International Journal of Food Science and Nutrition
ISSN: 2455-4898; Impact Factor: RJIF 5.14
Received: 18-12-2020; Accepted: 04-01-2021; Published: 01-02-2021
www.foodsciencejournal.com
Volume 6; Issue 1; 2020; Page No. 51-55

Production of healthy and nutritive yogurt paneer cake using natural flavours

Subramanian K*, Rajeswari R, Jemima Romola CV
Department of Biotechnology, P.S.R Engineering College, Sivakasi, Tamil Nadu, India

Abstract
The consumption of probiotic components in India is significantly reduced compared to other countries. Unfortunately, usage of packed and readymade food items is increasing every year in India. This type of food habit change is responsible for getting health issues in some of the Indian population. A novel idea developed to ignoring this health issue by supplementation probiotic components in dietary sources. In this attempt we prepared a yogurt paneer cake using qualitatively improved milk yogurt by microbial strain selection, paneer and natural edible flavour developed from Royal jelly (RJ), Bee pollen grains (BPG) and coconut milk. The biochemical analysis of the yogurt revealed that yogurt prepared from milk with Bee pollen grains powder have higher protein content and good texture compared to yogurt prepared from other mode of preparation. The microbial load of probiotic bacteria was found between 6-7 Log CFU-g^{-1} in prepared cake at the time of consumption. The shelf life of the yogurt was estimated as 21 days. The natural flavour imparted in paneer by adding coconut milk, custard apple extract and powdered moringa leaves. The paneer with custard apple extract was widely preferred during sensory test. The shelf life of paneer with natural flavours was estimated as 10 days. The yogurt from milk with coconut milk and paneer from milk with custard apple extract was selected for the production of yogurt paneer cake. The shelf life of the yogurt paneer cake was estimated as 2 days under refrigeration.

Keywords: yogurt paneer cake, natural flavours, biochemical, analysis

Introduction
Consumption of probiotic components either directly or indirectly is not significantly present among the present Indian population. The intake of probiotic component containing eatables like cheese, yogurt, fermented products, honey, royal jelly, etc. have valuable health benefits. Yoghurt is one of the most important fermented product obtained through symbiotic growth of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* in a sterile environment at the temperature of 36°C–42°C for 3–8 hrs [1]. During milk fermentation and both bacterial strains must remain active in the final product. This association have wide significant role in improving lactose intolerance, anticholesterolemic impacts, reducing risk of cancers [1] and other benefits related to probiotic bacteria [2]. Therefore we were added this yogurt as one of the raw material for preparation cake.
Bee pollen grains (BPG) are a male gametophyte of flowers contains valuable level of proteins, carbohydrates, fats, minerals, vitamins, and phenolic substances [3]. Due to their nutritional enrichment property this also selected as a raw material in this cake preparation. Royal jelly (RJ) is a natural substance, produced from worker bees to feed the bee queens and young larvae which have high nutritional value, functional characteristics and biological properties [4] which can be added directly in food or as dietary supplement to import medication [5], since it has antibacterial, Antifatigue [6], antioxidant activity [7], anti-allergic [8], and anti-inflammatory [9] properties.
Paneer which is commonly called as Indian cottage cheese, made by curdling heated milk with vinegar or any other acidic foods have versatile flavour which makes it widely used in Indian cuisine. Paneer is marble white spongy in nature and has a sweetish nutty flavour and contains 53-55% moisture, 23-25% fat, 17-18% proteins, 2-2.5% lactose and 1.5-2.0% minerals [10]. Considering all these characteristic features we included these materials to prepare a Medicare cake.

