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Optimisation of Processing Parameters for the Preparation of Vegetable Curd from Decorticated Sesame Seeds (*Sesamum indicum*)

Dev Kumar Yadav*, Veerakyathappa H.C., Prakash Eknath Patki, and Gopal Kumar Sharma

DRDO-Defence Food Research Laboratory, Mysuru - 570 011, India
**E-mail: dev@dfrl.drdo.in:*

ABSTRACT

Current study aims to develop and optimise the processing parameters for the preparation of imitated curd by using vegetable milk extracted from decorticated sesame seeds (*Sesamum indicum*). Experiments were planned to optimise curdling process for sesame milk with respect to inoculum (1-5%), lactose (1-5%), added proteins (1-3%). It was established that total solid content in a range of 9-10%, dispersion stability with 30-45% scored well (7.8±0.2) on preferential sensory test i.e. nine-point hedonic scale. Sesame milk supplemented with 5% lactose, 1% electrolyte salt mix, 1% sucrose was subjected to fermentation (10±1.0) hrs at 35±2°C) with 5% inoculum (lactic acid bacteria culture) resulted into a vegetable curd with desired consistency (855.6±47.5 g.sec), acidity (0.57±0.02% lactic acid equivalent; LAE), flavor and overall acceptability (OAA). The Solid Not Fat (SNF) content of optimised vegetable curd was found to be 22.20±0.40%, with protein, fat and ash content as 6.70±0.06%, 6.91±0.06% and 0.92±0.01% respectively. The characteristics of vegetable milk (non-dairy) and toned milk (dairy) were well comparable on various aesthetic parameters (fluidity, color, flavor and taste). Proportional characterisation of vegetable (non-dairy) curd with dairy curd relating to its nutritional profile, color analysis, textural and rheological parameters established its similarity. Such dairy analogues may be considered as substitute to various dairy products where milk-based products are scanty or non-available.

Keywords: vegetable curd; acidity; sensory acceptability; optimisation

1. INTRODUCTION

It's a matter of fact that mammary gland secretion of any animal is basically to feed its own siblings, however, after civilisation mankind assumed its nutritional strength and started consuming milk as extracted from various animals viz. cow, goat, buffalo, camel etc. for appreciating its various nutritional benefits[1]. Processing interventions led to the evolution of various value-added dairy products such as condensed milk, flavoured milk, curd, butter milk, clarified butter, shrikhand, cheese, yoghurt, and ice-cream etc.[1]. These became unanimously acceptable to the majority of individuals owing to their well acceptable organoleptic profile and nutritional strength. Owing to various geo-climatic conditions and economic status of various countries in general, and developing nations in particular, huge population suffers from the unavailability of milk. This may be further attributed its unaffordable price, low production rate (during summer session), milk allergy (especially cow milk), high cholesterol, and lactose intolerance and unethical practices (adulteration) during production resulting in its substantial quality etc. Sensitivity to animal-milk protein (particularly cow) causes varying degrees of injury to the intestinal mucosal surface[2]. Moreover, milk is an animal product and has its dependency on the reared animals[3]. Hypersensitivity of an individual to animal milk may be attributed to either lactose or protein fractions present in milk[4, 5].

The challenges associated with the consumption of dairy milk led adoption of various research methodologies to develop imitated milk products or dairy milk substitutes by using edible portions of various plants *viz.* peanut (*Arachis hypogaea*), Soy (*Glycine max*), coconut (*Cocos nucifera*), almond (*Prunus dulcis*), rice (*Oryza sativa*), Bambara ground nut (*Vigna subterranea*), Decne (*Treculia africana*), sesame seeds (*Sesamum indicum*) chick peas (Cicer arietinum), pigeon peas (*Cajanus cajan*), black gram (*Vigna mungo*), mung beans (Vigna radiata), cowpea (*Vigna unguiculata*), sunflower (*Helianthus annuus*)[6-10] etc. The rising cognizance about nutritional benefit of plant-based foods by health-conscious users has transformed attention of various investigators for developing vegetable milk and its value added products[11'12]. As plant based imitated milk extracts are free from cholesterol and other dairy proteins inherently therefore, their edible portions suitable for milk preparation may be included as complements of pre-school and school children's diet or may be adopted as vegan substitute for dairy products for allergy and taste susceptive individuals of all age groups. Several researchers have worked on the development of vegetable milk from legumes and oil seeds[13,14]. Among the sources of vegetable milk, soybean has received considerable attention and various

