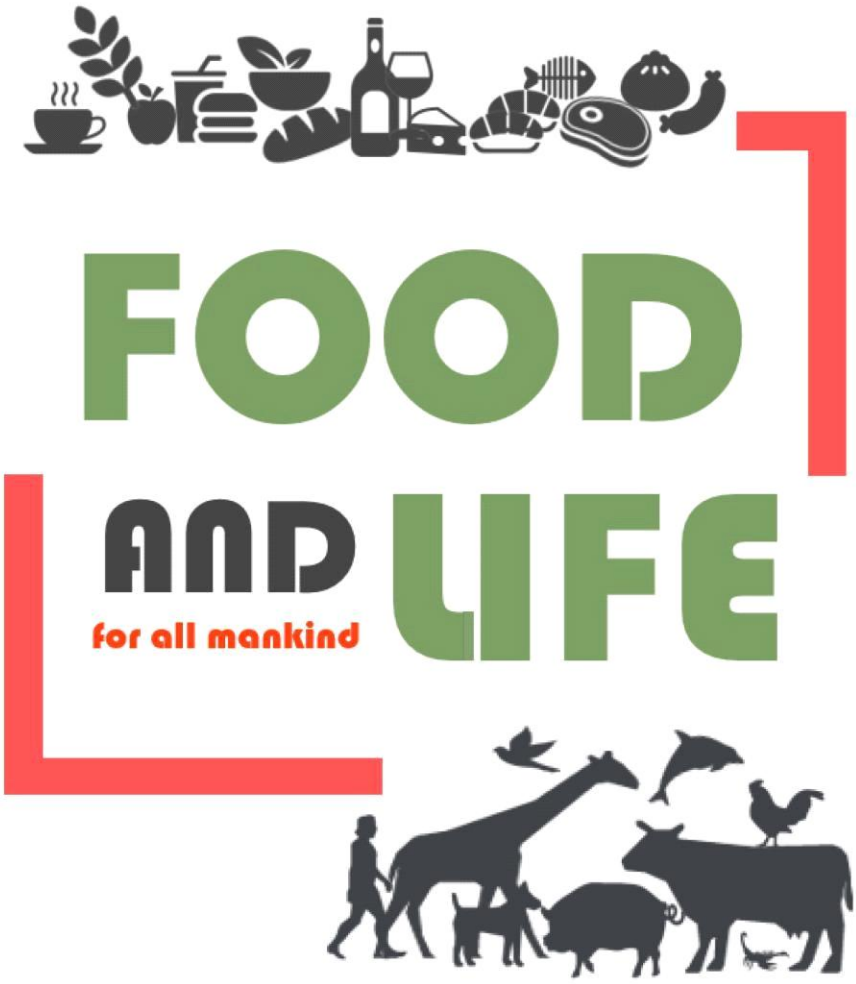

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Antioxidant potential of *Lycium barbarum* and *Delphinium denudatum* to improve shelf life of acid-coagulated milk model system

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Abstract

Acid coagulation of milk model viz., channa is one of the common processing methods of various dairy products. Channa is a traditional dairy product used as the base for many sweets. Being nutrient dense, they are in peril to lysis of proteins and lipids and have lower shelf-life. The present study aimed to prepare functional channa exploring antioxidants potential of two locally available fruit and flower *Lycium barbarum* and *Delphinium denudatum* respectively to make them functional dairy products with better shelf-life. The value-added channa was optimized for its processing with the incorporation of 3% *L. barbarum* and 0.25% *D. denudatum*. The value added channa was optimized and standardized for its processing and subjected to an *in vitro* antioxidant profile analysis of total phenols, total flavonoids content, 2,2-diphenyl-1-picrylhydrazyl, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid and Ferric reducing antioxidant property assays and product storage profile. The value-added channa prepared with 3% *L. barbarum* and 0.25% *D. denudatum* was having a shelf life of more than three weeks stored at refrigeration temperature. Thus, functional channa can be prepared with the fortification of 3% *L. barbarum* and 0.25% *D. denudatum* with enhanced shelf-life.