Materials and Methods
Fresh cow milk was collected from Hatsun Agro foods limited, Madurai, Tamilnadu, India heated to 90 ± 2 ºC for 10 minutes then allowed to cool to 42 ºC and used in this experiment. Bee pollen grains (BPG) and royal jelly (RJ) were obtained from Shamee bee farm, Rajapalayam, Tamilnadu. Fresh coconut milk was obtained from locally procured coconut. Yogurt starter containing *Lactobacillus delbrueckii* and *Streptococcus thermophiles* (1:1) was obtained from National Centre for Microbial Resource (NCMR), Pune, India. The ripened custard apple and fresh moringa leaves were obtained from local market.
The fresh coconut milk was prepared from non-mature coconut by grinding the coconut flesh with pre refrigerated coconut water, then filtered and store in a bottle. The prepared coconut milk and standardized milk was taken in the ratio of 3:1 in a vessel and heated to 90 ± 2 ºC for 10 minutes then cool to 42 ºC and used for further experiments.

Yogurt preparation
Toned cow's milk with ~3% fat, Standardized cow's with ~4.5% fat and Frees milk (FM) with ~5.5% fat were heated to 90 ± 2 ºC for 10 minutes, allowed to cool to 42 ºC and used for yogurt preparation.
T1- Toned milk (TM) 100ml + 3% yogurt starter
T2- Toned milk 100ml + 3% yogurt starter + 0.6 RJ
T3- Toned milk 100ml + 3% yogurt starter + 0.8% BPG
T4- Standardized milk (SM) 100ml + 3% yogurt starter
T5- Standardized milk 100ml + 3% yogurt starter+ 0.6 RJ

T6- Standardized milk 100ml + 3% yogurt starter + 0.8% BPG
T7- Full cream milk (FCM) 100ml + 3% yogurt starter
T8- Full cream milk 100ml + 3% yogurt starter + 0.6 RJ
T9- Full cream milk 100ml + 3% yogurt starter + 0.8% BPG
T10- Mixed milk (3:1) 100ml + 3% yogurt starter
T11- Mixed milk (3:1) 100ml + 3% yogurt starter + 0.6 RJ
T12- Mixed milk (3:1) 100ml + 3% yogurt starter + 0.8% BPG
150 ml plastic beaker were used for yogurt preparation and sample incubated at 42 ºC until it reached pH ~4.6. Then it is refrigerated at 4 ± 1 ºC for further analysis.

Paneer preparation with coconut milk
The paneer was prepared by mixing the standardized milk 300 ml with 200ml of extracted coconut milk and stir the mixture for sometimes. The milk mixture was heated to 90 ± 2 ºC for 10 minutes and then cooled to 70 ºC. Then the milk mixture was coagulated by adding 1% citric acid (mild heat was applied until the coagulum and the whey separates completely) and the coagulum was collected by filtering the mixture using muslin cloth. The coagulum was then pressed at 2-4 kg/cm^2 for 15 minutes to drain the maximum moisture in the coagulum. Then it was dipped in chilled water for 2-3 hours. After that the water content is removed by using muslin cloth. Then 2% salt solution was sprinkled over the coagulum called as paneer. The prepared sample was packed with plastic cover in hygienic condition and stored in a refrigerator at 3 ºC for further use.

Paneer preparation with custard apple extract
The custard apple flesh was removed and homogenised in a blender for 3 minutes to get a concentrated extract and stored in the refrigerator. 350 ml of standardized cow milk mixed with 150 ml of custard apple extract and stir the mixture for sometimes. Added 100g sugar to this mixture and heated to 90 ± 2 ºC for 10 minutes and then cooled to 70 ºC. Then the prepared mixture was coagulated by adding 1% citric acid (mild heat was applied until the coagulum and the whey separates completely) and used for further preparation.

Paneer preparation with powdered moringa leaves:
The fresh moringa leaves were collected and dried under indirect sunlight to remove the moisture content from the leaves. The dried moringa leaves were grinded into fine powder. Then it was packed aseptically for further use. 50 g moringa leaf powder added in 500 ml of standardized cow milk and stirred well. Further process is similar to previous paneer preparation method.

