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Developing a Low-fat Drinking Yoghurt by Incorporating Green Tea (*Camellia sinensis*) Extract as a Functional Ingredient

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ABSTRACT

Background: Nowadays people are health-concerned and consume more of functional foods. Thus, this study is focused on developing a drinking yoghurt incorporated with green tea (*Camellia sinensis*) extract as a functional ingredient.

Methods: Green tea extract (GTE) was obtained by brewing 02 g of dried green tea leaves in 100 mL of water at 90°C for 30 minutes. Drinking yoghurts were prepared with standardized sterilized milk incorporated with 0%, 10%, 15%, 20% and 25% (v/v) of GTE. Physio-chemical, microbial and sensory analysis were conducted to evaluate the quality attributes of the developed product. It was stored under refrigerated conditions (4°C) for 15 days.

Result: Sensory data showed that 25% green tea extract incorporated drinking yoghurt had the best organoleptic properties. It had the significantly ($p<0.05$) highest ash and lowest fat contents compared to the control. Significantly ($p<0.05$) higher pH and lower titratable acidity values were observed in 25% green tea extract incorporated yoghurt compared to the control on 15th day of storage. The microbial analysis did not show the presence of coliform. In conclusion, yoghurt incorporated with 25% (v/v) green tea extract has better organoleptic properties and nutritional value while it can be stored under refrigerated conditions for 15 days without any quality deterioration while producing at affordable price.

Key words: Drinking yoghurt, Green tea extract, Microbial analysis, Physio-chemical analysis, Sensory evaluation.

INTRODUCTION

Functional foods have the potential to provide health benefits beyond the essential nutrients. Fermented dairy products such as yoghurts are the most common functional foods among all the other functional foods available in the market. Yoghurt products are an excellent source of protein, calcium, phosphorus, vitamin B2, vitamin B1, vitamin B12, folate, niacin, magnesium, Potassium and zinc (McKinley, 2005). As yoghurt is rich in calcium; it is good for bone health. The potassium present in yoghurt helps to eradicate excess of sodium from the body and helps in controlling blood pressure. The probiotics present in yoghurt helps to increase the immunity of the body (Weerathilake *et al.,* 2014). The types of yoghurt vary according to the fat content, texture and the added flavours to the yoghurt (Fidaleo *et al.,* 2015). Different yoghurt products were tested by many researchers to ensure the nutritional and health benefits according to the current consumer demand. Low fat frozen flavoured yoghurt with added carrot pulp are high in the organoleptic properties and nutritional value (Agarwal and Prasad, 2013). The aloevera gel added yoghurt rich with vitamin C, phytonutrients and found to be a good probiotic beverage with enhanced taste (Govindammal *et al.,* 2017). Further, Narayana and Kale (2019) showed the possibility of using spirulina in probiotic yoghurt which has a great importance in dairy industry. Drinking yoghurt is a growing area of interest due to the consumer demand for portable, hand-held meals that fit for a busy lifestyle. Moreover, drinking yoghurt can deliver all the health and nutritional benefits of regular set yoghurt or stirred yoghurt in an easily consumable way (Allgeyer *et al.,* 2010). Fortification of yoghurt with different types of fruits has already gained popularity and preferred markedly over plain yoghurt. Mango yoghurt drink prepared with 6% mango pulp cought highest consumer acceptance (Raut *et al.,* 2015).

