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Shelf-life assessment of herbal Greek yoghurt during refrigerated storage

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Abstract
The shelf-life assessment of control Greek yoghurt and concentrated lemongrass extract (CLE) added Greek yoghurt, was carried out at refrigeration temperature (7±1ºC) up to the period of 42 days and the samples were analyzed for changes in pH, total phenol content (TPC), antioxidant activity (ABTS, DPPH and FRAP assay) and microbial quality. The pH decreased significantly ($P<0.05$) during storage, with a rapid decrease in control as compared to developed product. CLE added Greek yoghurt showed the highest total phenolic content and antioxidant activity (ABTS, DPPH, FRAP), followed by control sample, although these values decreased during storage. The coliform counts (cfu/g) were absent throughout the storage period, whereas yeast and moulds (cfu/g) were present within limits in developed product up to 35 days. The CLE added Greek yoghurt remained acceptable for up to 35 days, while control sample was acceptable up to 28 days when stored at refrigeration temperature.

Keywords: Lemongrass, Greek yoghurt, herbal, antioxidant, shelf-life, microbial quality

Introduction

The oxidation products from biological interactions or exogenous chemicals in the body and nutrition are produced by reactive oxygen species (ROS), which comprise free radicals such as superoxide anion radical (O^{2-}), hydroxyl radical (OH^{-}), and singlet oxygen [1, 2]. Both free radicals and active oxygen species, like the oxygen molecule, display paradoxical tendencies. They are vital for the creation of physiologically active and required molecules and are significant mediators in signal transduction. They are also known to be poisonous and to cause a variety of diseases [3, 4].

ROS are implicated in several common in-*vivo* regulatory processes, according to mounting evidence. An overabundance of free radicals can overpower defensive enzymes like superoxide dismutase, catalase, and peroxidase by oxidising membrane lipids, cellular proteins, DNA, and enzymes. This can lead to fatal cellular effects (apoptosis) by halting cellular respiration. Moreover, oxidative stress has been found to be a significant cause of some age-specific diseases. Lipid peroxides and low molecular weight molecules formed in the later stages of the oxidative process are the factors accountable for these illnesses. For example, multiple studies have found that the brains of Alzheimer's patients exhibit increased oxidative damage to all main classes of macromolecules [5, 6]. Other clinical conditions such as diabetes, rheumatoid arthritis, and atherosclerosis are associated with signs of severe damage from free radicals [5, 7, 8].

Over 200 million tonnes of milk is anticipated to be produced in India each year. Dairy products are made from about 55% of the country's milk production. Fermented milk products are expected to account up roughly 7% of the total volume of milk produced. Fermented milk products were developed worldwide to keep milk solids from deteriorating, and they have persisted for centuries in developing countries. Among other organoleptic characteristics, fermented milk products are popular because of their distinct flavour, revitalising taste, and improved digestion. Fermented milk products can easily have their contents changed to meet various dietary requirements. Fermented milk products have been demonstrated to have dietetic relevance due to their many nutritional and therapeutic benefits [3, 4, 9].

Greek yoghurt is a popular dairy product and it differs from other yoghurts because it goes through a straining process to remove the whey. Strained Greek yoghurt is lower in sugar than regular yoghurt. Removing the whey produces thicker, creamier yoghurt with a tart taste. Some manufacturers add thickening agents to regular yoghurt, and market it as Greek-style yoghurt. According to empirical data, value-added goods with extra health advantages are progressively making up the average Indian's food basket [9, 10].

Cymbopogon citratus, or lemongrass, belongs to the Gramineae family of grasses. It is a one-meter-tall perennial grass that grows in clumps. The leaf blade has a maximum length of 50 cm and a maximum width of 1.5 cm. Both ends of it are tapered and linear. This tropical grass is indigenous to Sri Lanka and western India. It generates aromatic oil that is used in medicinal, fragrance, and flavouring. It has been employed for ages in South America and India and is claimed to have a range of therapeutic effects. Only a few lemongrass species are recommended for use in food and medicine, despite the fact that there are numerous species [3, 4].

Lemongrass extract is made from the leaves and shoots of the lemongrass plant and is used in a number of culinary products, including frozen foods and beverages. Lemongrass extract is said to provide several health benefits. The only essential oil used is that which is derived from either fresh or dried lemongrass leaves. Lemongrass is widely used as a crucial component in Asian cuisines due to its potent lemon flavour [3, 4].

