Optimization of process parameters for pickle *masala* flavored probiotic Greek yoghurt

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Received: 06 March 2020 / Accepted: 10 July 2020 / Published online: 27 October 2020
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Abstract: In this study, Greek yoghurt was developed using standardized homogenized milk and fermented with suitable indigenous starter culture with optimized process parameters and pickle masala as novel flavor. The product was prepared by fermenting with four culture. Suitability of starters was evaluated based on starter culture counts, pH, titratable acidity and sensory parameters of the Greek yoghurt. The C4 starter containing *Streptococcus thermophilus* MTCC 5460, *Lactobacillus bulgaricus* NCIM 2358 and *Lactobacillus helveticus* MTCC 5463 (probiotic strain) was found to be most suitable as it received significantly ($P < 0.05$) highest score for lactobacilli count (8.86 log CFU/ml), TS (23.41 %) and overall acceptability (8.88) compared to remaining. Among the straining period *viz.* 15 min, 30 min and 55 min, product prepared by straining for a period of 30 min was optimized based on pH (4.67), titratable acidity (% 0.89 LA) and overall acceptability scores (8.71). No significant effect of various straining period was observed on starter counts of the product. Pickle masala was used as novel flavor and it was added at the rate of 1 %, 1.5 % and 2 % of final product. The increased rate of pickle masala in Greek yoghurt affected adversely on starter count as well as sensory profile. So, 1% rate of addition

of pickle masala in Greek yoghurt as flavor was considered more suitable having lactobacilli count (9.05 log CFU/ml), streptococci count (10.37 log CFU/ml), pH (4.81), titratable acidity (0.90 % LA) and overall acceptability score (8.80) compared to other. The composition of optimized Greek yoghurt was TS (23.02 %), fat (7.92 %), protein (6.17 %), lactose (4.91 %), calcium (171 mg/100g) and ash (1.29 %).

Keywords: Greek yoghurt, Starter culture, Pickle masala, LAB

Introduction

It is generally observed that the fermented milks like yogurt were discovered accidentally when our forefathers used to store milk in sheep skin bags to preserve it. The yoghurt making is evolved over centuries and now a days varieties with a range of flavors, forms and textures are commercially available (Weerathilake et al. 2014). One such variety is Greek yoghurt and also known differently according to their origin, such as Labneh in Eastern Mediterranean, Torba in Turkey, Stragisto in Greece, Ymer in Denmark, Skyr in Iceland and Chakka in India (Aryana and Olson, 2017; Uduwerella, et al. 2017; Tamime et al. 2014). It is prepared from the yoghurt by removing part of whey by straining for some period of time and most popular in Levantine, Eastern Mediterranean, Middle Eastern, Central Asian and South Asian people. Traditionally, nomads in the Middle East used the containers made from animal skin for the production of yoghurt and the yoghurt was left in these skins until it was consumed. While the yoghurt was hanging in the animal skin some of the liquid phase would have been absorbed into the skin, while some of the whey that had seeped through the skin would have been lost by evaporation. In this way concentration of the product took place and the new product was referred to as concentrated/strained yoghurt. This product has better keeping quality than normal yoghurt, mainly as a result of the higher concentration of lactic acid (Tammie and Robinson, 2007) and low moisture content.

Greek yogurt is having creamier texture (Uduwerella, 2018) and thicker consistency with slightly sour taste. It is rich in protein content (Tamime and Robinson, 2007), lower carbohydrates (as decomposed by the starter culture), improved bioavailability of

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calcium, source of vitamin B 12 and health promoting microorganisms (Panahi et al. 2016). Greek yoghurt is popularly consumed as starter culture associated with it may also have the capacity to survive digestion, reach the gastrointestinal tract and ultimately provide several health benefits (Marco et al. 2017). Due to these reasons the demand of Greek yoghurt is increasing day by day and health conscious people have started to include it in their regular diet. Bell et al. (2018) suggested fermented foods like Greek yoghurt should be introduced to children early in life and incorporated into their everyday meal plans. This helps in boosting the immunity, improved digestion and increase the bioavailability of micronutrients.