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protocols have been developed for its domestic as well as for its commercial production[15].

Recently researches are oriented towards reconnoitering the consumption of oilseeds in general and sesame seed sespecially, for new food uses owing to their desirable functional characteristics. Dehulled/decorticated sesame seeds are considered to be an economical source of various functional and health promoting nutrients[16]. Soy based imitated dairy products limits their consumption mainly due to the presence of 'Beany and Astringent' flavour, presence of anti-nutritional factors, low solids yield, low dispersion stability, and flatulence factors inherently associated with it. The production of sesame-based imitated dairy products can surmount the challenges that limit consumption of imitated dairy products from soy and other plant sources. The nutritional profiling of decorticated sesame seeds establish following composition: 45-63% crude fat, 19-31% protein fraction, 14-20% carbohydrates and 3-5% minerals while establishing them as suitable inventory of such applications. Sesame seeds are rich in sulphur-containing amino acids but limits in essential amino acid lysine. Presence of significant amounts of antinutritional components such as oxalic (80-85mg/100g) and phytic acids (25-30mg/100g) also lures consideration for various pretreatments for these seeds before using them for milk extraction. As oxalic acid is present in the hulls, decortication is an effective mechanical operation to achieve significant oxalate reduction. The fat fraction of sesame seed seems quite balanced with 14% saturated, 39% mono-unsaturated and 46% poly unsaturated fatty acids. Its carbohydrate fractions are inclusive of 3.2% glucose, 2.6% fructose, 0.2% sucrose along with presence of significant amount of dietary fibre. Various physiological benefits of sesame seeds were established in experimental animals/humans model systems[16] inclusive of antioxidant activity, blood pressure, & serum lipid lowering properties. Fermentation being age old technique for improving taste, preserving food and fostering various health benefits was used to improve various plant based milk substitutes. In anticipation to processing possibilities and nutritional strength of decorticated sesame seeds, present investigation was undertaken to develop non-dairy vegetable curd, and the processing parameters were optimised. Effect of various factors such as inoculum, lactose concentration, and protein supplementation was studied to understand their effect of curdling of vegetable milk. The resulted optimised vegetable curd was subjected to comparative proximate composition analysis, hunter color profile, textural properties, rheological characteristics, and sensory attributes with dairy curd[17].

2. MATERIALS AND METHODS

2.1 Raw Materials

Good quality white Nylon till i.e. sesame seed, salt mixture (electrolyte powder), and sugar were purchased from the local market of Mysore, Karnataka, India. The chemicals used for the analytical work were of AR/LR grade (HIMEDIA, SD- Fine chemicals and Ranbaxy).

2.2 Processing Methods

2.2.1 Pretreatment

Sesame seeds were observed carefully for the physical parameters like colour, appearance, infestations, etc. The seeds were first cleaned and washed thoroughly in hot water at 80 °C and cooked for 5 min in boiling water (1:5, Seed: Water) followed by periodic soaking of 10 h each in 0.1% NaOH (80 °C) and 2% brine solution (80 °C) subsequently cooking for 5 min in water.

2.2.2 Preparation of Vegetable Milk

Pretreated seeds* were used for milk extraction. Cooked seeds (100g) with hot water (1:5) were grinded using heavy duty blender at 1500 rpm for 15 min to extract the milk followed by filtration using double layer of muslin cloth in hygienic conditions, finally making the volume of extract to 500-600 ml. Extracted milk was concentrated by agitated boiling to increase the total solid content for proper curdling during fermentation.

