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Nutritional qualities of Synbiotic cottage cheese using *Lactobacillus acidophilus* (*la-5*) and *Lactobacillus casei* (*Ncdc-298*) with pectin

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Abstract
Cottage cheese is a fresh curd cheese obtained from skim milk, with mild flavour, low fat and high protein content. A study was conducted to estimate the nutritional qualities in the synbiotic cottage cheese using *L. acidophilus* (*LA-5*) and *L. casei* (*NCDC-298*) with pectin. The product was prepared and stored at 4 °C for 14 days during which the nutritional parameters were studied on day 0, 3, 7 and 14. The protein content was found highest for T4, 14.51 per cent and lowest for the control 13.84 per cent, while the fat content was highest for T1, 2.12 per cent and lowest for T3, 1.16 per cent, further the lowest moisture content of 63.39 per cent was observed for T4 than control and other treatments, similarly the energy value of 142.81 kcal/100gm was observed for T4 than control and other treatments on 14 th day of storage. It was concluded that developed synbiotic cottage cheese had better nutritional qualities than that of the control.

Keywords: *Lactobacillus acidophilus* (*LA-5*), *Lactobacillus casei* (*NCDC-298*), pectin, moisture, ash, protein, fat, gross energy

Introduction
Cottage cheese has a good potential for delivery of probiotic microorganisms into human intestine due to its specific chemical and physical properties like higher pH value and lower titratable acidity, higher buffering capacity, greater fat content and denser matrix of the texture, as well as high nutritional value.
Lactobacillus acidophilus is widely recognized to have probiotic effects and is one of the most commonly suggested organisms for dietary use (Shah, 2007) [7]. *Lactobacillus casei* is documented to have a wide pH and temperature range, and complements the growth of *Lactobacillus acidophilus.* The health beneficial properties of probiotic bacteria include anticarcinogenic, stimulation of immune system, alleviation of lactose intolerance, serum cholesterol reduction, nutritional enhancement (like calcium absorption and production of B complex vitamins) and prevention of diarrhoea caused by *E. coli*, *Salmonella* and *Shigella*.
Pectin is a prebiotic, that is a soluble dietary fibre which exerts physiological effects on the gastrointestinal tract, such as reducing glucose absorption, enhancing hypocholesterolaemic effect and improving gastric emptying.
The term "synbiotic" refers to food ingredients or dietary supplements combining prebiotics and probiotics in a form of synergism, that beneficially affect the host by improving the survival and implantation of live microbial dietary supplements in the gastrointestinal tract.
In the present-day scenario, there is an increasing demand for low calorie foods with high therapeutic properties against common health problems like obesity, diabetes, hypertension, coronary heart diseases, etc. The young population is trending towards new varieties of healthy foods having good palatability, low fat content and high health benefits. The development of functional food with the addition of prebiotic and probiotic is an emerging need of the hour to deal with the challenges in modern biology.

Material and Method
Skim milk: Fresh whole milk was received from Livestock Farm Complex, Veterinary College and Research Institute, Namakkal, Tamil Nadu, India. The skim milk was obtained from whole milk by centrifugation method after separation of cream.

Pectin: Neotea pectin powder was purchased from Neoteric DCBA Ideas, Tamil Nadu, India and was preserved in a moisture proof pack for incorporation in cottage cheese.

Starter culture: Cheese starter culture, *Lactobacillus acidophilus* (*LA-5*) and *Lactobacillus casei* (*NCDC-298*) was procured in freeze dried form, from National Collection of Dairy cultures, National Dairy Research Institute, Karnal, India for the preparation of synbiotic cottage cheese.

Preparation of synbiotic cottage cheese: Cottage cheese was prepared as per Blanchette *et al.* (1996) [3], with certain modifications. In each treatment, fresh whole milk was preheated to 40°C and the cream was separated to obtain skimmed milk. Pectin was added @ 0.1 to 0.5 per cent to the skimmed milk and it was pasteurized at 80°C for 10 minutes then cooled down to 32°C. Calcium chloride was added to it at 0.02 per cent level and stirred well and kept it for 10 minutes. Cheese culture or the probiotics were added at the rate of 5% and incubated at 37- 40 °C for 40 minutes to 1 hour for development of acidity. After development of acidity, rennet was added at 0.1-0.2 g per litre of milk and incubated at 37 °C until a firm coagulum was formed. The coagulum was then cut into small cubes and cooked up to 53-56 °C for 90 minutes. The whey released during the cooking process was drained using a muslin cloth and the curd cubes were washed with 21 ^{0}C and 5 ^{0}C water to remove excess acidity. The excess water was drained off the cubes and then it was salted at 0.6%. The developed cottage cheese was packed in polystyrene cups and stored at 4 °C.
Synbiotic cottage cheese with 0.3 per cent pectin was found to be the optimum level of incorporation based on sensory evaluation and thus it was used for studying the nutritional parameters.