Syneresis in yogurt
Syneresis (whey content) in yogurt was measured by centrifugation method. 20 g of yogurt sample was taken and centrifuged at 10000 rpm for 20 minutes at 4 ºC. The clear supernatant collected and weighed.

Biochemical analysis in yogurt and paneer
1 ml of yogurt/paneer sample was thoroughly mixed with 9 ml of distilled water. Then 3 drops of 0.1% phenolphthalein solution was added to the yogurt suspension. The yogurt suspension was then titrated using 0.1 M NaOH. The mixture was stirred continuously and titration was continued until the indicator changed to a definite pink colour lasting for 30 seconds. The volume of NaOH required to neutralise the yogurt was noted. The content of acid (lactic acid percentage equivalent) can be calculated using the following formula.

$$\text{Acidity (\%)} = \frac{10\ X\ V_{NaoH}\ X\ 0.009\ X\ 0.1}{W} \text{ X } 100$$

The yogurt/paneer sample was treated with ammonia and ethyl alcohol. Ammonia is used to dissolve the protein and ethyl alcohol helps to precipitate the protein. Fat is then extracted by the addition of diethyl ether and petroleum ether then solvent evaporated from the sample and the residue was weighed.
The clean dry empty dish and lid was heated in oven maintained at 100 ± 2 ºC for one hour and cooled in desiccator and weighed.0.1 mg of yogurt/paneer sample was weighed. The lid was replaced and weighed again.1-2 drops of phenolphthalein solution was added to the yogurt/paneer sample in the dish. The yogurt/paneer sample was then neutralised with 0.1 N sodium hydroxide solution to a faint pink colour. The volume of 0.1N sodium hydroxide required to neutralize the sample was noted. The dish without the lid was placed on a boiling water bath until the water is removed from the sample. The under surface of the dish was wiped and placed in the oven maintained at 100 ± 2 ºC for 3 hours. The dish along with the lid was removed and cooled in a desiccator and weighed. Heating was continued and reweighed at hourly intervals until successive weighing do not vary by more than 0.5 mg. Deducted weight of sodium hydroxide added to neutralize the sample from the residue after drying. The total solids (TS) present in the sample was calculated by using the following formula

$$\text{Estimation of sodium hydroxide added (a)} = \frac{N\ X\ T.V\ X\ 40}{1000\ X\ 2}$$

$$\text{Total solids \%} = \frac{100\ (W_2 - a)}{W_1}$$

Where N = Normality of NaOH, T. V = Titer value, W_2 = Weight in g of residue left after drying, W_1= Weight in g of the prepared sample taken Solid Non Fat (SNF) was calculated from fat content removed samples followed by same procedure. The total protein content in the yogurt and paneer sample was determined by Kjeldhal method and moisture content estimated according to the method of AOAC **(**1984**)**.

Microbiological analysis in yogurt and Paneer
Microbiological analysis of the yogurt and paneer samples was carried out by prescribed method of Bureau of India Standard **(**BIS, 1989**)**. These samples were examined for presence of yeast and mould using chloramphenicol yeast and glucose agar. The yogurt and paneer sample were diluted (10^4 dilution) and plated into the sterile petri plate containing chloramphenicol yeast and glucose agar were incubated for 3-5 days at 37 ºC. The white spongy growth of yeast and mould was observed after 3 days of incubation. The colony formed in the incubated plate was counted using colony counter. Coliform test was also carried out by using violet red bile agar and incubated at 42-44°C for 24 hours. Development of pink colonies indicates the presence of coliform in the samples.