Lactic acid bacteria (LAB) are known for their widespread occurrence and diverse commercial use. Several species of LAB are industrially formulated to produce fermented food as starter cultures and enhance human health through probiotics. Probiotics alter the intestinal microflora balance, inhibit the growth of pathogen (Wulandari et al. 2020), modulate immune response and can act in various other ways as health promoters (Trush et al. 2020). Probiotics strains of lactobacillus are usually given generally regarded as safe (GRAS) status, since most are isolated either from traditional dairy products or the gastrointestinal tract of healthy individuals (Salminen et al. 1998). The technological application of probiotic organisms in fermented dairy products aims to combine the potential health benefits of the bacteria with their ability to grow in milk, resulting in a nutritionally healthy and desirable product for the consumers. Therefore, apart from health claims, probiotic strains intended for use in fermented dairy products should be selected on the basis of their overall effect in the products as many probiotic bacteria used today in yoghurt grow poorly in milk when compared with some common starter cultures.

Wide variety of Greek yoghurt with different flavours are available in Indian markets and sold under the brand names like Amul, Danone, Epigamia, Nestle, Chobani, Barambah etc. None of the commercially available Greek yoghurt are manufactured using Indian origin starter culture. Consumers always search for the novelty and uniqueness in the product. Taking these points in mind, the study was conducted with the objective to develop Greek yoghurt fermented by indigenous starter having probiotic potential, with underexplored novel flavor.

Materials and Methods

Collection of LAB and chemicals

The four combination of LAB were taken from the Culture Collection of Dairy Microbiology Department, Sheth M. C. College of Dairy Science, Anand Agricultural University, Anand, Gujarat, India C1- *Streptococcus thermophilus* MTCC 5460 + *Lactobacillus helveticus* MTCC 5463; C2- *Streptococcus thermophilus* MTCC 5460 + *Lactobacillus rhamnosus* MTCC

5946; C3- *Streptococcus thermophilus* MTCC 5460 + *Lactobacillus bulgaricus* NCIM 2358 (yoghurt culture) and C4- *Streptococcus thermophilus* MTCC 5460 + *Lactobacillus bulgaricus* NCIM 2358 + *Lactobacillus helveticus* MTCC 5463. Sterilized reconstituted skim milk with 12% TS was used for the propagation of lactic culture and further stored at $5 \pm 2^\circ\text{C}$. All the chemicals and reagents utilized in the study were purchased either from Hi-media (Mumbai), Merck (Germany) or Loba.

Procurement of milk and pickle masala

The standardized homogenized milk (Amul T special milk) and pickle masala (Vasant achar masala and taste maker) were purchased from the local market of Anand city of Gujarat state (India).

Preparation of Greek yoghurt

The flow chart to prepare Greek yoghurt is shown in Figure 1.

Determination of Starter culture count

Lactobacilli count of Greek yoghurt was determined as per the method described by Kathiriya et al. (2016). Eleven grams of sample was taken out from the plastic container and added to 99 ml phosphate buffer flask. This made 1:10 dilution. Similarly, required numbers of serial dilutions were prepared. One ml diluted sample from appropriate tubes was transferred to labeled petri plates (performed in triplicates) then 15-20 ml of molten and cooled (45°C) MRS agar was poured to respective petri plates. The content was mixed thoroughly by tilting and rotating the plates and allowed it to solidify and then additional layer (5-7 ml) of the same agar was poured over the solidified medium. Again allowed it to solidify, then incubated anaerobically at 37°C for 48 h in inverted position. Typical colonies were calculated and the counts were expressed as log CFU/ml. For Streptococci count, the procedure is same as the lactobacilli count. But M17 growth media was used instead of MRS agar for the enumeration of streptococci (Kathiriya et al. 2016).

Measurement of pH

The pH of the Greek yoghurt was measured as per the procedure described in Indian Standard (1961) with a calibrated pH meter (OAKTON pH700, India). Well mixed 10 ml Greek yoghurt sample was put into a beaker and then pH was measured by immersing the pH meter probe into the fermented milk sample at 25°C . Standard buffer solution of pH 4, 7 and 9 was used to calibrate the pH meter before measuring the sample.