The following three combinations of treatment and control have been prepared for this study.
C-Control (Skim milk + Commercial cheese culture)
T1-Skim milk + Commercial cheese culture with 0.3 per cent pectin
T2-Skim milk + *Lactobacillus acidophilus* (*LA-5*) culture with 0.3 per cent pectin
T3-Skim milk + *Lactobacillus casei* (*NCDC-298*) culture with 0.3 per cent pectin
T4-Skim milk + *Lactobacillus acidophilus* (*LA-5*) + *Lactobacillus casei* (*NCDC-298*) culture with 0.3 per cent pectin

Nutritional parameters
Moisture: As per AOAC (2005) [1], 5 g of sample was taken in a dry petri dish and placed in an oven at 105°C for overnight. The samples were desiccated and weighed. The dish was returned into the oven for another half hour and again cooled and reweighed.

$$\text{Moisture \%} = \frac{\text{Fresh weight of sample - Dry weight of sample}}{\text{Fresh weight of sample}} \times 100$$

Fat: 5 g of cottage cheese sample was taken and the fat content was determined by Soxhlet extraction method as stated by Ogunlade *et al.*, (2017) [5].

Protein: 2 g of cottage cheese sample was taken and protein was estimated by Kjeldahl nitrogen method (Ogunlade *et al.*, 2017) [5].

Ash: 5 g of cottage cheese sample was weighed into porcelain crucible previously ignited and weighed. The material was ignited in the fume cupboard until no fumes was seen charred of the organic matter and then transferred into muffle furnace at 550 °C for 3 hr., and cooled in a desiccator and weighed (Egbenni *et al.*, 2010) [4].

$$\text{Total ash \%} = \frac{\text{(Weight of ash + Crucible weight)–Empty weight of crucible)}}{\text{Weight of sample}} \times 100$$

Gross energy: Gross energy of cottage cheese was calculated from other proximate parameters (Egbenni *et al.*, 2010) [4].

Statistical analysis: The data were analyzed by two way ANOVA in SPSS (version 20.0).

Results and Discussion
Moisture content: It was observed that the moisture content gradually decreased during storage for all the treatments (Table 1). The lowest moisture content was 63.39 ± 0.28 for T4 on day 14 of storage and the highest was observed for T1 (81.87 ± 0.20) on day 0 of storage. The moisture content varied significantly (P< 0.05) between all the treatments during storage except between day 3 and 7 of storage. The moisture content of synbiotic cottage cheese was found to decrease significantly (P< 0.05) between day 0 and 14 of storage period, which might be due to increase in acidity that would have caused the protein matrix in the curd to contract and squeeze out the moisture content (syneresis). The mean moisture content of various treatments during the storage ranged from 63.39 to 81.87 per cent, which is in close agreement with Swati *et al.* (2018) [9] and Egbenni *et al.* (2010) [4] they observed the mean moisture content of 68.33 and 64.00 per cent, respectively for cheeses produced from raw milk.
In a similar study, Araujo *et al.* (2010) [2] developed a synbiotic cottage cheese with *Lactobacillus delbrueckii* UFV H2b20 and inulin, and found moisture content of 81.01 and 79.22 per cent for synbiotic cheese and control cheese, respectively.

Table 1: Moisture content (%) of synbiotic cottage cheese during storage at 4 °C

Treatments	Storage period (days)			
	0	3	7	14
C	75.89 ± 0.34^{Cc}	75.32 ± 0.35^{Bc}	75.10 ± 0.36^{Bc}	74.19 ± 0.35^{Ac}
T1	81.87 ± 0.20^{Ce}	81.36 ± 0.23^{Be}	81.10 ± 0.09^{Be}	80.29 ± 0.32^{Ae}
T2	79.43 ± 0.13^{Cd}	79.09 ± 0.33^{Bd}	78.89 ± 0.29^{Bd}	78.28 ± 0.19^{Ad}
T3	68.39 ± 0.19^{Cb}	68.02 ± 0.09^{Bb}	67.67 ± 0.14^{Bb}	67.41 ± 0.05^{Ab}
T4	64.72 ± 0.30^{Ca}	64.27 ± 0.26^{Ba}	63.90 ± 0.23^{Ba}	63.39 ± 0.28^{Aa}