Tea is the most widely consumed beverage around the world which only seconds to water. In green tea production, tea leaves are picked and immediately subject to steaming to prevent fermentation. By this steaming process, the enzymes responsible for breaking down the pigments are destroyed and the green colour of the leaves are retained. Due to this process, green tea has higher polyphenol content (Nakagawa, 1970). These polyphenols mainly attribute to the health-promoting effects of green tea. Green tea

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polyphenols include flavanols, flavandiols, flavonoids and phenolic acids. Most of these polyphenols are flavanols and known as catechins. The mainly found catechins are epicatechin, epigallocatechin, epicatechin gallate and Epigallo Catechin Gallate (EGCG). Green tea has many health benefits associated with the antioxidative property of polyphenols. EGCG is the most effective catechin in the antioxidative ability among all the other green tea catechins (Khan and Mukhtar, 2007). Antioxidants help to improve the immune system of the body, thus lower the risk of infections, cardiovascular diseases and prevent cancers. Apart from that green tea has many other health benefits such as protection against Alzhemers disease and Parkinson's disease, helps to lower and stabilize blood sugar levels by increasing the insulin sensitivity, lower the low-density lipoprotein (LDL) levels in the body without affecting high-density lipoprotein (HDL) levels, lower the chance of the oral cavities and other dental problems by killing the bacteria in the oral cavity, facilitates weight loss by reducing body fat percentage, EGCG stimulates hair cell production and hair growth, helps to control high blood pressure and lower the risk of osteoporosis and related fractures (Ziaedini *et al.* 2010). Other than that caffeine and amino acids are also important compounds present in green tea (Lamothe *et al.* 2014).

Yoghurt and other fermented dairy products have considerable economic importance all over the world due to its high nutritional value. This nutritional value can be further diversified by adding nutraceutical ingredients like green tea polyphenols, targeting consumers with various health issues. So that incorporation of green tea into drinking yoghurt is a good solution for those who do not like the astringent taste of plain green tea. Therefore, this study is focused on developing a drinking yoghurt incorporated with green tea extract as a functional ingredient.

MATERIALS AND METHODS

Preparation of green tea extract

Standardized full cream milk, sterilized non-fat milk, skim milk powder, starter culture, gelatin and sugar were supplied by Milco (Pvt) Ltd, Narahenpita, Sri Lanka. Green tea powder was supplied by Heritage Teas (Pvt) Ltd, Wellampitiya, Sri Lanka. The green tea powder (2 g/100 mL of water) was infused in deionized hot water (90°C) for 30 minutes. Then the content was filtered using a sieve and allowed to cool.

Production of drinking yoghurt incorporated with green tea extract

Experiment consisted of five treatments namely, plain drinking yoghurt as the control, addition of green tea extract at the rate of 10%, 15%, 20% and 25% v/v. First, the Punnet square calculation was done to find out the required amount of sterilized non-fat milk and full cream milk for the standardization of milk (1.5% fat). Then the required amounts of all the ingredients were measured. Sugar, gelatine and skim milk powder were mixed, separately. Then the standardized milk was pre-heated using the double boiling method. The mixture of sugar, skim milk powder and gelatine was gradually added when the temperature rose to 60°C-70°C. The mixture was stirred well until all the ingredients were dissolved. Then the mixture was filtered using a sieve. The filtered mixture was pasteurized at 90°C for 4 minutes. Then the mixture was cooled up to 44°C and green tea extract and starter culture (20 g/L) were added. The drinking yoghurt mixture was stirred until all ingredients mix well. Then it was incubated at 44°C until pH dropped to 5. When pH was 5, the sample was taken out and refrigerated. When the sample was suffienciently cooled, taken out of refrigerator and stirred to break the texture. Then it was poured into cups and stored in refrigerated conditions (4°C).

Sensory evaluation

Each drinking yoghurt sample was evaluated using 30 un-trained panellists for the colour, aroma, taste, texture and overall acceptability using a five-point hedonic scale. The samples were served in regular size (80 g) white colour cups with randomly selected three digits numbers.

Nutritional and physiochemical analysis

pH of the drinking yoghurt samples was measured daily during the period of shelf life of 15 days using a pH meter. Titratable acidity of the samples was measured in three day intervals by titrating with 0.1% NaOH during the storage period. Total solids, moisture, ash, crude fat and crude protein contents of the samples were measured as described in official methods of analysis of the association of official analytical chemists (AOAC, 2003).