Measurement of titratable acidity

The titratable acidity (% Lactic acid) of Greek yoghurt was estimated by the procedure described in IS:1479 (Part I, 1960). Ten gram of sample was taken into a porcelain dish and an equal

Fig. 1 Flow chart for the preparation of Greek yoghurt

volume of distilled water was added to it. Then 1 ml of phenolphthalein indicator was added and it was titrated against 0.1[N] NaOH till the appearance of light pink color, which persisted for 30 seconds in the solution. Titratable acidity was calculated as follow

$$\text{Acidity (\% Lactic acid)} = \frac{9 \times V \times N}{X}$$

Where, V = Volume of 0.1[N] NaOH required for the titration; N = Normality of NaOH solution and X = quantity of product taken for titration

Determination of total solids (TS)

The TS of product was determined by oven drying method (AOAC, 2012). Heat the clean dry empty dish in oven maintained at $100 \pm 2^\circ\text{C}$ for one hour, cool in a desiccator and weigh. Weigh about 5 g product. Add 1-2 drops of phenolphthalein solution to the sample in the dish and neutralise with 0.1 N sodium hydroxide solution to a faint pink colour. Note the volume of 0.1N sodium hydroxide required to neutralize the sample. Place the dish on a boiling water bath until the water is removed from the sample. Wipe the under-surface of the dish and place in the oven maintained at $100 \pm 2^\circ\text{C}$, for 3 h. Remove the dish and cool in a desiccator and weigh. Continue heating and re-weighing at hourly intervals until successive weighings do not vary by more than 0.5 mg. Deduct half weight of the 0.1N sodium hydroxide added

to neutralize the sample from the residue after drying and calculate total solids using following formula (FSSAI Lab manual 1, 2012).

$$a = \frac{N \times TV \times 40}{1000 \times 2}$$

$$\text{Total solids (\% w/w)} = \frac{(W2 - a)}{W1} \times 100$$

Where, N = Normality of NaOH; TV = Titre value; W2 = Weight in g of residue left after drying; W1 = Weight in g of the prepared sample taken and a = Half of the volume of 0.1 N Sodium hydroxide added

Sensory analysis of the product

The Greek yoghurt was subjected to the sensory evaluation by trained panel of nine judges on nine point hedonic scale (Stone and Sidel, 2004) with sensory parameters viz. flavor, body and texture, color and appearance and overall acceptability. The product was served at 10°C .

Compositional analysis of the product (Fat, Protein, Lactose, Calcium and Ash)

Fat percentage of Greek yoghurt was determined by Gerber method as per adopting the procedure FSSAI Lab manual 1 (2012). The protein content of Greek yoghurt was determined by kjeldahl method described in AOAC (1990). The lactose content of Greek yoghurt was determined by Lane Eynon method as per FSSAI

Lab manual 1 (2012). The calcium content of Greek yoghurt was determined by following the method mentioned in the BIS handbook (BIS: Part XI, 1981). Total ash was determined according to method described by Ranganna (1986).

Statistical analysis

All the data were subjected to statistical analysis using Completely Randomized Design (CRD) as per the methods described in Steel and Torrie (1980). The significance was tested at 5 % level using mean value, co-efficient of variance (% CV) and critical difference (CD). The values for starter counts were log transformed before analysis. The results were presented as mean $\pm$ SD of the replicates. All the observations were taken in triplicates.

Results and Discussion

In order to minimize the fat losses during straining, homogenized milk was taken for the preparation of Greek yoghurt in our experiment. Desai et al. (1985) reported that fat losses in the whey were reduced during the manufacture of strained yoghurt by homogenization of the milk before addition of starter cultures during fermentation stage.

Selection of suitable starter culture

Each starter culture was inoculated at the rate of 2 % in previously heat treated and cooled standardized homogenized milk to prepare Greek yoghurt. The prepared product was subjected to lactic count, pH, titratable acidity, TS and sensory analysis in order to select the best suited starter culture to prepare Greek yoghurt.