*Oil seeds were screened to remove damaged seeds, odd material such as dust/straw/stones followed by cleaning with water. Subsequently sesame seeds were soaked in 0.1% lye solutions at pH 11.5 (Treatment-1)/ 2% H_2O_2 solution at pH 9.5 (Treatment-2) for 8 h in (1:5) ratio, washed to make it free from alkali/peroxides. This step was followed by soaking seeds in 2 % saline solution at pH 7.0 for 12 h in (1:5) ratio. The treated seeds were cooked using steam under pressure for 15 min. This treatment to sesame seeds was given to remove its oxalate content and to remove its bitterness.

2.2.3. Preparation of Curd

Vegetable milk was added with 5 % of lactose, 1 % electrolyte salt mix, and 1 % sucrose, which play a major role in curdling as well as in sugar salt balance were added. Then milk was homogenised in grinder for about 5 min for the uniform distribution of added ingredients, followed by cooking for 5 min. Then milk was cooled down and finally 5 % of starter culture (Fresh curd from dairy) which is a source of Lactic Acid Bacteria for curdling was added. It was incubated at room temperature (35±2°C) for 10-12 h.

2.3. Analytical Determinations

2.3.1 Physico-chemical Analysis

The experimental samples of vegetable curd were analysed for % titratable acidity, moisture, and total ash as per method given by AOAC[17]. Total protein was estimated by Kjeldhal method[18] using Gerhardt nitrogen digestion (Turbotherm) and distillation (Vapodest) auto apparatus (Bio incorporation, India). Crude fat, total solids and SNF were analysed as per the method given by FSSAI (2015)[19]. The pH was measured using a digital pH meter (METTLER TOLEDO Five easy plus- FEP20) as per methods explained by Steinkraus[20], *et al.*

2.3.2 Rheological Analysis

The rheological parameters were determined using rotational Viscometer[21]. The temperature of the system was set and maintained at ambient temperature (25 °C) for the flow curve. The flow curves of the milk and curd were determined using shear rate ranging from 0-100 s^{-1}.

2.3.3 Texture Profile Analysis of Vegetable Curd

The Textural profile analysis of the sample was carried out by using a digital texture analyser (TAHd^{plus}) stable micro system UK. As the product was delicate, hence 50 kg load cell was used. The textural parameters studied were firmness (g), consistency (g·sec), cohesiveness (g) and index of viscosity (g·sec). The experiment was carried out in compression mode using A/ BE d-40 (back extrusion rig). The test parameters were as follows, pretest speed = 1 mm/sec, test speed = 1 mm/sec, post test speed = 10 mm/sec, the distance travelled = 30 mm in a sample container having a 100 g sample with sensible trigger force = 10 g. The experiment was carried out in triplicates. Before the experiments the instrument was calibrated for force and height.

2.3.4 Colour Measurements (CIE System)

Colour values were assessed using a Miniscan XE Plus, Model 45/0-S, Hunter Lab, Hunter Associates Laboratory Inc., Reston, VA, USA. Hunter L (lightness), a (redness-greenness), and b (yellowness-blueness) values were measured. The measurements were done by D-65 illuminate and 10° observer. Standardisation of instrument for calibration was performed using standard black and white tile followed by measurement of samples. The values were recorded by a software program Easy match QC. Colour measurements were taken in triplicates, and average values were taken for calculation.

2.3.5 Sensory Evaluation

The optimised vegetable milk and prepared vegetable curd of 10 to 12h fermentation time was analysed organoleptically as well as by determination of titratable acidity (TA) as % lactic acid equivalent (LAE), pH and TSS of the sample. The test panel consisted of 30 semi trained members. The panelists evaluated the vegetable milk compared with normal dairy toned milk and vegetable curd compared with normal dairy curd samples for its colour, taste, flavour, consistency and overall acceptability. The nine-point hedonic scale grading was given and followed as 9= Like extremely to1= Dislike extremely.

