

Figure 2: *In vitro* antioxidant profile of *Delphinium denudatum* in heat concentrated milk model system (Khoa) (Mean ± S.E.)
*Mean ± SE; n = 6 for each treatment.

Sensory attributes of variants of *Lycium barbarum* fortified value added Khoa.

When food quality is assessed employing human sensory organs, the evaluation is sensory or subjective. It is the only method for getting the correct opinion of the target population and consumer acceptance of the product. Sensory evaluation of the product was conducted weekly, including colour and appearance, softness texture, flavour and taste, and overall acceptability. The colour and appearance of any product decide its degree of liking by consumers. The flavour is one of the essential parameters in the acceptability of a dairy product.

Figure 3 depicted sensory attributes of variants of *Lycium barbarum* fortified value added Khoa which revealed that the level of 4% *Lycium barbarum* in khoa had been liked by panelists to adjudge the best level of incorporation which may be due to combined effects from flavour compounds of *Lycium barbarum.*

Therefore, the addition of nut-based material leads to higher overall acceptability of khoa at 4% of *Lycium barbarum*. Similar reporting was done while working on the sensory profile and

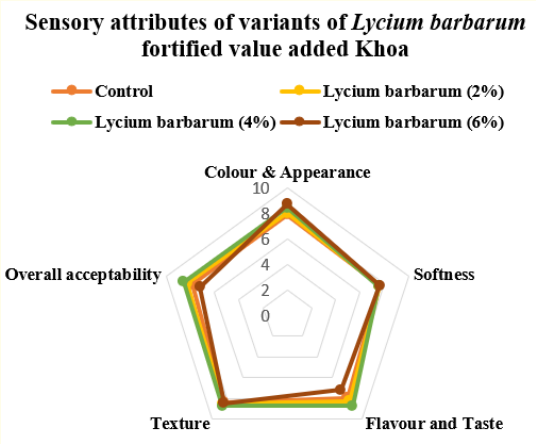

Figure 3: Sensory attributes of variants of *Lycium barbarum* fortified value added Khoa (Mean ± S.E).

*Mean ± SE with different superscripts in a row wise (lower case alphabet) differ significantly ($p < 0.05$) n = 21 for each treatment.

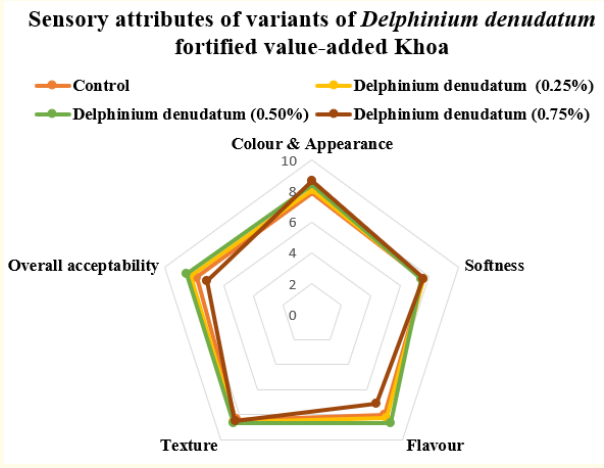

Figure 4: Sensory attributes of variants of *Delphinium denudatum* fortified value-added Khoa (Mean ± S.E)

*Mean ± SE with different superscripts in a row wise (lower case alphabet) differ significantly ($p < 0.05$) n = 21 for each treatment.

consumer acceptability of prebiotic white chocolate with *Lycium barbarum* [10]. [26] stated *Lycium barbarum* and honey affect the sensory quality of the yoghurt and showed that the use of *Lycium barbarum* improved the sensory acceptance of consumers and acted as an enhancer of probiotic levels in yoghurt. The results were in concordance with the findings of [19] who worked on herbal and spiced paneer and the result revealed that using malic acid oregano, the overall acceptability was higher.