Keywords: channa, *Delphinium denudatum*, milk model system, *Lycium barbarum*

Introduction

The acidified milk gels produced using organic acids are the oldest and the most popular food cuisines produced throughout the entire world. The popularity of fermented milk acid-coagulated cheese varieties (channa, cream, cheese, kaladi, yogurt), is due to various health claims health benefits. Irrespective of the commercial importance of these acidified milk products, there is not much information products such as fresh regarding formation, structures and physico-chemical properties of acid- coagulated milk products (Sharma et al., 2023). Channa, also known as paneer when pressed with same amount of whey, is a product made from cow or buffalo milk or a combination thereof by citric or lactic acid precipitation. It should not include contain more than seventy percent moisture and have a milk fat content of at minimum fifty percent of dry matter. It is considered the Indian counterpart of soft cottage cheese. It is a heat acid coagulated product with having spongy texture with a mildly acidic flavour and marble white colour. It is used as a base material for the preparation of a large variety of sweets such as Sandesh (sweet dish made from channa as base), paneer, cham, kheermohan, rasogolla etc. The product obtained from cow milk is velvety body soft with smooth texture which are highly desirable attributes for making channa-based sweetmeats, particularly rasogolla. Cow milk is preferred for channa making. Approximately, six percent of the total milk production in India is converted into channa through organic acid coagulation. It is a suitable food product for diabetes people due to its high protein and fat content with low sugar content. Channa prepared from an admixture of sweet cream buttermilk and buffalo milk using citric acid as a coagulant was suitable for rasogolla making based on rheological characteristics. Sandesh of acceptable quality also can be made from buffalo milk by standardized procedure (Sanyal et al., 2011). There are many synthetic chemicals used to enhance the shelf-life of channa, but those pose severe health hazards. *Lycium barbarum* (goji berry) and *Delphinium denudatum* (jadwar) are the two natural herb known for their antioxidant potential can be potent enough to enhance the commercial

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commodity i.e., acid coagulated milk model system (channa).

L. barbarum (goji berry) is a traditional Chinese herb of the family Solanaceae with popular name of "wolfberry" is derived from the Chinese character "gou", which is related to the word "wolf" (Amagase and Farnswort, 2011). It has been used as a functional food product from ancient times for medicinal and nutritional purposes. The popular name goji berries that contain bioactive moiety like scopoletin (cerebroside, β-sitosterol, flavonoids, amino acids, carotenoids, betaine, and vitamins) (Chen et al., 2015; Qian et al., 2017). The polysaccharide constitutes 5%-8% of dried fruit, carotenoids (zeaxanthin) about 0.03%-0.5% and phenols (caffeoylquinic acid, chlorogenic acid, caffeic acid, p-coumaric acid). *L. barbarum* polysaccharide (LBP) is the primary bioactive component which beholds a wide range of biological properties, including antioxidant and immuno-modulatory effects (Cheng et al., 2015). This fruit is a rich source of ascorbic acid at higher levels than those found in citrus fruits, vitamin E-which is rarely found in fruits but found in cereals, beta-stilosterol, which is an anti-inflammatory moiety, as well as a many essential fatty acids. These essential fatty acids are essential for the synthesis of hormones in our body and for the efficient working of central and peripheral nervous system, chaperone-a sesquiterpene which possess benefits for blood pressure and heart, reduces menstrual irritation, and is used in cervical cancer prohylaxix, solavetivone is a powerful anti-fungal and anti-bacterial moiety, physalin is a natural moiety that is active against leukaemia, betaine-used by the liver to synthesize choline, which calms anxiety, boosts memory, enhances growth and development, as well as maintain liver health.

D. 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 the presence of alkaloids like 3-hydroxy-2-methyle-4H-pyran-4-one, staphisagrine, delphinine, condelphine, panicutine, diterpinoid alkaloid 8, delpho-curarine, and acetylhetero-phyllisine (Rauf, 2013). When we are living in such a hostile environment where mutants of coronavirus are evident, and a lack of fighting drugs or molecules is not discovered, we have to be entirely dependent on our innate immunity which directly depends on the consumption of functional foods. The population explosion further enhances responsibility for the quality production of food. Improved milk and its products with antioxidant traits and functional foods of animal origin have been utilized to enhance immunity and counter recurrent occurrence of mutants of coronavirus. The designer milk product contains low fat and less lactose, more protein, modified level of fatty acids, and desired amino acid profiles. The importance of milk and its products is due to the presence of bioactive peptides, conjugated linoleic acid, omega-3 fatty acid, calciferols, selenium, and calcium. These constituents present in milk product, play a key role in the physiological development in human bodies. The consumer awareness regarding benefits of designer foods like milk and its products is almost non-existent worldwide and needs to be established to reach the benefits of designer food technologies to people in the near future. The main objective of the research was to design a organic acid coagulated milk fortified with plant extract thus making it functional as well as enhances its shelf life. What better method for our confectioneries and sweets to become immunogenic than this? The acid coagulated milk product viz., channa is vulnerable to have a low shelf-life. We chose *L. barbarum* and *D. denudatum* to be integrated as source of antioxidant and immunity enhancer. Therefore, the study aimed to utilize total antioxidant properties, total phenolic content and total flavonoid content of *L. barbarum* and *D. denudatum* in acid coagulated milk model system to increase its functionality and longer shelf-life.

Materials and Methods

Sources of materials

Raw milk

Hygienically milked full-fat buffalo milk was used standardized at 6% fat and 9% snf buffalo milk from the Instructional Livestock Farm Centre of the University. The standardized milk was further processed for making value added milk products.

Chemicals and media

All chemicals and media used in the quality analysis of the product were of analytical grade and the chemicals used in the product preparation were of food grade.