2.4 Statistical Analysis

Statistical software (Statistical, Ver 7.1 Series 1205) was applied for analysing the data collected as per experimental design. Assessment of statistical significance between the parameters were performed by 2-way ANOVA using Duncan's multiple range test while considering significance at $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1 Effect of Lactose and Inoculum on Curd Forming Properties of Vegetable Milk

Modern day life style has come up with various kinds of digestion problems and lactose intolerance is one of them. Lactose intolerance restricts the individual from consuming foods having lactose as one of its components like, milk and milk products. Introduction of conventional milk alternates can efficiently be utilised to overcome lacuna in digestion. Vegetable milk is one such substitute of conventional milk which can effectively be utilised as milk substitute. Appropriated curdling of the dispersed solids in any fluid (dairy / nondairy) can be achieved with proper ratio of total solids (13-14%), protein (4-5%), fat (3.5-4%), and lactose (4.5-5%)[22].

During current research vegetable milk was produced from decorticated sesame seeds and further utilised for the development of vegetable curd. During preliminary studies the role of varying concentration of inoculum, lactose, protein and total solid were analysed for desirable curdling of milk. The preliminary studies for standardising the concentration of inoculum and lactose was carried out in two different sets of experiment. In first set, 100 ml of vegetable curd was inoculated with varying concentration of inoculum (1-5%) while, keeping the concentration of lactose constant. The content was allowed to ferment for 10-12 h at room temperature (35±2°C) and analysed for the development of acidity during fermentation. Inoculum at 5% concentration was optimised as it had significant positive impact on curd formation. In second set of experiment the concentration of lactose was varied from 1-5% while keeping the inoculum concentration constant (5%) and allowed to ferment under specified conditions. A positive impact of increasing lactose concentration was observed on texture and acidity of the vegetable curd, the increased acidity levels were observed for curd having higher lactose content. This can be attributed to the activity of LAB microorganisms which tends to produce acids while utilise lactose as substrate[23]. The acidity of curd at lactose concentration of 1-5% varied from 0.570 to 0.634% (Table 1). On the basis of above results the inoculum and lactose concentration were optimised at 5% level each. However, the desirable curd formation was not achieved which might be due to the lack of protein and solid content required to achieve the proper curd formation in milk[24]. The product obtained during preliminary analysis was subjected to sensory evaluation. It tasted similar to sweet beverage rather like curd, possibly due to the lower concentration of lactose which had significant impact on fermentation capability of lactic acid bacteria. Similar results for the importance of lactose concentration in curd preparation were reported in previous researches[24].

Table 1. Effect of lactose on curd forming properties of vegetable milk with 5% inoculum

Sample	% Lactose (added)	% Acidity (lactic acid equivalent
S1	1.00	$0.570^{a}±0.003$
S2	2.00	$0.589^{b}±0.006$
S3	3.00	$0.590^{b}±0.006$
S4	4.00	$0.602^{c}±0.001$
S5	5.00	$0.634^{d}±0.100$

The values are mean (n=3±SD) and the value with different superscript in the same column are significantly different (p<0.05)

3.2 Effect of Protein content on Curd Forming Properties of Vegetable Milk

The protein and total solid content of the vegetable milk obtained from sesame were 2% and 9.0% respectively. The low protein content is also anticipated to negatively affect the curdling of vegetable milk after fermentation,[25] therefore; the other approach for proper curd preparation performed was the addition of dairy (casein and whey) as well as sesame protein isolates. Sesame protein isolates were prepared in the laboratory by isoelectric precipitation[26] while casein and whey protein isolates used were from commercial sources. Normally, protein content of the standard dairy milk ranges between 3.5-4.0%, hence these isolates were added in 1, 2 and 3% concentration while keeping the concentration of lactose and inoculum percentage constant (5%). The experiments were subjected for 10-12h of fermentation at ambient temperature conditions (35±2°C). On sensory evaluation of the sample it was realised that the samples have undergone purification rather than fermentation. This may be attributed to the poor solubility of added protein and its higher availability of selective fermentation, resulting in undesirable flavour leading to poor organoleptic acceptance[27]. Hence the protein concentration was optimised at 2% level.