Microbial analysis

Total coliform count and yeast and mould count of prepared yoghurt samples were analyzed on 1st day, 8th day and 15th day. MacConkey agar was used to enumerate coliforms and *E. coli* in yoghurt. Spread plate techniques were used and the plates were incubated at 37°C for 18 hours. Potato dextrose agar was used to enumerate yeast and mould counts in drinking yoghurt. Spread plate technique was used and the plates were incubated at 25°C for 5 days and colonies were counted manually.

Statistical analysis

Complete randomized design (CRD) was used as the experimental design of the research. The physicochemical data were analyzed using one-way ANOVA by Statistical Analysis System (SAS) version 9.0 (SAS Institute Inc. Cary, North Carolina, USA) with 95% confidence interval and the mean separation was done by using Least Significant Difference (LSD) test. Sensory evaluation data were analyzed using Minitab statistical software (Minitab, State Collage, Pearson Addison, Wesley, USA) with a 95% confidence interval under the Kruskal Wallis test.

RESULTS AND DISCUSSION

Nutritional properties

Table 1 shows the nutritional properties of green tea extract

(GTE) incorporated drinking yoghurts. Protein, total solids and moisture contents of drinking yoghurts incorporated with different levels of green tea extract were not significantly different ($p > 0.05$) among treatments (Table 1). However, the numerically highest protein content was observed from 10% GTE incorporated drinking yoghurt and the lowest protein content was observed from 25% GTE incorporated drinking yoghurt. According to the SLS standards, the minimum protein content for drinking yoghurt is 2.7%. In this study protein content of all the samples were higher than the minimum required value. This could be due to the usage of skim milk powder. Skim milk powder contains around 34% of protein (Thomas and Wansapala, 2017). The significantly highest moisture and lowest total solid contents were observed from 25% GTE incorporated drinking yoghurts while the highest total solids content was observed from 15% GTE incorporated drinking yoghurt. In general, finished dairy products has a higher total solid content than the milk due to the addition of skimmed milk powder and sugar (Kale *et al.* 2008). Most commercial drinking yoghurts have a moisture content range between 80 - 86% (Thomas and Wansapala, 2017). Therefore, the moisture content of all the samples were also within this range.

The observed fat and ash contents of the treatments were significantly different ($p < 0.05$) among treatments (Table 1). Fat content is an important factor that affects the texture of drinking yoghurt. According to the Sri Lanka Standards (SLSI, 2016), the minimum fat content of low-fat drinking yoghurt is 1.5%. The fat content of all the samples were within the standard level. The significantly higher fat percentage was observed from the control (0% GTE) compared to the 15, 20 and 25% GTE incorporated yoghurts. This can be due to the interaction between tea polyphenols and the yoghurt fat.

The significantly highest ($p < 0.05$) ash percentage was observed from 25% GTE incorporated drinking yoghurt while the lowest was observed from the control (0% GTE) ($p < 0.05$). The ash content has gradually increased with increasing level of green tea extract. Ash content represents the mineral content of the product. Normally tea contains 5 - 7% minerals, mainly calcium (Ca), potassium (K), phosphorus (P) and magnesium (Mg) and trace amounts of zinc (Zn), copper (Cu) and manganese (Mn) (Thomas and Wansapala, 2017). This could be the reason for observed higher ash contents in GTE incorporated samples. The highest was observed in yoghurt with 25% of GTE.

Shelf life evaluation

Table 2 shows variations in pH values of GTE incorporated drinking yoghurts during the storage period. The observed pH was significantly different ($p < 0.05$) among treatments. The initial pH of the treatments were ranged between 4.78 - 4.91. The pH on day 15 was ranged between 4.42 - 4.54. According to the SLS standards pH values of drinking yoghurt range between 4.2 - 4.6. In this study, the pH of samples varied within the range of SLS standards. The significantly lowest ($p = 0.0001$) pH value was observed from the control (0% GTE) throughout the storage life. The 25% GTE incorporated sample showed a higher pH throughout the storage life. During the storage period, pH had gradually decreased in all treatments. This indicates that lactic acid bacteria (LAB) were not inhibited during their growth or their survival by different levels of GTE concentrations (Jaziri *et al.* 2009). According to the findings of other similar studies, the addition of GTE significantly inhibited the growth of many pathogenic microbes including *Staphylococcus aureus, Escherichia coli* and *Salmonella enteritidis* but did not inhibit LAB (Jaziri *et al.*, 2009).