Lemongrass herbal tea is used as a sedative and an immunostimulant in India. Lemongrass was traditionally only known for its aromatic properties. But it also has various health perks, making it a priceless herb. Lemongrass extract is said to enhance the immune system, detoxify the liver, pancreas, kidney, bladder, and digestive tract, and have antioxidant, antibacterial, and antifungal properties.

Like citrus tastes, lemongrass flavour goes well with acidic foods. Thus, in present study the herbal Greek yoghurt was prepared with increased antioxidant activity by using lemongrass extract.

Materials and Methods

Milk: Fresh, chilled, raw cow milk of Kankrej breed was collected from the Livestock Research Station (LRS), SDAU, Sardarkrushinagar.

Lemongrass: Lemongrass (*Cymbopogon citratus*) grown in the Centre for Agro-Forestry, Forage Crops and Green Belt, SDAU was used for the study. Fresh green matured leaves of medium size were selected and cut from the plant.

Lactic cultures: Freeze dried DVS culture (Lyofast MS 059 ET) consisting of specifically selected blend of lactic acid bacteria used for preparing the fermented milk products was obtained from Sacco System, Italy.

Skim milk powder: Spray dried skim milk powder (SMP) was procured from Banas dairy, Palanpur, Gujarat and was used for standardization of solid not fat (SNF) level.

Sugar: Crystalline sugar procured from the local market was used as the sweetening material in the preparation of fermented product.

Method for preparation of lemongrass incorporated herbal Greek yoghurt

The herbal Greek yoghurt was prepared by adding different levels i.e. T1 (0.0%, control), T2 (1.0%), T3 (2.0%), T4 (3.0%) and T5 (4.0%) of concentrated lemongrass extract (CLE) possessing highest antioxidant activity. Optimization of level of addition of CLE was carried out through sensory evaluation using 9 points Hedonic scale by a panel of 7 semi-trained judges. The sensory attributes like flavour, body and texture, colour and appearance, product acidity and overall acceptability were used for sensory evaluation of Greek yoghurt. The scores were ascertained for each factor and expressed numerically. Finally, based on the sensory acceptability of the product, addition of CLE (3.0%) was considered as the best level for incorporation into Greek yoghurt [4].

The control Greek yoghurt as well as Greek yoghurt prepared with addition of CLE (3.0%), adjudged as best optimized product on the basis of sensory evaluation were packed in polypropylene cups with lid and stored at refrigeration temperature (7±1ºC). The samples were drawn after every 7 days till 42 days and analyzed for change in their sensory attributes *viz*. flavour, body and texture, colour and appearance, product acidity and overall acceptability, TPC and antioxidant activity (ABTS, DPPH and FRAP assay). The samples were also analyzed for changes in pH and microbial load.

Analysis of control and optimized Greek yoghurt

During storage study, the control and optimized Greek yoghurt samples were analyzed for sensory attributes *viz*. flavour, body and texture, colour and appearance, product acidity and overall acceptability. The pH was determined using digital bench top pH meter [11]. The samples were also analyzed for TPC [12] and antioxidant potential by ABTS assay [13, 14], DPPH assay [15] and FRAP assay [16]. Analysis of yeast and mould counts and the coliform counts of the samples were also ascertained [17].

Statistical analysis

A completely randomized design (CRD) with 3 repetitions was used for data analysis. The data are presented as means±standard error of mean (SEm). Analysis of variance (ANOVA) was used to determine the main effects of treatments [18, 19].

Results and Discussions

Changes in sensory attributes

The control Greek yoghurt as well as Greek yoghurt prepared with addition of CLE (3.0%) samples were stored at refrigeration temperature (7±1ºC) were monitored for changes in various sensory attributes *viz*. flavour, body and texture, colour and appearance, product acidity and overall acceptability after every 7 days till its acceptable sensory attributes up to the period of 42 days and the results are illustrated in Table 1.