3.3 Effect of Total Solids on Curd Forming Properties of Vegetable Milk

It was obvious from the above sets of experiments that for the proper curd formation from vegetable milk total solid content and optimum fermentation are vital. Separately increasing lactose or inoculum or protein will not be a suitable approach. Therefore, the total solid content of vegetable milk was enhanced (18-20%) by concentrating the vegetable milk (40- 50% moisture removal while agitated boiling). The milk was subjected to fermentation for 10-12h at ambient temperature conditions (35±2 ºC) with lactose, protein and inoculum concentration of 5, 5 and 2%, respectively. This resulted into proper curd texture and acidity (0.55±0.08% LAE). The product was subjected to sensory evaluation carried out by the sensory panel of 30 semi trained panel members. Even though the desirable texture in product was achieved but the taste was not very desirable as it scored less 6.5 on 9-point hedonic scale. To improve the overall acceptability of vegetable curd, salt mix (electrolyte-1.0%) and sucrose (1.0%) was added as it was assumed by the panelists that poor taste of the curd was due to improper salt and sugar balance. The overall aesthetic attributes such as flavour, consistency, taste, mouth feel and overall acceptability was comparable with dairy curd.

3.4 Proximate Composition Analysis of the Vegetable Curd

The proximate composition analysis of vegetable curd in comparison with dairy curd is as shown in Table 2. The moisture content of vegetable curd was lower (70.90%) as compared to dairy curd (87.85%) this can be attributed to higher moisture loss during the concentration process of vegetable milk, which was performed to achieve high solid content for proper curd formation. Total solid content of vegetable curd was 29.14±0.37% which was significantly ($p<0.05$) higher than the dairy curd. The higher solid content may also be attributed to added lactose, electrolyte mix and sucrose. The protein, fat, ash and solid-not-fat of vegetable milk were 6.70, 6.91, 0.92 and 0.57%. The high percentage of protein, fat and solid-not-fat can be attributed to moisture removal during concentrating process of and indigenous composition of vegetable milk (sesame milk). The fermentation process resulted in proper acidity and flavour development in the vegetable curd and resulted in improved overall acceptability[28].

Table 2. Comparative proximate component analysis of the vegetable curd

Proximate composition	Vegetable curd	Dairy curd
Moisture (%)	70.90^{a}±0.34	87.85^{b}±1.10
Total solids (%)	29.14^{b}±0.37	12.15^{a}±0.85
Crude protein (%)	06.70^{b}±0.06	03.20^{a}±0.20
Crude fat (%)	06.91^{b}±0.06	03.50^{a}±0.10
Total ash (%)	00.92^{b}±0.01	00.72^{a}±0.05
Titratable acidity (%) lactic acid equivalent (LAE)	00.57^{a}±0.02	00.60^{a}±0.02
SNF (%)	22.20^{b}±0.40	08.50^{a}±0.54

The values are mean (n=3±SD) and the value with different superscript in the same row are significantly different ($p<0.05$)

3.5 Colour Analysis

The colour of curd sample is vital in deciding the sensory acceptability of the sample. Therefore, the colour analysis was carried out by using a Miniscan XE Plus, Model 45/0-S, Hunter Lab, Hunter Associates Laboratory Inc., Reston, VA, USA. Hunter L (lightness), *a** (redness-greenness), and *b** (yellowness-blueness) values for curd samples were measured. The colour values are as mentioned in Table 3. The results showed that colour profile of vegetable curd was comparable to commercial dairy curd. The degree of brightness for vegetable and dairy curd was observed to be similar with L values of 84.25 and 84.59 respectively, whereas the vegetable curd was observed to have higher b* value (5.43). This signifies the slight yellowness of vegetable curd in comparison with dairy curd. The a* value for vegetable curd and dairy curd was in green coordinates having *a** values of 0.57 and 2.29 respectively. The greenish tinge in vegetable curd was lower than dairy curd, which might be due to the absence of whey protein in vegetable curd[29]. The results obtained were in accordance with the results for soy yoghurt.