Preparation of yogurt paneer cake

200 ml of yogurt from milk with coconut milk which contains bee pollen grains and sugar powder was taken in a bowl and whisked well for 2 minutes. Then 100 g of paneer with custard apple extract was crumbled with hand and added to the yogurt and mixed well. Then 4-5 tablespoon of water was taken in a bowl and two tablespoons of gelatin powder was added to it. This gelatin solution was microwave at low temperature for 11 minutes. Afterwards, the gelatin solution was added to the yogurt-paneer mix and folded well. The available biscuits were grinded into coarse powder mixed with melted butter was added and mixed well. This prepared sample was transferred cake ring and pressed firmly. Then the yogurt-paneer mix was poured and refrigerated for 4-5 hours. After 4-5 hours, a firm stable yogurt-paneer cake was found. The appearance and texture of yogurt paneer cake was shown in fig1.

Fig 1: Experimentally prepared yogurt paneer cake

Sensory evaluation

Sensory evaluation was carried out in order to find comparative analysis of sensory qualities of different combinational prepared cake samples is assessed by a panel of untrained individuals working in the laboratory. The sensory attributes such as appearance, mouth feel, sourness, consistency, aroma and acceptance of the samples were evaluated by scoring of 1-10 points scale.

Results and discussion

The syneresis measurement of all types of yogurt samples indicating that bee pollen grains added samples were expelled less whey when compared to other samples. This ultimately shows that the yogurt containing BPG gave better texture and appearance than the plain yogurt and yogurt containing royal jelly; also full cream yogurt with BPG is shown better texture than the other yogurt. The nutritive value analysis of yogurt samples revealed that yogurt containing BPG have higher protein content and lower fat level than other yogurt samples (Table 1). The protein content of yogurt with coconut milk is little low when compared to other yogurt types. Percentage of TS and SNF is higher in yogurt sample prepared with BPG than the other samples. The pH and acidity of BPG containing yogurt samples not significantly differed from control samples.

Table 1: Biochemical analysis of yogurt developed from various milk samples

Sl. no	Yogurt type	pH	Acidity (%LA)	Fat %	SNF %	TS %	Protein %	Syneresis %
Yogurt from Toned Milk (TM)								
1.	TM- C	4.58	0.71	3.00	9.00	12.00	3.33	15.9
2.	TM- RJ	4.55	0.67	3.00	9.54	12.54	3.53	13.2
3.	TM- BPG	4.50	0.72	3.00	9.72	12.72	3.60	9.4
Yogurt from Standardized Milk (SM)								
4.	SM- C	4.40	0.79	4.50	9.10	13.60	3.37	12.3
5.	SM- RJ	4.35	0.83	4.45	9.10	13.55	3.37	8.3
6.	SM- BPG	4.50	0.65	4.40	9.15	13.55	3.39	8
Yogurt from Full Cream Milk (FCM)								
7.	FCM- C	4.35	0.69	6.00	9.60	15.60	3.55	0.5
8.	FCM- RJ	4.50	0.69	6.00	9.70	15.70	3.59	0.3
9.	FCM- BPG	4.23	0.73	5.95	9.80	15.75	3.63	0.1
Yogurt from milk with Coconut Milk (CM)								
10.	CM- C	4.21	0.72	10.20	27.00	37.20	2.57	14.3
11.	CM- RJ	4.22	0.72	10.00	27.80	37.80	2.50	12.5
12.	CM- BPG	4.20	0.74	9.95	28.25	38.20	2.41	12.7

Table 2: Biochemical analysis of Paneer developed various milk samples.

Sl.no	Paneer- type	pH	Acidity (LA %)	Fat %	SNF %	TS %	Protein %
1	Paneer from milk with coconut milk	5.50	0.52	24.70	17.10	41.80	6.22
2	Paneer from milk with custard apple extract	5.80	0.72	12.50	28.10	40.60	5.89
3	Paneer from milk with powdered moringa leaves	5.70	0.70	24.60	17.10	41.70	6.22

Biochemical analysis

The biochemical analysis report of paneer samples revealed that the healthy materials incorporated in the paneer preparation have good impact on protein quantity, TS, SNF and fat content. Further the custard apple extract incorporated paneer sample showing significant lower fat level when compared with others.