Flavour is a crucial sensory component in judging Greek yoghurt's acceptability. The flavour of both the control and lemongrass added Greek yoghurt declined significantly ($P<0.05$) during the storage period, however the control sample's reduction was more pronounced than the CLE-added Greek yoghurt's (Table 1). Fermentation continues to change the taste ingredients even at low temperatures [20]. Greek yoghurt should also have a good body and a smooth texture. It was discovered that the body and texture scores considerably ($P<0.05$) decreased during storage, regardless of the control or experimental sample. The decrease in scores could be due to the loose body seen over the storage period [21]. The colour and appearance of the control and CLE-added Greek yoghurt differed noticeably after storage, and sample scores dramatically decreased ($P<0.05$) till the end of storage period. Similarly, during the course of the storage period, the sensory scores for the product acidity of control and CLE Greek yoghurt considerably ($P<0.05$) declined. A rise in acidity, which may be connected to the production of organic acids as a result of continuous fermentation during storage, could be the reason for the declining trend [22]. The overall acceptability scores significantly ($P<0.05$) decreased over the storage time in both control as well as developed product, in contrast to other sensory attributes; however, the control sample's decline was more rapid than that of the CLE Greek yoghurt samples.

Table 1: Effect of refrigerated storage on sensory attributes of Greek yoghurt

Control Greek yoghurt					
Storage Days	**Flavour**	**Body and Texture**	**Colour and Appearance**	**Product Acidity**	**Overall Acceptability**
0	8.12 ± 0.11^{fA}	8.03 ± 0.16^{eA}	7.26 ± 0.10^{fA}	7.67 ± 0.11^{gA}	7.59 ± 0.14^{gA}
7	7.79 ± 0.08^{eA}	7.83 ± 0.12^{deA}	7.15 ± 0.12^{fA}	7.33 ± 0.09^{fA}	7.24 ± 0.11^{fA}
14	7.63 ± 0.10^{eA}	7.74 ± 0.09^{dA}	6.95 ± 0.09^{efA}	6.89 ± 0.13^{eA}	6.85 ± 0.12^{eA}
21	7.42 ± 0.07^{cdA}	7.31 ± 0.10^{cA}	6.58 ± 0.13^{dA}	6.48 ± 0.07^{dA}	6.42 ± 0.09^{dA}
28	7.28 ± 0.10^{cA}	7.02 ± 0.12^{cA}	6.05 ± 0.10^{cA}	6.19 ± 0.14^{cA}	6.09 ± 0.15^{cA}

35	6.66±0.12^{bA}	6.53±0.14^{bA}	5.51±0.14^{bA}	5.84±0.10^{bA}	5.66±0.13^{bA}
42	5.78±0.07^{aA}	6.12±0.13^{aA}	5.14±0.11^{aA}	5.52±0.09^{aA}	5.24±0.10^{aA}
Optimized Greek yoghurt					
Storage Days	Flavour	Body and Texture	Colour and Appearance	Product Acidity	Overall Acceptability
0	8.49±0.11^{fB}	8.37±0.09^{fB}	8.16±0.13^{eB}	8.36±0.09^{eB}	8.55±0.13^{fB}
7	8.44±0.09^{fB}	8.19±0.11^{efB}	7.95±0.15^{eB}	8.23±0.14^{eB}	8.31±0.09^{eB}
14	8.19±0.14^{eB}	7.98±0.13^{eB}	7.42±0.07^{dB}	8.04±0.10^{deB}	7.96±0.09^{dB}
21	7.84±0.11^{dB}	7.54±0.08^{dB}	7.35±0.10^{cdB}	7.81±0.12^{dB}	7.53±0.11^{cB}
28	7.80±0.10^{cB}	7.23±0.11^{cB}	7.16±0.11^{cB}	7.48±0.09^{cB}	7.25±0.09^{bcB}
35	7.45±0.13^{bB}	6.95±0.12^{abB}	6.55±0.09^{bB}	7.17±0.14^{bB}	7.07±0.12^{bB}
42	7.06±0.08^{aB}	6.83±0.09^{aB}	6.17±0.13^{aB}	6.69±0.11^{aB}	6.55±0.14^{aB}
Means with different superscripts in each column (a, b, c, d, e, f, g) and (A, B) differ significantly (P < 0.05) from each other. Data are presented as means ± SEm (n = 18).					