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

Dried fruits of *L. barbarum* and dried roots of *D. denudatum*

were blended into a fine powder with a blender and used for the preparation of alcoholic aqueous extracts. The *L. barbarum* and *D. denudatum* extract were prepared by process of cold maceration for three days with intermittent stirring. The mixture was filtered and then the filtrate was collected. *L. barbarum* and *D. denudatum* were used as a source of antioxidants in the form of lyophilized extracts and finely blended powder. Three concentrations of each antioxidant viz. T_1 (2%), T_2 (3%) and T_3 (4%) of *L. barbarum* in chhana, and T_1 (0.15%), T_2 (0.25%) and T_3 (0.35%) of *D. denudatum* in channa as acid coagulated milk model.

Methods of preparation of channa

The fresh buffalo milk was filtered and standardized to 6% fat and 9% snf. For channa making, milk was heated to 82 degree centigrade for 5 minutes and then cooled to 70 degree centigrade. It was then coagulated by the slow addition of 2.5 g citric /lactic acid per kg of fresh milk and slow stirring of the mix to avoid foam formation which obstructs the visibility of the clear coagulation stage. When it was entirely coagulated, the coagulum were poured over a thin muslin cloth spread over receiving vessel. The muslin cloth containing the coagulated solids was then removed, tied up into a bundle without squeezing or applying pressure and hung it up to drain out whey completely and to cool the channa-pat.

Sensory evaluation

Seven semi-trained sensory evaluation panelists consisting of scientists evaluated for various sensory attributes using 9 points descriptive scale and every experiments have been repeated thrice (Sharma et al., 2023). In this, the extremely desirable scale is 9 and the extremely poor scale is 1. Coded samples were prepared for channa. Samples were then served to the evaluators. The water was provided between the two samples evaluation for rinsing of the mouth. Sensory performance was used for evaluating value-added channa.

***In vitro* antioxidant potential profile**

Ferric reducing antioxidant property (FRAP)

Sample (100 µL) (antioxidant extract and channa extract) is mixed with 3 mL of working Ferric reducing antioxidant (FRAP) reagent. (a) Acetate buffer 300 mM pH 3.6. A weighing of 3.15 g 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) 2, 4, 6-Tripyridyl-s-triazine (TPTZ) 10 Mm in 40 Mm HCl, 20 mM $FeCl_3$. (a, b, and c) In the ratio of 10:1:1 were mixed to prepare the working reagent at the time of use and absorbance (593 nm) is measured at immediately (zero minute) after vortexing. Subsequently, samples were placed at in water bath in thirty-seven degree centigrade. Then, absorption is taken after four minutes. Standards of ascorbic acid (100-1,000 µM) were processed in the similarly (Sharma et al., 2023).

Total phenolic content (TPC)

The total phenolic contents (TPC) of the antioxidant extract of a sample were determined (Sharma et al., 2023). Gallic acid was taken as standard. 0.1 mL of extract solution having thousand µg of the extract was then mixed with 46 mL of distilled water in a volumetric flask, with one ml Folin-Ciocalteu reagent was added to it. The mixture was thoroughly shaken then allowed to react for five minutes. After that, a three mL of 2% Na_2CO_3 was added. After two hours incubation, the absorbance was measured at 760 nm. The standard solutions of gallic acid was used following similar steps, to obtain standard curve. Total phenolic contents were expressed in terms of µg gallic acid equivalents per mg of the respective extract. Then, all tests were repeated out in triplicate.

Total flavanoids (TF)

Estimation of the total flavonoids was carried out using the method of Sharma et al. (2023). To half mL of sample, half mL of 2% $AlCl_3$, ethanol solution was added. After one-hour incubation at room temperature, the absorbance was taken at 420 nm. A yellowish color indicated the presence of flavonoids. Extract samples were assessed at a end concentration of 0.1 mg/mL. Total flavanoids (TF) was calculated as quercetin (mg/g) using equation based on the calibration curve: y=0.0256x, R^2=0.9813, where x was the absorbance and was the quercetin equivalent (mg/g).

2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

Various concentrations (80, 90 μg/mL) of antioxidant extracts and channa extracts were mixed with three mL of a alcoholic solution containing 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical (6×10^{-5} mol/L). The mixture was shaken vigorously and left to

stand for an hour in the dark. Its value is read at 517 nm using Eppendorf nanodrop UV-vis spectrophotometer. DPPH radical-scavenging was measured with by following equation (Sharma et al., 2021a):

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

2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radical scavenging activity