Sensory attributes of variants of *Delphinium denudatum* fortified value added khoa

The sensory attributes of variants of *Delphinium denudatum* fortified value added Khoa which revealed that the level of 0.5% *Delphinium denudatum* in khoa was found to be sensorily optimum by the panelists (Figure 4). The scores for flavour presented higher for 0.5% *Delphinium denudatum* in khoa. The overall acceptability of the product was judged and influenced only on the basis of the flavour of the product. It has been shown that the flavour of foods fortified with extract was the most sensitive indicator of their sensorial acceptability. These levels of extract incorporation in respective products i.e. khoa have been selected for further storage studies and microbiological profile analysis. The literature revealed that [8] who worked on herbal burfi prepared with 85% khoa with 15% stevia powder, was found to be in the category of 'like moderately' to 'like very much.'

Shelf-life analysis profile of *Lycium barbarum* and *Delphinium denudatum* extract in heat-concentrated milk model system (Khoa) on refrigeration storage

The TBARS and FFA value indicates lipid hydrolysis in dairy products and is expressed as the amount of malonaldehyde formed due to the oxidation of fatty acids with three or more double bonds during the storage period. In the present study, the treated products' TBARS and FFA values were within an acceptable range in contrast to the control product even on the 28th day of refrigeration storage. This was an indicator of the retardation of lipid oxidation in the treated product (Figure 5).

The storage parameters were within an acceptable range in *Lycium barbarum* treated product as compared to control was probably attributed to antioxidant and antilipolytic properties of active metabolites like polysaccharides, phenols (caffeic acid, chlorogenic acid, caffeoylquinic acid), carotenoids, betaine, cerebroside, β-sitosterol, flavonoids present in *Lycium barbarum.* The present study went hand in hand with the state of [9] who studied the effects of goji berry extract on some quality characteristics of common carp sausages. The findings of the result were further in congruence with [18] who added *Lycium barbarum* to extra virgin olive oil.

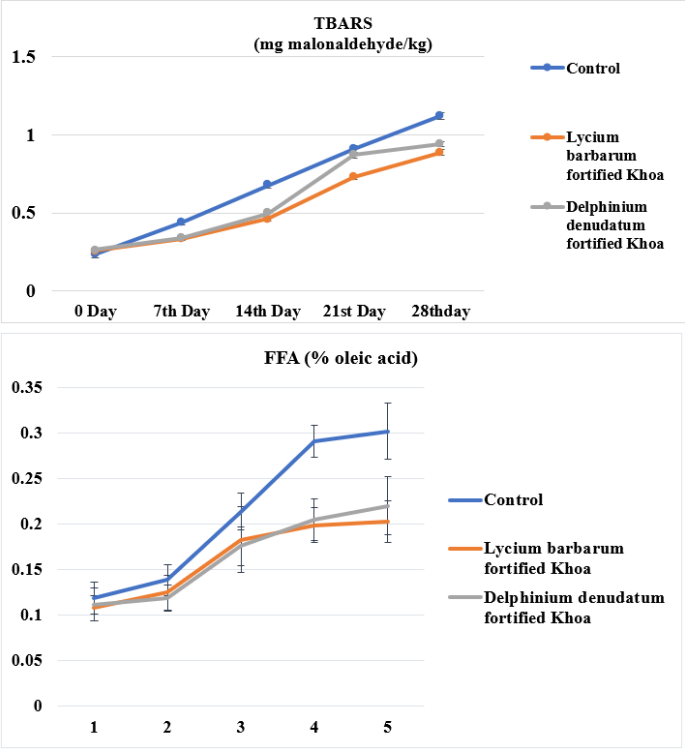

Figure 5: Shelf life analysis profile of herbal extract in heat concentrated milk model system (Khoa) on refrigeration storage at (4 ± 1°C) (Mean ± S.E.).

*Mean ± SE with different superscripts in a row wise (lower case alphabet) and column wise (Upper case alphabet) differ significantly ($p < 0.05$) n = 6 for each treatment.