From the Table 1 it is observed that lactobacilli counts were ranged from 8.39 to 8.86 log CFU/ml in Greek yoghurt prepared with various starter cultures. Lactobacilli counts of Greek yoghurt prepared by C4 starter was higher (8.86 log CFU/ml) followed by C3 (8.82 log CFU/ml), C1 (8.77 log CFU/ml) and C2 (8.39 log CFU/ml). No significant change in lactobacilli count was observed between C4 and C3 starter containing Greek yoghurt. While lactobacilli counts of C1 and C2 starter containing Greek yoghurt

differed significantly ($p < 0.05$). In case of streptococci count of Greek yoghurt prepared by various starters, the counts were in range of 9.20 to 10.17 log CFU/ml. Highest streptococci count was in C2 treatment (10.17 log CFU/ml) followed by C1 (10.16 log CFU/ml), C3 (9.69 log CFU/ml) and C4 (9.20 log CFU/ml). pH of the Greek yoghurt prepared with different starter cultures was found in the range of 4.87 to 4.49. C2 starter showed highest reduction in pH (4.49) followed by C3 (4.60), C4 (4.65) and C1 (4.87). No significant difference observed in C2, C3 and C4 treatment. The acidity (% LA) produced by the different treatments was in the range of 0.87 % LA to 0.97 % LA. The Greek yoghurt containing starter culture C3 showed highest acidity production (0.97 % LA) followed by C4 (0.94 %), C1 (0.92 % LA) and C2 (0.87 % LA). No significant difference observed between C3 and C4 treatment. Total solids (TS) of Greek yoghurt prepared with different starters was found in the range of 22.27 to 23.41 %. Among all the starters, the Greek yoghurt prepared with C4 showed the highest TS (23.41 %) followed by C3 (23.12 %), C1 (22.42 %) and C2 (22.27 %).

The sensory evaluation score of the Greek yoghurt prepared with different starters is shown in Fig. 2. Significant ($p < 0.05$) difference was observed in body and texture as well as overall acceptability scores of product while no significant difference observed in flavor and color & appearance of product prepared using different starters. In case of body and texture, C4 treatment received highest score (8.63) followed by C2 (8.21), C3 (7.83) and C1 (7.71). C4 again received highest overall acceptability score (8.88) followed by C3 (8.67), C1 (8.19) and C2 (8.08).

The Greek yoghurt prepared with starter C4 received highest score for lactobacilli count, TS and overall acceptability compared to remaining. The pH and acidity (% LA) value of product containing C4 starter was at par with the product containing other starter received highest score for pH and acidity (% LA). So the C4 is considered most suitable among all starters to prepare Greek yoghurt.

Total plate counts of 7 log to 8 log CFU/ml have been reported for strained yoghurt (Rosenthal et al. 1980; Yamani and Abu-Jaber, 1994; Al-Kadamany et al. 2003). In another study, conducted

Table 1 Effect of different starter on lactic count and chemical parameters of product

LAB Combination	Lactobacilli (Log CFU/ml)	Streptococci (Log CFU/ml)	pH	Acidity (% LA)	TS (%)
C1	8.77 $\pm$ 0.25	10.16 $\pm$ 0.18	4.49 $\pm$ 0.24	0.92 $\pm$ 0.04	22.42 $\pm$ 0.14
C2	8.39 $\pm$ 0.27	10.17 $\pm$ 0.32	4.87 $\pm$ 0.13	0.87 $\pm$ 0.02	22.27 $\pm$ 0.49
C3	8.82 $\pm$ 0.06	9.69 $\pm$ 0.86	4.60 $\pm$ 0.14	0.97 $\pm$ 0.04	23.12 $\pm$ 0.81
C4	8.86 $\pm$ 0.23	9.20 $\pm$ 0.30	4.65 $\pm$ 0.13	0.94 $\pm$ 0.05	23.41 $\pm$ 0.64
SEm	0.11	0.24	0.08	0.02	0.29
CD(0.05)	0.33	0.75	0.26	0.06	0.89
CV %	2.49	4.98	3.58	3.94	2.53

Data are presented as mean $\pm$ SD (n=3)

Fig. 2 Effect of different starter on sensory parameters of Greek yoghurt (C1: *Streptococcus thermophilus* MTCC 5460 + *Lactobacillus helveticus* MTCC 5463; C2: *Streptococcus thermophilus* MTCC 5460 + *Lactobacillus rhamnosus* MTCC 5946; C3: *Streptococcus thermophilus* MTCC 5460 + *Lactobacillus bulgaricus* NCIM 2358 (yoghurt culture) and C4: *Streptococcus thermophilus* MTCC 5460 + *Lactobacillus bulgaricus* NCIM 2358 + *Lactobacillus helveticus* MTCC 5463); Error bars show standard deviation (n=3)