Acidity in yogurt and paneer were estimated in order to find the sourness of sample while tasting. In this experiment all yogurt samples have acidity ranging from 0.6-0.7 which gives better sour taste while eating (Table 1). The acidity of paneer samples also showing similar range from 0.6-0.7 and this will support the taste quality.

Addition of RJ and BPG in the samples does not mean to alter the fat content of the yogurt. The yogurt from milk with coconut milk containing more fat than other yogurt types is recommended for underweight personalities. From fat analysis, toned yogurt containing BPG will be suggested for diet purposes. The fat analysis of paneer samples shown that paneer with custard apple extract contains less amount of fat compared to other flavoured paneer. Hence this will be suggested for diet purpose.

Total solids (TS) analysis

TS in the yogurt sample determine the texture of the yogurt. The yogurt from coconut milk with various milk samples has higher TS value which results in better texture of the yogurt. TS analysis report of various source yogurt samples was shown in Table 2**.** TS analysis in developed paneer samples shows insignificant differences. So we considered the texture of different sources of paneer samples might be same and sample selection for cake preparation based on the biological significant of source.

Microbiological analysis in yogurt and paneer

Yeast and mould test

The microbiological analysis was done to analyse the shelf life of product and to determine the level of contamination. The produced yogurt sample was stored under refrigeration for 21 days. The test was conducted to find any yeast and mould found in the stored yogurt and paneer samples at regular intervals (0^{th}, 7^{th}, 14^{th} and 21^{st} day). Through this experimental analysis the report revealed that presence of < 10 yeasts and < 1 mould/g are satisfactory and safe to use up to 14 days. Higher growth of yeast and mould was observed during 21^{st} day of storage. The yeast and mould test results for yogurt samples were shown in Table 3**.** The yeast and mould test for paneer sample was done periodically up to 15 days and the results revealed that initial five days the microbial growth is insignificant but later showing improvement in growth; after 15 days showed highly significant growth Table 4.

Table 3: Microbiological quality- Yeast and mould count in yogurt samples.

Sl.no	Yogurt sample	Storage days*			
		0	7	14	21
1.	TM- C	ND	ND	1.6	12.9
2.	TM- RJ	ND	ND	1.2	13.2
3.	TM- BPG	ND	ND	1.5	13.6
4.	SM- C	ND	1	1.8	13.8
5.	SM- RJ	ND	ND	1.3	12.8
6.	SM- BPG	ND	ND	1	12
7.	FCM- C	ND	ND	1.2	13.6
8.	FCM- RJ	ND	1	1.9	13.8
9.	FCM-BPG	ND	ND	1.4	12.9
10.	CM- C	ND	ND	1.3	13.7
11.	CM- RJ	ND	ND	1.2	13.1
12.	CM- BPG	ND	1	2	13.7

*Microbial count 10^4 cfu/g ND – Non detectable

Table 4: Microbiological quality- Yeast and mould count in Paneer

Sl.no	Paneer sample	Storage days*			
		0	5	10	15
1.	Paneer with coconut milk	ND	1	2.3	14
2.	Paneer with custard apple	ND	1.2	2.5	14.3
3.	Paneer with powdered moringa leaves	ND	1	2	14

*Microbial count 10^4 cfu/g ND – Non detectable

Coliform test

Presence of coliform bacteria is indicating the improper sanitation and unhygienic condition of edible products. Presumptive coliform was absent at all stages of storage. The coliform test report for yogurt and paneer was presented in Table 5 & 6 respectively**.**

Table 5: Microbiological quality- Coliform count for yogurt

Sl. no	Yogurt sample	Storage days*			
		0	7	14	21
1.	TM- C	ND	ND	ND	ND
2.	TM- RJ	ND	ND	ND	ND
3.	TM- BPG	ND	ND	ND	ND
4.	SM- C	ND	ND	ND	ND
5.	SM- RJ	ND	ND	ND	ND
6.	SM- BPG	ND	ND	ND	ND
7.	FCM- C	ND	ND	ND	ND
8.	FCM- RJ	ND	ND	ND	ND
9.	FCM-BPG	ND	ND	ND	ND
10.	CM- C	ND	ND	ND	ND
11.	CM- RJ	ND	ND	ND	ND
12.	CM- BPG	ND	ND	ND	ND