The physicochemical parameters ie TBARS and FFA were found to be within permissible limits in *Delphinium denudatum* treated product as compared to control was probably attributed to antioxidant and antilipolytic properties of active metabolites like hetisine, histidine, denudatine, delnudine present in *Delphinium denudatum*. There had been no reporting of the incorporation of *Delphinium denudatum* in the food system. [4] reported the preparation of an indigenous food product called burfi using underutilized ghee residue and honey containing the maximum amount of antioxidants, phenols, and flavonoid contents, and results revealed the FFA content increased from 1.04% at the initial level to 1.42% after 30 days, which remained within desirable limits.

Effect on the microbiological profile of *Lycium barbarum* and Delphinium denudatum fortified Khoa at refrigeration temperature

The Lycium barbarum treated khoa has shown its excellent antimicrobial properties by exhibiting its activity against both bacteria as well as fungus. In the present study, the microbial profile of *Lycium barbarum* treated khoa as compared to control indicated the exhibition of antimicrobial characteristics of extract which made the treated product with *Lycium barbarum* extract acceptable for a longer durationie. 28 days and thereby enhance the shelf life of the product (Table 1-6). This may be due to the fact that the extract might have acted as an oxygen barrier to microbial growth [9] reported that the goji berry extract was very effective in inhibiting microorganisms' growth in common carp. The results were found in concordance with reporting of [3] who worked on the antimicrobial potential of herbal burfi. [17] found the antimicrobial and antioxidant activities of *Lycium barbarum* flowers and revealed that the extract exhibited a moderate antimicrobial potential against G+ bacteria.

Parameters / Products	TPC (mg GAE/g)	TFC (mg QE/g)	DPPH (µg ascorbic acid/ml)	ABTS (TEAC µmol/g)	FRAP (% of $FeCl_3$/ mg)
Control	47.48 ± 1.63	26.75 ± 2.46	89.42 ± 5.71	78.60 ± 3.26	18.73 ± 0.55
Lycium barbarum fortified khoa	25.87 ± 1.86	14.43 ± 2.87	15.39 ± 0.54	09.43 ± 0.86	3.94 ± 0.77

Table 1: *In vitro* antioxidant profile of *Lycium barbarum* in heat concentrated milk model system (Khoa) (Mean ± S.E.).

*Mean ± SE; n = 6 for each treatment.

Parameters / Products	TPC (mg GAE/g)	TFC (mg QE/g)	DPPH (µg ascorbic acid/ml)	ABTS (TEAC µmol/g)	FRAP (% of $FeCl_3$/mg)
Control	46.48 ± 0.64	41.79 ± 1.89	91.23 ± 1.64	37.42 ± 0.83	11.23 ± 0.44
Delphinium denudatum fortified khoa	21.95 ± 1.84	17.31 ± 1.89	12.43 ± 0.76	11.86 ± 0.93	4.04 ± 0.18

Table 2: *In vitro* antioxidant profile of *Delphinium denudatum* in heat concentrated milk model system (Khoa) (Mean ± S.E.).

*Mean ± SE; n = 6 for each treatment.

Control	*Lycium barbarum (2%)*	*Lycium barbarum (4%)*	*Lycium barbarum (6%)*
Colour and Appearance			
7.89 ± 1.075	8.00 ± 1.104	8.48 ± 1.102	8.72 ± 1.086
Softness			
7.56 ± 0.076	7.56 ± 0.101	7.52 ± 0.106	7.63 ± 0.101
Flavour and Taste			
7.99 ± 0.119^{a}	8.25 ± 0.081^{ba}	8.72 ± 0.098^{a}	7.16 ± 0.098^{a}
Texture			
8.39 ± 0.159	8.55 ± 0.123	8.68 ± 0.129	8.46 ± 0.126
Overall acceptability			
7.85 ± 0.085^{a}	8.26 ± 0.088^{ba}	8.57 ± 0.086^{c}	7.19 ± 0.096^{a}

Table 3: Sensory attributes of variants of *Lycium barbarum* fortified value added Khoa (Mean ± S.E).