Table 3. Comparative colour analysis of vegetable curd

Sample	L	a*	b*
Vegetable curd	84.25^{a}±0.02	0.57^{a}±0.01	5.43^{a}±0.05
Dairy curd	84.59^{b}±0.10	2.29^{b}±0.01	4.91^{b}±0.04

The values are mean (n=10±SD) and the value with different superscript in the same column are significantly different ($p<0.05$)

3.6 Comparative Rheological Analysis of Vegetable Curd

The apparent viscosity (η) against the shear rate (γ s^{-1}) was represented to study the rheological behaviour of vegetable curd. Fig. 1 illustrates the viscosity variation in final product as a function of shear rate. The apparent viscosity was observed to depend on the shear rate. The initial apparent viscosity of vegetable and conventional curd was 838 and 742 (Pa) and reduced drastically at shear rate of 5.26 s^{-1}. This indicates the

Non- Newtonian behaviour[30] of curd. The apparent viscosity was observed to be negatively affected by shear rate. This clearly indicates that curd samples exhibited shear thinning behaviour with apparent viscosity characteristics. In curd samples, the apparent viscosity decreased rapidly with increasing shear rate until about 5.26-5.28 s^{-1} and then decreased slowly with shear rate to the maximum shear rate of 100 s^{-1}. Comparatively vegetable curd showed higher initial apparent viscosity values than commercial dairy curd samples[30].

Table 4. Comparative texture analysis of the vegetable curd

Batch	Firmness (g)	Consistency (g.sec)	Cohesiveness (g)	Index of viscosity (g.sec)
Vegetable curd	33.5^b±1.2	855.6^b±47.5	25.0^b±2.4	54.7^b±5.7
Dairy curd	28.7^a±2.4	736.1^a±64.9	19.2^a±1.2	34.7^a±4.5

The values are mean (n=3±SD) and the value with different superscript in the same column are significantly different (p<0.05)

Table 5. Comparative sensory analysis of the vegetable curd on nine-point hedonic scale

Sample	Taste	Flavour	Consistency	Appearance	Overall acceptability (OAA)
Vegetable curd	7.8^a±0.2	7.8^a±0.0	8.0^a±0.1	7.7^a±0.1	7.8^a±0.2
Dairy curd	8.2^b±0.1	8.2^b±0.1	8.1^a±0.1	8.2^b±0.0	8.2^b±0.1

The values are mean (n=3±SD) and the value with different superscript in the same column are significantly different (p<0.05)

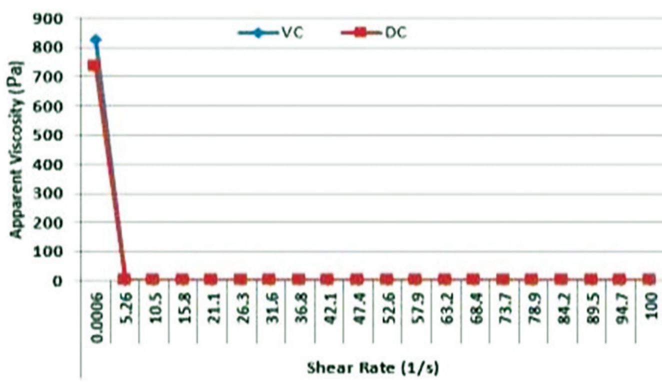

Figure 1. Comparative rheological analysis of vegetable curd.