Table 2 Effect of straining period on lactic count and chemical parameters of product

Straining Period (Min)	Lactobacilli (Log CFU/ml)	Streptococci (Log CFU/ml)	pH	Acidity (% LA)	TS (%)
15	9.09 ± 0.32	10.97 ± 0.18	4.83 ± 0.13	0.75 ± 0.10	21.76 ± 0.67
30	9.32 ± 0.49	10.37 ± 0.79	4.67 ± 0.25	0.89 ± 0.08	23.99 ± 0.94
55	9.25 ± 0.45	10.47 ± 0.47	4.50 ± 0.08	1.08 ± 0.12	25.30 ± 0.86
SEm			0.07	0.04	0.37
CD(0.05)	NS	NS	0.23	0.14	1.14
CV%			3.55	11.03	3.51

Data are presented as mean ± SD (n=3) NS -Non significant

by Serhan et al. (2016) reported around 7 log CFU/ml starter culture counts in the concentrated yoghurt (labneh) made using goat milk. Ozer and Robinson (1999) reported that lactobacilli counts and streptococcus counts at the end of incubation period (pH 4.3) was 8 log CFU/ml and 9 log CFU/ml respectively. In this study also the starter count was in the range of 8 to 10 log CFU/ml. Aboudonia et al. (1992) and Amer et al. (1997) reported the use of different starter culture combinations for making strained yoghurt affects the overall quality of the product. We obtained the similar results when Greek yoghurt prepared with different starters. In one of the study acceptable strained yoghurt was prepared using *L. delbrueckii* subsp. *bulgaricus* in combination with *Enterococcus faecalis* instead of using *L. delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* (El-Samragy et al. 1988). We also tried to prepare Greek yoghurt by utilizing starter other than *Lactobacillus bulgaricus* and *Streptococcus*

thermophilus and superior quality of Greek yoghurt was obtained.

Optimization of straining period

Greek yoghurt prepared using C4 starter was strained for different time periods viz. 15 min, 30 min and 55 min. The product strained at different time intervals was subjected to lactic count, pH, titratable acidity, TS and sensory analysis order to get most suitable straining period for Greek yoghurt preparation.

Table 2 shows that starter count i.e. lactobacilli and streptococci counts of product prepared by straining for different time period, has no significant difference. Whereas significant ($p < 0.05$) pH drop was observed with increasing straining time from 15 min to 55 min. The pH of product strained at 15 min, 30 min and 55 min are 4.83, 4.67 and 4.50 respectively. While no significant drop in

Fig. 3 Effect of straining for different period of time on sensory parameters of Greek yoghurt; Error bars show standard deviation (n=3)

pH was found in the product when straining period was increased from 15 min to 30 min. Also no significant drop in pH was found in the product when straining period was increased from 30 min and 55 min but the pH was reduced significantly ($p < 0.05$) when product made by straining for 15 min and 55 min. Similar trend was observed in titratable acidity. The acidity was increased significantly ($p < 0.05$) with increasing the straining period. Titratable acidity of the product strained for 15 min, 30 min and 55 min are 0.75 % LA, 0.89 % LA and 1.08 % LA respectively. While no significant increase in titratable acidity observed when straining period was increased from 15 min to 30 min. The titratable acidity was increased significantly ($p < 0.05$) when straining period further increased to 55 min. TS of the product was also increased significantly ($p < 0.05$) with increasing the straining period. TS of the product strained for 15 min, 30 min and 55 min are 21.76 %, 23.99 % and 25.30 % respectively.

The sensory evaluation score of the Greek yoghurt prepared by straining for different period of time is shown in Fig. 3. The color and appearance scores of Greek yoghurt prepared by straining for 15 min, 30 min and 55 min were non significant. Significant ($p < 0.05$) difference was observed in flavor, body and texture as well as overall acceptability scores. The scores for flavor was highest in Greek yoghurt prepared by 30 min straining period (8.46) followed by 55 min (8.26) and 15 min (7.70). The scores for body and texture was highest in Greek yoghurt prepared by 30 min straining period (8.64) followed by 55 min (8.45) and 15 min (8.07). The scores for overall acceptability was highest in Greek yoghurt prepared by 30 min straining period (8.71) followed by 55 min (8.20) and 15 min (8.08).