2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radical scavenging activity was totalled by the method of Sharma et al. (2021a). The stock solutions included ABTS and potassium persulphate. The working solution was prepared by mixing two stock solutions in equal quantities allowing them to react overnight at room temperature in the dark condition. Dilution of the sample was done by mixing ABTS solution with ethanol for obtaining an absorbance of 0.706±0.005 units at 734 nm. Preparation of fresh ABTS solution was done for each assay. The various concentrations (20-100 μg) of the antioxidant plant extracts and 1 mL each of the samples were reacted with ABTS solution. The absorbance was measured at 734 nm after seven mins using a Eppendorf UV-visible spectrophotometer. ABTS radical scavenging activity was totalled according to the following equation:

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

Physico-chemical properties

Thiobarbituric acid reacting substances (TBARS) value

For estimation of thiobarbituric acid reacting substances (TBARS) value of the dairy products. Channa containing the antioxidants, the procedure used was given by Kaur et al. (2016). Ten g sample was finely blended with 50 mL of 20% Tri-chloro-acetic acid (TCA) in homogeniser/blender for two minutes followed by allowing the resultant extract to stand for ten minutes. The extract was filtered in a test tube through the Whatman filter paper. Three mL of this extract was mixed with an equal volume of 0.1% (w/v) TBA reagent. Simultaneously, three 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 half an hour. At 532 nm, the absorbance value of the samples was read and TBA value was calculated by comparing the test sample readings with that of the standard graph made by using known concentrations of malon-aldehyde. For standard graph preparation, 0.3055 g of 1,1,3,3, TEP was dissolved in hundred mL of ninety five percent absolute alcohol and a final concentration of one mg malon-di-aldehyde/mL was used for further preparation of solution. The 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 three g/mL of malon-di-aldehyde. The standard graph was prepared from this solution by using different concentrations of malon-di-aldehyde.

Free fatty acid (% oleic acid)

Free fatty acid (FFA; % oleic acid) values were estimated following (Ganie et al., 2016). Twenty-five g of a sample containing the antioxidant was blended with one hundred thirty-seven mL of chloroform for two minutes in presence of one teaspoon of sodium sulphate. The extract was filtered using Whatman filter paper no. 12. An aliquot of twenty-five mL of extract was transferred to conical flask. Then, 10 drops of 0.2% phenolphthalein indicator were added to it. The sample was titrated with 0.1 N 90% potassium hydroxide to (pink color) as end point. Another twenty-five mL of the extract was placed in a pre-weighed beaker for calculation at 80℃ in a drying oven. FFA could be calculated as:

$$\text{FFA (\% oleic acid)} = \frac{(0.1\ \text{mL}\ 0.1\ \text{N alc. KOH} \times 0.282 \times 100)}{\text{Weight of fat}}$$

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 calculated (Sharma et al., 2023).

Sample preparation

Ten g of the samples were taken aseptically and blended with 90 mL of 0.1 per cent sterile peptone in a pre-sterilized blend flask. Ten-fold serial dilution of sample was made in pre-sterilized tubes. The sample preparation was done following standard protocol on laminar.

Total plate count

23 g of plate count agar (code no. M091) was prepared in one litre distilled water. The media was boiled to dissolve the suspension and final pH was adjusted to 7.0. The media was then sterilized by autoclaved for 15 minutes and then cooled to 45℃. For the plating of the sample, the pour plate technique was followed. 1 mL of the inoculum was taken in duplicate and media was poured up to 2/3rd level of the pre-sterilized petriplates. These prepared plates were incubated at 35℃ for a day. The incubated plates that showed 30-300 colonies were counted and measured in Log_{10}CFU/ g of sample.

Psychrophilic count

The sample was prepared similarly as for the total plate count. The prepared plates were then incubated at 4℃ for half a month and the colonies were counted and measured as Log_{10}CFU/ g of sample.

Coliform count

42 g of violet red bile agar (code no. 049) was suspended in one litre of distilled water and then boiled to dissolve the media completely followed by cooled to 35℃ with final pH to 7.4. An overlay technique was used for the inoculation of suitable sample dilution was done and plates were incubated at 35℃ for one day. The colonies were counted and the results were measured in Log_{10}CFU/g.

Yeast and mould count

39 g of potato dextrose agar (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 (12 1℃) for half an hour. The final pH was adjusted to 3.5 at 25℃. The pour plate with overlay technique was followed for inoculation and the plates were incubated at 37℃ for one week. The colonies were enumerated and expressed as Log_{10}CFU/g.

Statistical analysis

Data obtained for different parameters (n=6 for *in vitro* antioxidant profile and storage or shelf life profile and n=21 sensory evaluation) were compiled and analyzed using Software Package Statistical Study version 21.0 (IBM, Armonk, NY, USA). Duncan's multiple range test using a significance level of 0.05 were used for comparisons. The results are presented in Tables as mean±SE and level of significance with varying superscript.