Means (n=6) bearing different uppercase superscripts between columns differ significantly (P< 0.05)
Means (n=6) bearing different lowercase superscripts between rows differ
significantly (P< 0.05)

Fat content: The results (Table 2) showed a mild increment in fat content up to day 14 of the storage. The highest fat per cent (2.416 ± 0.42) was observed on the day 14 of storage for control and the lowest fat content was observed for T3 (1.166 ± 0.01) on day 0 of storage. There was no significant (P< 0.05) difference between control and T1 and between T3 and T4 but T2 differed significantly from all the other treatments. Mild increment of fat content was noticed during storage period, which might be due to relative decrease in moisture content during storage period. The fat content of the synbiotic

cottage cheese ranged from 1.166 to 2.416 per cent, which is in close proximity with results obtained by Blanchette *et al.* (1996) [3], where they observed the fat content in cottage cheese made with dressing fermented by Bifidobacteria to be 4.2 per cent. Similarly, Araujo *et al.* (2010) [2] developed a synbiotic cottage cheese with *Lactobacillus delbrueckii* UFV H2b20 and inulin, and found a fat content of 1.00 and 3.95 per cent for synbiotic cheese and control cheese, respectively.

Table 2: Fat content (%) of synbiotic cottage cheese during storage at 4 °C

Treatments	Storage period (days)			
	0	3	7	14
C	1.968 ± 0.03^{c}	2.032 ± 0.01^{c}	2.040 ± 0.03^{c}	2.416 ± 0.42^{c}
T1	2.129 ± 0.01^{c}	2.130 ± 0.03^{c}	2.140 ± 0.02^{c}	2.143 ± 0.02^{c}
T2	1.614 ± 0.02^{b}	1.623 ± 0.02^{b}	1.623 ± 0.02^{b}	1.629 ± 0.02^{b}
T3	1.166 ± 0.01^{a}	1.178 ± 0.01^{a}	1.186 ± 0.01^{a}	1.193 ± 0.01^{a}
T4	1.176 ± 0.01^{a}	1.177 ± 0.01^{a}	1.185 ± 0.01^{a}	1.190 ± 0.01^{a}

Means (n=6) bearing different lowercase superscripts between rows differ significantly (P< 0.05)

Protein content: The results (Table 3) showed the lowest protein content of 13.84 ± 0.29 per cent for the control on day 0 of storage, which increased to 14.50 ± 0.30 per cent on day 14 of storage. The highest protein per cent (14.51± 0.13) was observed for T4 on day 0 of storage which increased to 14.85 ± 0.35 per cent on day 14 of storage. There was no significant difference between day 0, 3 and 7 of storage but there was a significant (P< 0.05) difference between day 0 and 14 of storage. The control differed significantly (P< 0.05) from other treatments. The protein content was noticed to increase mildly during the storage period, which might be due to relative decrease in the moisture content. The mean protein content of various treatments during the storage ranged from 13.84 to 14.97 per cent. These findings agree with Swati *et al.* (2018) [9], who found the protein content to be 15.35 per cent in cottage cheese made with kiwi fruit enzyme. The results obtained by Blanchette *et al*. (1996) [3] are in close proximity with the results of this study; they reported a protein content of 12.20 to 13.00 per cent for different fermented cream dressing.

Table 3: Protein content (%) of synbiotic cottage cheese during storage at 4 °C

Treatments	Storage period (days)			
	0	3	7	14
C	13.84 ± 0.29^{Aa}	14.09 ± 0.30^{ABa}	14.16 ± 0.27^{ABa}	14.50 ±0.30^{Ba}
T1	14.35 ± 0.21^{Ab}	14.51 ± 0.21^{ABb}	14.83 ± 0.28^{ABb}	14.97 ±0.31^{Bb}
T2	14.43 ± 0.21^{Ab}	14.63 ± 0.22^{ABb}	14.74 ± 0.37^{ABb}	14.83 ± 0.45^{Bb}
T3	14.37 ± 0.18^{Ab}	14.58 ±0.23^{ABb}	14.79 ±0.24^{ABb}	14.97 ± 0.31^{Bb}
T4	14.51 ± 0.13^{Ab}	14.64 ± 0.19^{ABb}	14.72 ± 0.29^{ABb}	14.85 ± 0.35^{Bb}

Means (n=6) bearing different uppercase superscripts between columns differ significantly (P< 0.05)
Means (n=6) bearing different lowercase superscripts between rows differ significantly (P< 0.05)