*Mean ± SE with different superscripts in a row wise (lower case alphabet) differ significantly ($p < 0.05$) n = 21 for each treatment.

Control	Delphinium denudatum (0.25%)	Delphinium denudatum (0.50%)	Delphinium denudatum (0.75%)
Colour and Appearance			
7.89 ± 1.075	8.05 ± 1.106	8.42 ± 1.108	8.68 ± 1.105
Softness			
7.56 ± 0.076	7.57 ± 0.101	7.47 ± 0.111	7.60 ± 0.108
Flavour			
7.99 ± 0.119^{a}	8.25 ± 0.079^{ba}	8.66 ± 0.098^{a}	7.13 ± 0.096^{a}
Texture			
8.39 ± 0.159	8.55 ± 0.178	8.65 ± 0.181	8.48 ± 0.179
Overall acceptability			
7.85 ± 0.085^{a}	8.23 ± 0.080^{ba}	8.52 ± 0.092^{c}	7.14 ± 0.091^{a}

Table 4: Sensory attributes of variants of *Delphinium denudatum* fortified value-added Khoa (Mean ± S.E).

*Mean ± SE with different superscripts in a row wise (lower case alphabet) differ significantly ($p < 0.05$) n = 21 for each treatment.

Parameters / Products	0 Day	7th Day	14th Day	21st Day	28thday
TBARS (mg malonaldehyde/kg)					
Control	0.233 ± 0.018^{Ae}	0.439 ± 0.017^{Ad}	0.675 ± 0.015^{Bc}	0.908 ± 0.023^{Bb}	1.119 ± 0.020^{Ba}
Lycium barbarum fortified Khoa	0.257 ± 0.014^{Be}	0.335 ± 0.013^{Ad}	0.461 ± 0.016^{Ac}	0.727 ± 0.014^{Ab}	0.886 ± 0.017^{Aa}
Delphinium denudatum fortified Khoa	0.263 ± 0.010^{Ae}	0.341 ± 0.014^{Ad}	0.496 ± 0.015^{Ac}	0.869 ± 0.018^{Ab}	0.940 ± 0.016^{Aa}
FFA (% oleic acid)					
Control	0.119 ± 0.018^{Ae}	0.139 ± 0.017^{Ad}	0.214 ± 0.020^{Bc}	0.291 ± 0.018^{Bb}	0.302 ± 0.031^{Ba}
Lycium barbarum fortified Khoa	0.108 ± 0.014^{Ae}	0.125 ± 0.019^{Ad}	0.183 ± 0.036^{Ac}	0.199 ± 0.019^{Ab}	0.203 ± 0.023^{Aa}
Delphinium denudatum fortified Khoa	0.112 ± 0.018^{Ae}	0.119 ± 0.014^{Ad}	0.176 ± 0.021^{Ac}	0.205 ± 0.023^{Ab}	0.220 ± 0.032^{Aa}

Table 5: Shelf life analysis profile of herbal extract in heat desiccated milk model system (Khoa) on refrigeration storage at (4 ± 1°C) (Mean ± S.E.).

*****Mean ± SE with different superscripts in a row wise (lower case alphabet) and column wise (Upper case alphabet) differ significantly ($p < 0.05$) n = 6 for each treatment.