Changes in total phenolic content (TPC) and antioxidant capacity (ABTS & DPPH)

The changes in TPC and antioxidant capacity (ABTS, DPPH, FRAP assay) of control as well as CLE incorporated Greek yoghurt were determined after every 7 days up to the period of 42 days and the results are shown in Table 2. The results delineated significant (P<0.05) decrease in TPC (µM GAE/100 g) of control and CLE Greek yoghurt from 32.14±0.03 and 59.23±0.04, of fresh product to 10.13±0.03 and 42.31±0.05 of 42nd day stored product, respectively. Similarly, the antioxidant capacity (µM TE/100 g) of control and CLE Greek yoghurt measured by ABTS assay was significantly (P<0.05) decreased from 11.81±0.03, 21.33±0.04 at 0 day to 4.11±0.02, 10.48±0.03 at 42nd day, respectively, whereas DPPH values were observed to decrease significantly (P<0.05) from 17.85±0.04, 44.38±0.06 at 0 day to 8.15±0.01, 35.57±0.05 at 42nd day, respectively. Also, the FRAP values decrease significantly (P<0.05) from 13.61±0.03, 29.14±0.05 at 0 day to 6.25±0.01, 19.67±0.04 at 42nd day, respectively. Even minute amounts of phenolic compounds found in fruits and medicinal plants may have beneficial effects on human health, including antioxidant, antibacterial, anti-inflammatory, and anticarcinogenic action. They are also linked to a lower mortality risk from cardiovascular diseases, despite the fact that the TPC and antioxidant capacity of CLE-incorporated Greek yoghurt decreased during the storage period [23, 24].

Table 2: Effect of refrigerated storage on TPC and antioxidant capacity (ABTS, DPPH, FRAP) of CLE incorporated herbal Greek yoghurt

Storage period (days)	Total phenolic content (µM GAE*/100 g)		ABTS - Antioxidant capacity (µM TE#/100 g)		DPPH - Antioxidant capacity (µM TE#/100 g)		FRAP - Antioxidant capacity (µM TE#/100 g)	
	Control	CLE incorporated herbal *Greek yoghurt*	Control	CLE incorporated herbal *Greek yoghurt*	Control	CLE incorporated herbal *Greek yoghurt*	Control	CLE incorporated herbal *Greek yoghurt*
0	32.14 ± 0.03^{gA}	59.23 ± 0.04^{gB}	11.81 ± 0.03^{gA}	21.33 ± 0.04^{eB}	17.85 ± 0.04^{gA}	44.38 ± 0.06^{eB}	13.61 ± 0.03^{gA}	29.14 ± 0.05^{gB}
7	28.11 ± 0.02^{fA}	57.15 ± 0.03^{fB}	10.42 ± 0.02^{fA}	20.82 ± 0.03^{eB}	16.41 ± 0.03^{fA}	44.33 ± 0.04^{eB}	12.43 ± 0.03^{fA}	28.53 ± 0.04^{fB}
14	23.64 ± 0.02^{eA}	54.87 ± 0.05^{eB}	9.75± 0.05^{eA}	19.24 ± 0.04^{eB}	15.08 ± 0.03^{eA}	44.29 ± 0.05^{eB}	11.27 ± 0.04^{eA}	26.34 ± 0.06^{eB}
21	21.83 ± 0.03^{dA}	49.65 ± 0.06^{dB}	8.52 ± 0.02^{dA}	17.57 ± 0.04^{dB}	14.16 ± 0.02^{dA}	42.51 ± 0.03^{dB}	10.09 ± 0.02^{dA}	25.51 ± 0.04^{dB}
28	17.46 ± 0.04^{cA}	47.34 ± 0.03^{cB}	7.14 ± 0.03^{cA}	15.61 ± 0.01^{cB}	12.37 ± 0.04^{cA}	40.16 ± 0.02^{cB}	9.31 ± 0.02^{cA}	23.92 ± 0.04^{cB}
35	13.92 ± 0.02^{bA}	45.48 ± 0.04^{bB}	5.03 ± 0.03^{bA}	13.43 ± 0.02^{bB}	10.64 ± 0.03^{bA}	38.94 ± 0.04^{bB}	8.53 ± 0.03^{bA}	21.74 ± 0.03^{bB}
42	10.13 ± 0.03^{aA}	42.31 ± 0.05^{aB}	4.11 ± 0.02^{aA}	10.48 ± 0.03^{aB}	8.15 ± 0.01^{aA}	35.57 ± 0.05^{aB}	6.25 ± 0.01^{aA}	19.67 ± 0.04^{aB}
Means with different superscripts in each column (a, b, c, d, e, f, g) and in row (A, B) differ significantly (LSD test, P < 0.05) from each other. Data are presented as means ± SE (n = 3). * - GAE- gallic acid equivalent, # - TE- trolox equivalent								