The straining period has no significant effect on starter count. The overall acceptability score of 30 min straining treatment was

significantly ($p < 0.05$) higher compared to remaining treatments. The overall acceptability score in sensory evaluation of product prepared by 15 min is the lowest among three treatments. This can be correlated with the comparatively less reduction in pH, lesser titratable acidity (% LA) and low TS (%) of the product prepared by 15 min than 30 min and 55 min. Straining the curd for 55 min has adversely affected the pH, titratable acidity and sensory parameters of Greek yoghurt. Hence it was concluded that Greek yoghurt prepared by straining for 30 min is more acceptable than the remaining treatments.

Vargas *et al.* (2008) reported that longer the draining of the yogurt in the cloth bags, the higher the total solids of the final product. Thus, moisture content is related to syneresis. Similar result was also observed in this study, the total solid content in the product was increased with increasing the straining period from 15 min to 55 min. Tamime (1977) reported that use of various strains of yoghurt starter culture affects the rate of whey drainage during straining. The sensory properties of Greek yoghurt made by traditional method (straining in muslin cloth bag) are excellent (Robinson, 20002) compared to other methods. Preparation of Greek yoghurt by straining in muslin cloth bag is still preferred in Middle East, as the investment in mechanized system of production are quite high (Lange, 2020).

Optimization of rate of addition of pickle masala

Greek yoghurt was prepared using C4 starter and strained for 30 min with different rate of addition of pickle masala viz. 1%, 1.5% and 2%. The product containing different proportion of pickle masala was subjected to starter count, pH, titratable acidity, TS and sensory analysis in order to select most appropriate rate of addition of pickle masala.

Fig. 4 Effect of different concentration of pickle *masala* on sensory parameters of Greek yoghurt; Error bars show standard deviation (n=3)

Table 3 Effect of varying rate of addition of pickle masala on lactic count and chemical parameters of product

Rate of pickle masala addition (%)	Lactobacilli (Log CFU/ml)	Streptococci (Log CFU/ml)	pH	Acidity (% LA)	TS (%)
1.0	9.05±0.16	10.37±0.18	4.81±0.07	0.90±0.06	23.02±0.17
1.5	8.73±0.16	10.01±0.28	4.67±0.05	0.98±0.03	23.13±0.31
2.0	8.48±0.32	10.00±0.23	4.62±0.09	0.98±0.07	23.54±0.22
SEm	0.10	0.11	0.03	0.025	0.11
CD(0.05)	0.32	0.32	0.10	0.076	0.33
CV%	2.62	2.32	1.52	5.78	1.03

Data are presented as mean ± SD (n=3)

Table 3 shows that with increasing rate of addition of pickle masala from 1 % to 2% has significantly ($p<0.05$) reduced the lactobacilli and streptococci count. The lactobacillus count was in the range of 8.48 log CFU/ml to 9.05 log CFU/ml. streptococcus count was in the range of 10.00 log CFU/ml to 10.37 log CFU/ml. Significant ($p<0.05$) pH reduction was observed with increasing rate of pickle masala addition from 1 % to 2 %. The pH of product containing 1 %, 1.5 % and 3 % are 4.81, 4.67 and 4.62 respectively. Similar trend was observed in titratable acidity. The acidity was increased significantly ($p<0.05$) with increasing the rate of pickle masala addition. Titratable acidity of the product containing 1 %, 1.5 % and 3 % are 0.90 % LA, 0.98 % LA and 0.98 % LA respectively. TS of the product was also increased significantly ($p<0.05$) with increasing rate of pickle masala addition. TS of the product containing 1 %, 1.5 % and 3 % are 23.02 %, 23.13 % and 23.54 % respectively.