Treatments	0 Day	7th Day	14th Day	21st Day	28th Day
Total Plate Count (log_{10}cfu/g)					
Control (Khoa)	2.59 ± 0.17^{Aa}	3.31 ± 0.19^{Bb}	3.40 ± 0.16^{Cc}	4.09 ± 0.19^{Bd}	4.53 ± 0.019^{Be}
Lyciumbar barum fortified Khoa	2.69 ± 0.18^{Aa}	2.38 ± 0.20^{Ab}	2.62 ± 0.14^{Ac}	3.16 ± 0.12^{Ad}	3.37 ± 0 .014^{Ae}
Delphinium denudatum fortified Khoa	2.39 ± 0.17^{Aa}	2.73 ± 0.23^{Ab}	2.89 ± 0.17^{Bc}	3.62 ± 0.15^{Ad}	3.83 ± 0.012^{Ae}
Psychrotropic Count (log_{10}cfu/g)					
Control (Khoa)	ND	ND	ND	1.18 ± 0.021^{Ba}	1.63 ± 0.018^{Bb}
Lycium barbarum fortified Khoa	ND	ND	ND	0.56 ± 0.024^{Aa}	0.80 ± 0.026^{Ab}
Delphinium denudatum fortified Khoa	ND	ND	ND	0.55 ± 0.023^{Aa}	0.81 ± 0.022^{Ab}
Coliform Count (log_{10}cfu/g)					
Control (Khoa)	ND	ND	ND	ND	ND
Lycium barbarum fortified Khoa	ND	ND	ND	ND	ND
Delphinium denudatum fortified Khoa	ND	ND	ND	ND	ND
Yeast and mould count (log_{10}cfu/g)					
Control (Khoa)	ND	ND	ND	ND	1.13 ± 0.196^{B}
Lycium barbarum fortified Khoa	ND	ND	ND	ND	0.23 ± 0.042^{A}
Delphinium denudatum fortified Khoa	ND	ND	ND	ND	0.32 ± 0.037^{A}

Table 6: Effect on the microbiological profile of *Lycium barbarum* and *Delphinium denudatum* fortified Khoa at refrigeration temperature (Mean ± SE).

*****Mean ± SE with different superscripts in a row wise (lower case alphabet) and column wise (Upper case alphabet) differ significantly ($p < 0.05$) n = 6 for each treatment.

The microbial load of *Delphinium denudatum* fortified khoa was much lesser than the standard levels even on and after 28 days of refrigeration storage as compared to the control. The incorporation of *Delphinium denudatum* not only contributed to the safety of foods but also prevented microbial spoilage. This may be attributed to the presence of bioactive components, beta-sitosterol, flavonoids (quercitin, kaempferol, rutin), and carotenoids in the extract acted as an oxygen barrier to microbial growth. [16] demonstrated that *Delphinium denudatum* possessed antimicrobial properties. The results found were in concordance with the findings of [32], who studied the antibacterial activity of *Delphinium denudatum* against no. of microorganisms including *Staphylococcus aureus, Bacillus cereus, Escherichia coli*, and *Pseudomonas aeruginosa*. The results found were congruent to those reported in the studies of [28]. [34] reported the effect of Aloe vera as a preservative agent in flavoured pasteurized milk and showed its antimicrobial property. Similar results were quoted by [23] who evaluated the properties of turmeric-incorporated paneer prepared from different types of milk, i.e., cow milk, buffalo milk, and mixed milk.

Therefore, *Lycium barbarum* and *Delphinium denudatum* extract are additives that preserve food from "farm to plate" and militate against oxidative deterioration on storage and further processing. Due to their low volatility and high stability, the antioxidants aid to maintain the level of colour, taste, nutrients, texture, freshness, functionality, aroma, and appeal to consumers.

Conclusions

Therefore, the value addition in our traditional products with natural antioxidants is utmost required in today's date. It will not only enhance the product's shelf life but also make the traditional dairy product a functional nutraceutical-grade food. The extract prepared possessed significant bioactivity with total phenolic and total flavonoid content. It also possessed significant radical scavenging activity as evaluated with DPPH, ABTS and FRAP assays. The khoa prepared after fortification had significant radical scavenging capacity as evaluated by DPPH, ABTS and FRAP assay. It was sensory assessed to be best prepared at 4% *Lycium barbarum* and 0.5% *Delphinium denudatum* in khoa. The storage profile of the product was also evaluated to be retard lipid oxidation and antimicrobial activity of khoa prepared at 4% *Lycium barbarum* and 0.5% *Delphinium denudatum*. The Khoa prepared with the fortification of extract of *Lycium barbarum* and *Delphinium denudatum* was found to be acceptable even on the 28th day of storage at refrigeration in contrast to control.

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