Changes in pH

The control Greek yoghurt as well as Greek yoghurt prepared with addition of CLE (3.0%) samples stored at refrigeration temperature (7±1°C) were analyzed for changes in pH after every 7 days till its acceptable sensory attributes and the results are illustrated Fig. 1. The results depicted decreasing trend in the pH values of all samples during the entire period of storage study, however rapid decrease was observed in the control Greek yoghurt as compared to CLE Greek yoghurt. According to Phattayakorn and Wanchaitanawong (2009) [25], gram-positive bacteria are vulnerable to antimicrobial activity. This may be connected to the slower rate of pH reduction of produced products, which may have been brought on by phenolic compounds' inhibitory action on the starter cultures.

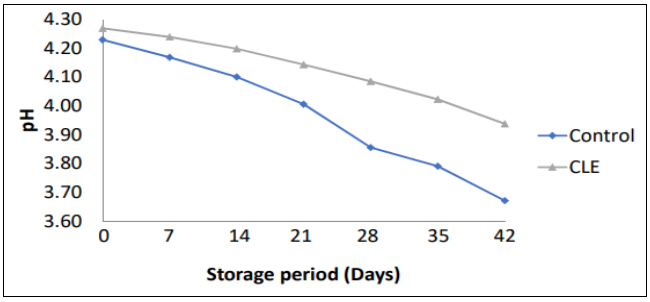

Fig 1: Changes in pH of control and CLE incorporated herbal Greek yoghurt during storage at refrigeration temperature (7±1°C)

Microbiological analysis of control and CLE incorporated herbal Greek yoghurt

The microbiological analysis (coliforms and yeast and moulds) of control as well as CLE incorporated herbal Greek yoghurt samples stored at refrigeration temperature (7±1ºC) was performed after every 7 days till their acceptable attributes. The effect of storage on yeast and mould counts of control and CLE Greek yoghurt are given in table 3. The results of microbiological analysis revealed that the coliform counts (cfu/g) were not detected/absent in both control as well as CLE incorporated herbal Greek yoghurt samples during the entire period of storage study at refrigeration temperature. The fermented dairy product's low pH and strong acidity prevented coliform from growing in them [26, 27].

Table 3: Effect of refrigerated storage on yeast and mould counts of control and CLE incorporated herbal Greek yoghurt

Storage period (days)	Yeast and Moulds (log cfu/g)	
	Control Greek yoghurt	CLE incorporated herbal Greek yoghurt
0	0.00 ± 0.00^{aA}	0.00 ± 0.00^{aA}
7	2.97 ± 0.32^{bA}	0.00 ± 0.00^{aB}
14	6.11 ± 0.81^{cA}	3.12 ± 0.26^{bB}
21	13.49 ± 1.34^{dA}	7.04 ± 0.59^{cB}
28	27.56 ± 2.43^{eA}	14.56 ± 1.06^{dB}
35	53.74 ± 3.10^{fA}	30.00 ± 2.81^{eB}
42	89.46 ± 5.24^{gA}	58.85 ± 3.17^{fB}
Means with different superscripts in each column (a, b, c, d, e, f, g) and in row (A, B) differ significantly (LSD test, $P < 0.05$) from each other. Data are presented as means ± SEm (n = 3).		

Conclusion

The present study concludes that Greek yoghurt prepared with addition of CLE (3.0%) had 35 days shelf life, when stored at refrigeration temperature (7±1ºC). Storage has led to decline in sensory quality as well as physico-chemical parameters. During storage study, the average scores for flavour, body and texture, colour and appearance, product acidity and overall acceptability of all samples were significantly decreased. Similarly, the decrease in pH, TPC and antioxidant capacity (ABTS, DPPH, FRAP) was also recorded.

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