The sensory evaluation score of the Greek yoghurt prepared with different rate of addition of pickle masala is shown in Fig. 4. The body and texture scores of Greek yoghurt prepared by 1 %, 1.5 % and 2 % pickle masala were non- significant. Significant ($p<0.05$) difference was observed in flavor, color and appearance

as well as overall acceptability scores. The scores for flavor was highest in Greek yoghurt prepared by 1 % pickle masala (8.80) followed by 1.5 % (8.30) and 2 % (7.20). The scores for body and texture was highest in Greek yoghurt prepared by 1.5 % pickle masala (8.60) followed by 1 % (8.90) and 2 % (8.20). The scores for overall acceptability was highest in Greek yoghurt prepared by 1 % pickle masala (8.80) followed by 1.5 % (8.40) and 2 % (7.80).

Addition of pickle masala affected adversely on starter count as well as sensory profile of the Greek yoghurt. So 1% rate of addition of pickle masala as flavoring agent was considered as more suitable compared to 1.5 % and 2 %.

The streptococci count and lactobacilli counts were around 8 log CFU/ml in fresh fruit flavored Greek yoghurt (Abou et al. 2013). Upadhyay et al. (1984) and Upadhyay et al. (1985) found a positive correlation between the chemical changes and microbial counts of sweetened strained yoghurt and a sensory evaluation of fresh samples. While we observed the inverse relation between chemical changes and starter counts of pickle flavored Greek yoghurt. The reason behind getting such result is not known

Table 4 Compositional analysis of optimized Greek yoghurt

Constituents	Concentration
Fat (%)	7.92 ± 0.22
Protein (%)	6.17 ± 0.17
Lactose (%)	4.91 ± 0.28
Calcium (mg/100g)	171.00 ± 0.82
Ash (%)	1.29 ± 0.15
Total Solids (%)	23.02 ± 0.17

Compositional analysis of optimized Greek yoghurt

The optimized Greek yoghurt was prepared from standardized homogenized milk (4.5% fat and 8.5% SNF) with C4 starter culture. Straining was carried out for 30 min and the pickle masala was added at the rate of 1% of strained curd weight. The product was subjected to TS (%), fat (%), protein (%), lactose (%), calcium (mg/100g) and ash (%) analysis. The composition of the product is shown in Table 4.

Composition of Greek yoghurt varies, containing usually 23 to 25 % TS (Abu-Jdayil et al. 2002) and 6 to 11 % fat (Nsabimana et al. 2005, Tamime and Robinson, 2007). According to the Codex Standard for Fermented Milk (Codex Alimentarius, 2003) strained yogurt should have minimum of 5.6 % protein. Tamime et al. (2014) analysed 109 commercial concentrated fermented milks with different geographical origins. The protein content in these commercial products varied from approximately 4.5 to 8%. In a similar study, Desai et al. (2013) collected 24 samples of Plain commercial Greek yogurts from across the United States. They reported that Protein and TS of all the samples ranged from 5.8 to 10.6 % and from 15 to 23.8 %, respectively. Ozer and Robinson (1999) reported that concentrated yoghurt prepared by traditional method contains 5.66 % lactose. Serhan et al. (2016) reported that ash content of concentrated yoghurt (labneh) prepared from cow milk was 1.16 %. While Calcium levels in 38 samples of strained yoghurts ranged between 43 to 921 mg/100 g (Abou Jaoude et al. 2010). Greek Yoghurt optimized in this study contains 23.02 % TS, 7.92 % fat, 6.17 % protein, 4.91 % lactose, 1.29 % ash and 171 mg/100g calcium. All the compositional parameters of Greek yoghurt prepared in this study are either in the range or nearer to the results reported by above mentioned authors.

Conclusion

The Greek yoghurt can be prepared at large scale using indigenous starter culture (*Streptococcus thermophilus* MTCC 5460, *Lactobacillus bulgaricus* NCIM 2358 and *Lactobacillus helveticus* MTCC 5463) with 30 min straining in muslin cloth bag and containing 1% pickle masala as novel flavor. The viable cells of probiotic strain *Lactobacillus helveticus* MTCC 5463 were more than 8 log CFU/ml in the optimized product. To make the manufacturing of Greek yoghurt more feasible at large scale

production, instead of straining it in muslin cloth trials can be taken by centrifugation, ultrafiltration or other less time consuming methods.

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