3.7 Textural Profile Analysis of Vegetable Curd

Firmness is a textural property basically governs the moderate resistance to deformation and falls on the same property spectrum as that of hardness/softness based on the magnitude of the force needed for deformation. The vegetable curds experienced comparatively greater force than dairy curd (Table 4). The difference can be explained based on the characteristic properties of soluble and insoluble proteins and fat matrix. The softer texture of vegetable curd was established as the function of protein, fat and lower chain fatty acids[31,32]. Consistency in textural profile relates to the 'firmness', 'thickness' or 'viscosity' of a liquid or semi-solid fluid. The consistency of vegetable curd was higher than dairy curd which may be due to firm setting and curdling of solids after fermentation[33]. During second compression of texture analysis of curd, the selected probe senses the pull force which is measured as the cohesiveness and explained as the rate at which the material disintegrates under mechanical action[34]. Vegetable curd was more cohesive than the dairy curd and can be related to the good mouth feel, as depicted by sensory analysis (9-point hedonic scale). Due to higher cohesiveness the retention time of curd over tongue was more fostering more perception. Index of viscosity is the parallel term to the consistency and similar pattern for this parameter was also observed[35].

The comparative sensory analysis of vegetable curd was carried out for various organoleptic attributes such as taste, flavour, consistency, appearance and overall acceptability on a preferential sensory test i.e. nine point hedonic scale3. The results showed (Table 5) that vegetable curd with overall acceptability score (7.8±0.2) has comparable sensory characteristics[36].

4. CONCLUSIONS

The study was conducted to establish a non-dairy alternative of milk and milk product, suitable for the consumers having lactose intolerance. The study concludes that, increasing protein, fat, lactose as separate component will not facilitate proper curdling of vegetable milk, rather a whole matrix in concentrated form produces synergistic effect and facilitates the desirable curdling and production of optimum texture during preparation of vegetable curd from decorticated sesame seeds milk. Finally, the optimised curd with highest sensory score was obtained from a batch having, inoculum (5%), lactose (5%), sucrose (1%), and electrolyte mixture (1%). The optimised vegetable curd was accepted readily on organoleptic grounds, as the sensory score for the vegetable curd was above 7 on nine-point hedonic scale. The study resulted in a non-dairy fermented product with well acceptable sensory profile which can be an efficient alternative to the conventional curd and encounter the terrain specific requirement of troops posted at far-flung areas where supply of animal milk (dairy or local supply) is scanty due to its perishable life and availability on time.

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CONTRIBUTORS

Mr Dev Kumar Yadav, Scientist 'D' is presently working in Grain Science and Technology Division at DRDO-Defence Food Research Laboratory, Mysuru. He has published more than 28 publication in various peer reviewed journals. His research areas includes modification of starches for specific food applications, development of microwave based disinfestation process, instant foods based on cereals, pulses and Millets, development of imitated milk products from decorticated sesame seeds, design and development of ration storage system for Service specific applications.
He is the lead writer of the present paper and help to research work as per plan and objective.

Mr Veerakyathappa, H.C. has done his BTech (Food science and technology) from University of agriculture sciences Bengaluru. And post graduate diploma in Food Analysis and Quality Assurance from DRDO-Defence Food Research Laboratory, Mysuru.
He supported the experimental execution of the present research work.

Mr Prakash Eknath Patki was working as Scientist G and was Head of Division in Fruits and Vegetable Technology at DRDO-Defence Food Research Laboratory, Mysuru. He has worked in the development of instant foods based on cereals, pulses, and millets. He has published 40 research papers and10 patents to his credit.
He has contributed meticulously for the suitable content and layout of the manuscript.

Dr Gopal Kumar Sharma, Sc 'G', obtained his PhD (Chemistry) from Lucknow University. He had worked as Associate Director at DRDO-Defence Food research Laboratory, Mysuru. He has significantly contributed in separation and identification of various flavours and off flavour compounds from various processed foods, development of convenience foods, and indigenisation of low cost processed equipment.
His contribution includes the planning and guiding of the work.