Technical programme

Our study included a series of experimental steps which were characterized for verification by the application of various analytical tests. Initially, extracts of *L. barbarum* and *D. denudatum* were prepared. On preparation, they were subjected to analysis for their *in vitro* antioxidant potential through estimation of their TPC and TF. Their radical scavenging activity was estimated through DPPH, ABTS and FRAP assays. Followed to this preparation and standardization of channa were done. Optimization and standardization of the process of incorporation of different levels of extracts i.e., 2%, 3%, 4% of *L. barbarum* and 0.15%, 0.25%, 0.35% *D. denudatum* to chhana were done to make acid coagulated milk model (channa) functional. Product quality profile was analyzed which consisted of sensory evaluation of the product, and the *in vitro* antioxidant profile of antioxidant extracts incorporated channa in terms of TPs, TFs and radical scavenging activity through DPPH, ABTS and FRAP assays. Based on the sensory evaluation, final levels of incorporation of *L. barbarum* and *D. denudatum* to channa, to make it functional were selected for further experimental proceedings. The prepared channa was subjected to refrigerated storage at 4℃ and was analyzed on weekly interval times for three weeks. The prepared channa was analyzed for product storage profile which included physico-chemical tests viz. estimation of FFA and TBARS and microbiological profile on week intervals.

Results and Discussion

The study is to unravel effects of herbs and nuts, rich in bioactive compounds on the quality and shelf life of acid coagulated milk model i.e., channa. In order to expedite the characteristic properties of *L. barbarum* and *D. denudatum*, nuts and herbs respectively are amalgamated in channa to improve the functional property and extend its shelf life.

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

Table 1 depicted that the *L. barbarum* extract has shown a high amount of TPC and TF. The extract was also exhibiting high radical scavenging activity in terms of DPPH, ABTS and FRAP aasay. The decline trend TPC, TF and the DPPH, ABTS and FRAP assays was observed when incorporated into channa.

Table 1. *In vitro* antioxidant profile of *Lycium barbarum* in acid coagulated milk model system (channa)

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 channa	25.78±1.79	15.93±2.78	14.30±0.86	10.41±1.38	3.78±0.19

Mean±SE; n=6 for each treatment.
TPC, total phenolic content; TFC, Total flavonoid content; DPPH, 2,2-diphenyl-1-picrylhydrazyl; ABTS, 2,2'-azino-bis(3-ethylbenzothiazoline- 6-sulfonic acid; TEAC, Trolox equivalent antioxidant capacity; FRAP, Ferric reducing antioxidant.

The total phenolic assay is based Folin-Ciocalteau method. Folin-Ciocalteau reagent contains phosphotungstic/phosphomolybdic acid complexes. There is a transfer of electrons from phenol functional group to form a bluish chromophore complex (phosphotungstic/phosphor-molybdenum) and the maximum optical density (OD) depends on the concentration of phenolic compounds. The molecule 1, 1-diphenyl-2-picrylhydrazyl (a,a-diphenyl-b_picryl-hydrazyl; a stable free radical in which delocalisation of the free electron occurs result in the molecule does not dimerize. The delocalization of electrons is responsible for deep violet color. When DPPH solution come in contact with a substrate Arbuzov-Huisgen (AH) that can donate an atom of hydrogen, this gives rise to the reduced form with the loss of this violet color. ABTS measures in color loss when an antioxidant moiety is added to the blue-green chromophore ABTSÆ+ (2,2-azino-bis(3-ethylbenz_ thiazoline-6-sulfonic acid). The antioxidant moiety reduces ABTSÆ+ to ABTS and decolorize it. ABTSÆ+ is a sturdy 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 analogue of vit. E. FRAP method is used to measure the capacity of the antioxidant moiety to lower ferric iron and TPTZ to ferrous form at acidic pH. This decrease in the trend of total flavonoid content (TFC), TF, DPPH, ABTS and FRAP assays may be attributed to the resistance offered by channa food matrices thereby lowering the exhibition of antioxidant activity of *L. barbarum* in khoa and channa. Islam et al. (2017) stated that all goji berries are rich in phenolics. The results were witnessed to be in non-agreement with the findings of Taneva and Zlatev (2020) who showed that enriched yoghurt with berries have higher phenolic content and higher radical scavenging activity. Similar results were quoted by Byambasuren et al. (2019) who found varieties of bioactive elements like flavonoids, carotenoids, polyunsaturated fatty acids having antioxidant properties. Skenderidis et al. (2019) concluded the *L. barbarum* was having higher content of carb and phenols than *L. chinense* Mill. Fruits. Gao et al. (2017) noted the pharmacological activities of LBP and other major components of *L. barbarum* and demonstrated significant antioxidant activities. The antioxidant and antimicrobial activities of *L. barbarum* flowers, as an alternative resource of naturally-occurring antioxidant moiety was revealed (Mocan et al., 2015). The exhibition antioxidant activity of *L. barbarum* significantly get reduced on incorporation channa, an acid coagulated milk model.