Ash content: The results (Table 4) of this study showed a mild increment in the ash content during the storage for all the treatments. The highest ash content (2.34 ± 0.07 per cent) was found for T3 and the lowest (0.66 ± 0.02 per cent) was observed for T2 on day 0 of storage. The ash content differed significantly (P< 0.05) between various treatments and between day 0 and 14 of storage. The ash content was found to have mild increment during the storage period for all the treatments which ranged from 0.66 to 2.50 per cent, it might be attributed to addition of calcium chloride and salt in the cottage cheese and reduction in moisture content. The findings of present study are supported by Rana *et al.* (2017) [6], who observed the mean ash content in cottage cheese made from buffalo milk with different levels of papaya latex to range from 2.75 to 3.79 per cent Similarly, Blanchette *et al.* (1996) [3] reported a range of ash content from 0.97 to 1.09 per cent for cottage cheese with different fermented cream dressing.

Table 4: Ash content (%) of synbiotic cottage cheese during storage at 4 °C

Treatments	Storage period (days)			
	0	3	7	14
C	1.99 ± 0.03^{Ac}	2.01 ± 0.05^{ABc}	2.09 ± 0.05^{BCc}	2.13 ± 0.13^{Cc}
T1	1.01 ± 0.02^{Ab}	1.04 ± 0.01^{ABb}	1.06 ± 0.03^{BCb}	1.10 ± 0.03^{Cb}
T2	0.66 ± 0.02^{Aa}	0.72 ± 0.01^{ABa}	0.78 ± 0.05^{BCa}	0.83 ± 0.04^{Ca}
T3	2.34 ± 0.07^{Ae}	2.40 ± 0.05^{ABe}	2.44 ± 0.07^{BCe}	2.50 ± 0.04^{Ce}
T4	2.17 ± 0.04^{Ad}	2.24 ± 0.04^{ABd}	2.28 ± 0.06^{BCd}	2.38 ± 0.05^{Cd}

Means (n=6) bearing different uppercase superscripts between columns differ significantly (P< 0.05)
Means (n=6) bearing different lowercase superscripts between rows differ significantly (P< 0.05)

Gross energy content: The results (Table 5) revealed that the energy content increased with the storage period. On day 0 of storage, the calculated gross energy was found to be highest for T4 (138.24 ± 1.17 kcal/100g) and lowest for control (74.35 ± 0.80 kcal/100g). Further, on the day 14 of storage the gross energy content of the control sample was 82.38 ± 2.09 kcal/100g and T4 was 142.81 ± 1.26 kcal/100g. There was a significant (P< 0.05) difference between the treatments but no significant difference was noticed between day 3 and 7 of storage. The gross energy content (kcal/100g) of synbiotic cottage cheese was observed to increase throughout storage period which may apparently be due to decrease in moisture content and relative increase in other proximate parameters. These findings are similar to the results obtained by Swati *et al.* (2018) [9], who found the gross energy to be 149.15 kcal/100g in cottage cheese made with kiwi fruit enzyme, and Smit *et al.* (2004) [8] who compared the energy values of different dairy products obtained by various methods and found a gross energy of fat free cottage cheeses to be 373 kJ/100 g.

Table 5: Gross energy content (kcal/100g) of synbiotic cottage cheese during storage at 4 °C

Treatments	Storage period (days)			
	0	3	7	14
C	74.35 ± 0.80^{Ac}	76.66 ± 1.03^{Bc}	77.38 ± 2.58^{Bc}	82.38 ± 2.09^{Cc}
T1	79.07 ± 0.62^{Aa}	81.01 ± 0.95^{Ba}	82.01 ± 2.41^{Ba}	85.11 ± 1.33^{Ca}
T2	87.68 ± 0.60^{Ab}	89.15 ± 1.29^{Bb}	89.39 ± 1.21^{Bb}	91.68 ± 0.64^{Cb}
T3	122.88 ± 0.76^{Ad}	124.16 ± 0.52^{Bd}	125.42 ± 0.59^{Bd}	126.27 ± 0.29^{Cd}
T4	138.24 ± 1.17^{Ae}	139.78 ± 1.49^{Be}	141.14 ± 0.82^{Be}	142.81 ± 1.26^{Ce}

Means (n=6) bearing different uppercase superscripts between columns differ significantly (P< 0.05)
Means (n=6) bearing different lowercase superscripts between rows differ significantly (P< 0.05)

Conclusion

In the present study, it was observed that the synbiotic cottage cheese prepared using a combination of prebiotic pectin and probiotics *L. acidophilus* (*LA-5*) and *L. casei* (*NCDC-298*), had better results for nutritional qualities than the control. Further, there was a favourable decrease in moisture content with a relative increase in the other parameters were observed during the storage.

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