*Microbial count 10^4 cfu/g ND – Non detectable

Table 6: Microbiological quality- Coliform count for Paneer

Sl. no	Paneer sample	Storage days*			
		0	5	10	15
1.	Paneer with coconut milk	ND	ND	ND	ND
2.	Paneer with custard apple extract	ND	ND	ND	ND
3.	Paneer with powdered moringa leaves	ND	ND	ND	ND

*Microbial count 10^4 cfu/g ND – Non detectable

Health cake

Compiling of all health aspects of the selected ingredients the health cake prepared and served to selected local people. The tastes, texture, flavour and feelings of consumer are very supportive and recommending for commercial availability.

Conclusion

The yogurt with probiotics was produced by incorporating Royal jelly and Bee pollen grains have good health impact. The biochemical analysis of yogurt samples with bee pollen grains is improving consistency and texture and also rich in protein. Even though, royal jelly is most expensive they produce less amount of protein and consistency than BPG. Hence the production of yogurt with probiotics using BPG is cost effective. The shelf life of yogurt is about 21 days**.** The additional flavouring agents like coconut milk, custard apple extract and powdered moringa leaves give medicinal values. The shelf life of paneer varied based flavouring agent**.** Additional work to be carried out to find the self-life of yogurt panner cake for recommending commercial application.

References

1. Saara Laiho, Roderick PW, Williams, Astrid Poelman, Ingrid Appelqvist, Amy Logan *et al*. Effect of whey protein phase volume on the tribology, rheology and sensory properties of fat-free stirred yoghurts. Food hydrocolloids. 2017; 67:166-177.
2. Rahmawati Irma Sarita, Worapot Suntornsuk. Effects of fermentation and storage on bioactive activities in milks and yoghurts. Procedia Chemistry. 2016; 18:53-62.
3. Pascoal A, Rodrigues S, Teixeira A, Feás X, Estevinho LM. Biological activities of commercial bee pollens: antimicrobial, antimutagenic, antioxidant and anti-inflammatory. Food and Chemical Toxicology. 2014; 63:233-239
4. Howe SR, Dimick PS, Benton AW. Composition of freshly harvested and commercial royal jelly. Journal of

Apicultural Research. 1985; 24(1):52-61.
5. El-Nekeety AA, El-Kholy W, Abbas NF, Ebaid A, Amra HA, Abdel-Wahham MA *et al*. Efficacy of royal jelly against the oxidative stress of fumonisin in rats. Toxicon. 2007; 50 (2):256-269.
6. Kamakura M, Mitani N, Fukuda T, Fukushima M. Antifatigue effect of fresh royal jelly in mice. Journal of nutritional science and vitaminology. 2001; 47(6):394-401.
7. Je-Ruei Liu, Yuan-Chang Yang, Li-Shian Shi and Chi-Chung Peng. Antioxidant properties of royal jelly associated with larval age and time of harvest. Journal of agricultural and food chemistry. 2008; 56 (23):11447-11452.
8. Iwao Okamoto *et al*. Major royal jelly protein 3 modulates immune responses in vitro and in vivo. Life Sciences. 2003; 73(16):2029-2045
9. Kohno K *et al.* Royal jelly inhibits the production of proinflammatory cytokines by activated macro phages. Biosci Biotechnol Biochem. 2004; 68 (1):138-145.
10. Arora KL, Sabikhi L, Kanawjia KS. Manufacture of paneer from substandard buffalo milk. Indian J. Dairy Biosci. 1996; 7:71-75.