In vitro antioxidant profile of *Delphinium denudatum* in acid coagulated milk model system

The invitro antioxidant profile of *D. denudatum* extract had a remarkably high level of TFC and TF. The TFC, TF, and antioxidant activity in terms of DPPH, ABTS and FRAP assays decreased when *D. denudatum* was fortified into acid coagulated milk model system (channa). It may be due to the binding of hydroxyl and ketone groups of phenols and flavonoids with the food matrix of channa (Table 2). The results were in similar trend with findings of Ferreira et al. (2017), who demonstrated that *D. denudatum* has the bioactive compound beta-sterol, which has antioxidant properties. However, there had been no reporting of the incorporation of *D. denudatum* in any food product. The results were in concordance with Fadiloglu and Çoban (2019) who developed antioxidant-enrich fruit-augmented probiotic yoghurts. The result revealed that the antioxidant of antioxidant-rich fruit-augmented probiotic yoghurts decreased. The exhibition antioxidant activity of *D. denudatum* significantly get reduced on incorporation channa, an acid coagulated milk model.

Table 2. *In vitro* antioxidant profile of *Delphinium denudatum* in acid coagulated milk model system (channa)

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 channa	21.73±0.95	16.38±1.87	13.37±0.92	11.65±0.89	3.23±0.36

Mean±SE; n=6 for each treatment.
TPC, total phenolic content; TFC, Total flavonoid content; DPPH, 2,2-diphenyl-1-picrylhydrazyl; ABTS, 2,2'-azino-bis(3-ethylbenzothiazoline-6- sulfonic acid; TEAC, Trolox equivalent antioxidant capacity; FRAP, Ferric reducing antioxidant.

Sensory attributes of variants of *Lycium barbarum* fortified value added channa

When food quality is assessed employing human senses, the evaluation is subjective. It is the procedure for getting the correct opinion of consumer acceptance of the outcome product. Sensory evaluation of the outcome product was conducted in terms of color and appearance, texture, flavour and taste, and acceptability. Colour and appearance of any product decides its degree of liking by the consumers. Flavour is one of the essential parameters in the acceptability of a dairy product. Table 3 presented sensory attributes of variants of *L. barbarum* fortified value-added channa in which the level of 3% *L. barbarum* in channa had been liked by panelist to adjudge the best level of incorporation which may be due to combined effects from flavour compounds from *L. barbarum.* Therefore, the addition of fruit-based material lead to higher overall acceptability of channa at 3% of *L. barbarum* respectively. Similar reporting was done while working on the better score of consumer acceptability of prebiotic white chocolate with *L. barbarum* (Ferreira et al., 2017). Rotar et al. (2015) stated *L. barbarum* and honey affect the sensory quality of yoghurt and showed that use of *L. barbarum* improved sensory acceptance of consumers and acted as enhancer of probiotic levels in yoghurt. The results were in concordance with the experiments of on herbal and spiced paneer by Neethu and Nair (2020) revealed that the using malic acid oregano, the overall acceptability was higher. Therefore, channa preparation has been optimized successfully for its preparation with incorporation of 3% of *L. barbarum.*

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

Table 4 depicted sensory attributes of variants of *D. denudatum* fortified value added channa which revealed that the level of 0.25% *D. denudatum* in channa was found to be sensorily optimum by the panelists. The flavour of the product is the key attribute of selection of channa as the rest of the parameters remained unchanged. The scores for flavour presented higher in channa for 0.25% *D. denudatum* revealed higher scores. The overall acceptability of the product was

Table 3. Sensory attributes of variants of *Lycium barbarum* fortified value-added channa

Variables	Control	*Lycium barbarum* (2%)	*Lycium barbarum* (3%)	*Lycium barbarum* (4%)
Colour & appearance	7.89±1.075	8.15±1.092	8.43±1.108	8.69±1.104
Sourness	7.65±0.82	7.86±0.94	7.98±0.92	8.03±0.96
Softness	7.56±0.076	7.56±0.107	7.48±0.102	7.63±0.108
Flavour and taste	7.99±0.119^{a}	8.24±0.071^{ab}	8.69±0.097^{a}	7.19±0.098^{a}
Texture	8.39±0.159	8.54±0.188	8.66±0.172	8.49±0.176
Overall acceptability	7.85±0.085^{a}	8.24±0.082^{ab}	8.57±0.090^{c}	7.20±0.099^{a}

$^{a-c}$Mean±SE with different superscripts in a row wise differ significantly (p<0.05) n=21 for each treatment.

Table 4. Sensory attributes of variants of *Delphinium denudatum* fortified value-added channa

Variables	Control	*Delphinium denudatum* (0.15%)	*Delphinium denudatum* (0.25%)	*Delphinium denudatum* (0.35%)
Colour & appearance	8.47±0.050	8.56±0.099	8.55±0.079	8.56±0.080
Sourness	7.62±0.81	7.84±0.110	7.98±0.102	8.08±0.103
Softness	7.56±0.076	7.56±0.101	7.51±0.111	7.68±0.105
Flavour and taste	7.99±0.119[a]	8.25±0.082[ab]	8.65±0.099[a]	7.20±0.098[a]
Texture	8.39±0.159	8.54±0.189	8.69±0.114	8.42±0.172
Overall acceptability	7.85±0.085[a]	8.24±0.0780[ab]	8.58±0.091[c]	7.19±0.098[a]

[a-c]Mean±SE with different superscripts in a row wise differ significantly (p<0.05) n=21 for each treatment.

judged and influenced only on flavour of the outcome product. It has been shown that the flavour of foods fortified with extract was most sensitive indicator of their sensorial acceptability. These levels of extract incorporation in channa have been selected for further storage studies and microbiological profile analysis. The results were found in concordance with the findings of Vithushana and Jayaweera (2020) noted Aloe vera effect as preservative on the sensory attributes of flavoured pasteurized milk and revealed that the product was towards the category of "extremely-like". The literature revealed that Goyal et al. (2015) 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.' Therefore, channa preparation has been optimized successfully for its preparation with incorporation of 0.25% *D. denudatum.*

Shelf life analysis profile of *Lycium barbarum* and *Delphinium denudatum* extract in acid-coagulated milk model system (channa) on refrigeration storage

As TBARS is employed for monitoring secondary oxidation products, i.e., aldehydes or carbonyls, which may contribute to the off-flavour of the dairy product. Similarly, FFA estimation uses the titration method to determine the fatty acid conversion during lipid oxidation, which is responsible for dairy product deterioration. In the present study, both TBARS and FFA values have increased on successive storage days in the control and the *L. barbarum* treated product. However, the TBARS and FFA values were under the permissible limit in *L. barbarum* treated product, fit for human consumption even on the 21st day of storage (Table 5). It could be attributed to antilipolytic and antioxidant properties of active metabolites like quinic acid, quercetin in the form of 3-o-hexose coumaric esters, and quercitin 3-0- hexose- rhamnose present in *L. barbarum*. This was in congruence with the findings of Fadiloglu and Çoban (2019), who studied goji berry extract on quality traits of common carp sausages and demonstrated that goji berry retarded TBA values and lipid oxidation of common carp sausages. The result was further supported by Montesano et al. (2019), who added *L. barbarum* to extra virgin olive oil.

These physicochemical parameters, i.e., TBARS and FFA were found within the acceptable range in *D. denudatum* treated product compared to control, safe for consumers until three weeks of storage at four degree centigrade. This may be attributed to the anti-lipolytic and antioxidant properties of active metabolites like lycoctonine, aconitine, atisine, beatchine present in *D. denudatum.* There had been no reporting of the incorporation of *D. denudatum* in the food system to date. The results were in congruence with the result outcome of Kaur et al. (2016) who reported that the natural antioxidants extracted from pomelo peel added to fat and oil reduced the FFA values after incorporation and hence rancidity is prevented. Therefore, channa prepared with fortification of 3% *L. barbarum,* and 0.25% *D. denudatum* exhibited reduction in lipolysis and proteolysis and become less rancid at three weeks of storage period at refrigeration temperature.

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

The microbiological profile of *L. barbarum,* and *D. denudatum* treated channa at refrigeration temperature. *L. barbarum* treated channa has critically shown its antimicrobial

Table 5. Shelf life analysis profile of herbal extract in acid-coagulated milk model system (channa) on refrigeration storage at (4±1℃)

Products	Parameters (day)			
	0	7th	14th	21st
TBARS (mg malonaldehyde/kg)				
Control	0.243±0.011^{Ad}	0.437±0.009^{Ac}	0.673±0.014^{Bb}	1.110±0.022^{Ba}
Lycium barbarum fortified channa	0.267±0.012^{Bd}	0.326±0.012^{Ac}	0.464±0.011^{Ab}	0.857±0.016^{Aa}
Delphinium denudatum fortified channa	0.261±0.014^{Ad}	0.338±0.013^{Ac}	0.569±0.017^{Ab}	0.939±0.015^{Aa}
FFA (% oleic acid)				
Control	0.115±0.028^{Ad}	0.147±0.011^{Ac}	0.211±0.012^{Bb}	0.309±0.029^{Ba}
Lycium barbarum fortified channa	0.107±0.013^{Ad}	0.119±0.017^{Ac}	0.175±0.015^{Ab}	0.176±0.028^{Aa}
Delphinium denudatum fortified channa	0.109±0.014^{Ad}	0.117±0.018^{Ac}	0.185±0.017^{Ab}	0.229±0.054^{Aa}

$^{a-d}$Mean±SE with different superscripts in a row wise and ^{A,B}column wise significantly (p<0.05) n=6 for each treatment. TBARS, thiobarbituric acid reacting substances; FFA, free fatty acid.

properties by showing its activity against the aerobic and anaerobic count, psychotropic as well as antifungal activity. The *L. barbarum* extract incorporated channa acted as an oxygen barrier to microbial growth. The microbial load showed an increasing trend on successive storage days in the extract treated product along with the control. However, the microbial load was within an acceptable range in a treated product even after third week of storage (Table 6). These findings were supported by Fadiloglu and Çoban (2019) studied goji berry extract on common carp sausages quality upto four weeks of storage at 2℃ and concluded that goji berry extract inhibited the microbial population in extract treated product. The results observed were in the line with Ganie et al. (2016) who worked on the garlic extract in extending the paneer shelf-life.

Microbial counts are very important indicative to assure a proper shelf life. The current study considered the plate count, psychotroph count, coliform count, and yeast and mould count for microbial analysis of *D. denudatum* incorporated acid coagulated product (channa). The increase in trend was observed *in* the total plate count, psychotroph count, coliform and yeast - mould count irrespective of the treatment/control group. However, it was found that the microbial count was lowered in *D. denudatum* treated product and has been within the acceptable range in *D. denudatum* treated product even on three weeks of storage (Table 6). This could be because, the antimicrobial and anti-fungal agents namely lycoctonine, aconitine, atisine, beatchine present in *D. denudatum* might have reduced their growth and hence count was within acceptable limit. These outcomes were further supported by Sharma et al. (2021a) studied the antibacterial activity of *D. denudatum* against no. of micro-organisms (Ahmad and Arvind, 2016). The results were further favoured by Neethu and Nair (2020) those worked on herbal-spiced paneer and the result revealed that the incorporation of malic acid oregano reduced the microbial load. Similar results of were reported on increased the shelf life of the livestock products (Ahmad and Arvind, 2016). Similar results were quoted by Sharma et al. (2021b) who evaluated the traits of turmeric fortified paneer made from various types of bovine milk, resulted that the fortification of turmeric (0.6%) by weight and packaged in aluminium foil enhance the product shelf life up to two weeks storage at refrigerated temperature. Therefore, *L. barbarum* and *D. denudatum* extract are additives that preserve food from "farm to plate" and mitigate oxidative spoilage on storage and further processing. The property of low volatility and high stability of *L. barbarum* and *D. denudatum* extract as antioxidants aid to maintain the level of sensorial traits to consumers. Thus, channa prepared with fortification of 3 % *L. barbarum,* and 0.25% *D. denudatum* exhibited less microbial load and better shelf-life at three weeks of storage period at

Table 6. Effect on the microbiological profile of *Lycium barbarum* and *Delphinium denudatum* fortified channa at refrigeration temperature

Treatments	0 day	7th day	14th day	21st day
Total plate count (Log_{10}CFU/g)				
Control (channa)	2.54 ± 0.13^{Aa}	3.30 ± 0.12^{Bb}	4.05 ± 0.14^{Bc}	4.47 ± 0.019^{Bd}
Lycium barbarum fortified channa	2.64 ± 0.13^{Aa}	2.37 ± 0.21^{Ab}	3.22 ± 0.10^{Ac}	3.31 ± 0 .014^{Ad}
Delphinium denudatum fortified channa	2.33 ± 0.12^{Aa}	2.71 ± 0.21^{Ab}	3.77 ± 0.18^{Ac}	3.80 ± 0.012^{Ad}
Psychrotropic count (Log_{10}CFU/g)				
Control (channa)	ND	ND	1.24 ± 0.012^{Ba}	1.65 ± 0.015^{Bb}
Lycium barbarum fortified channa	ND	ND	0.56 ± 0.027^{Aa}	0.84 ± 0.020^{Ab}
Delphinium denudatum fortified channa	ND	ND	0.54 ± 0.021^{Aa}	0.82 ± 0.018^{Ab}
Yeast and mould count (Log_{10}CFU/g)				
Control (channa)	ND	ND	ND	1.93 ± 0.196^{B}
Lycium barbarum fortified channa	ND	ND	ND	0.25 ± 0.045^{A}
Delphinium denudatum fortified channa	ND	ND	ND	0.58 ± 0.067^{A}

$^{a-d}$Mean±SE with different superscripts in a row-wise and ^{A,B}column-wise differ significantly (p<0.05) n=6 for each treatment. ND, not detected.

refrigeration temperature.

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 channa 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 3% *L. barbarum* and 0.25% *D. denudatum* in channa. The storage profile of the product was also evaluated to retard lipid oxidation and antimicrobial activity of chhana prepared at 3% *L. barbarum* and 0.25% *D. denudatum*. The chhana prepared with the fortification of extract of *L. barbarum* and *D. denudatum* was found to be acceptable for more than 21 days of storage at refrigeration in contrast to control. Thus, functional channa can be prepared with the fortification of 3% *L. barbarum* and 0.25% *D. denudatum*.

Conflicts of Interest

The authors declare no potential conflict of interest.

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Ethics Approval

This article does not require IRB/IACUC approval because there are no human and animal participants.

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