The type of culture used and the storage temperature are the main reasons for the pH variation in drinking yoghurts during the storage period. When the storage temperature goes beyond 4°C, the fermentation bacteria grow rapidly and decrease the pH value (Thomas and Wansapala, 2017). In this study, the same starter culture and same storage conditions (4°C) were maintained for all the treatments during the storage period. The pH level of 4 is considered as the minimum level of product deterioration (Thomas and Wansapala, 2017). Below pH 4, the product will give a high acidic taste which is not suitable for consumption. In this study, all the samples had a pH value greater than 4 at the end of day 15. All the drinking yoghurt samples were immediately transferred from incubator to refrigerator at pH 5. This can lead to a high pH value due to the inhibition of fermenting microbes (Thomas and Wansapala, 2017). Therefore, even after 15 days, the pH ranged between 4.42 - 4.54.

Table 2 shows the variation of the titratable acidity of GTE incorporated drinking yoghurts during the storage

Table 1: Nutritional composition of green tea extract incorporated drinking yoghurts.

Treatments	Fat (%)	Protein (%)	Ash (%)	Total solids (%)	Moisture (%)
T1 (25%)	1.61^{bc}	4.21	1.75^{e}	17.99	82.01
T2 (20%)	1.61^{bc}	4.24	1.56^{cd}	18.42	81.58
T3 (15%)	1.60^{c}	4.21	1.50^{c}	18.99	81.01
T4 (10%)	1.64^{ab}	4.25	1.32^{b}	18.26	81.74
T5 (0%)	1.65^{a}	4.23	1.24^{a}	18.76	81.23
SE	0.01	0.01	0.02	0.45	0.45
P value	0.0011	0.3489	0.0001	0.5568	0.5568

^{a,b,c,d,e}Differing superscripts within a column indicate means that were significantly different ($P < 0.05$).

period. During the storage period, the titratable acidity gradually increased in all five treatments. The activity of lactic acid bacteria, which converts lactose into lactic acid could be the reason for the increase in titratable acidity. According to the Sri Lanka standards, the titratable acidity of all types of yoghurts should be within 0.8 - 1.25% (SLSI, 2016). Therefore, all treatments were within the acceptable range during the storage period.

The significantly highest (p = 0.0001) titratable acidity was observed from the control (0% GTE) drinking yoghurt on day 1 while the lowest titratable acidity was observed from 25% GTE incorporated drinking yoghurt on day 1, 12 and 15. It shows that *Bifidiobacterium* and *Lactobacillus spp.* which are responsible for the acidity development are less severely affected by tea phenolics (Thomas and Wansapala, 2017). Initially, the titratable acidity was lower in all treatments, but finally, it came to the acceptable level.

Microbial count

The coliform counts were checked during the storage period of day 1, 8 and 15. Zero coliform counts were observed in all GTE incorporated treatments and in control during the storage period. According to Sri Lanka standards 824 (SLSI, 2016), the coliform count should not exceed 1CFU/g. Since the coliform was not positive either in GTE incorporated treatments or in control, it indicates that the hygienic measures that were undertaken during the production and storage process were at a satisfactory level.

Table 2 shows the yeast and mould counts of prepared drinking yoghurts during the storage period. The highest yeast and mould count was reported from 25% and 15% GTE incorporated drinking yoghurts. However, according to the Sri Lanka standards 824 (SLSI, 2016), the allowable limits for yeast and mould is $<10^3$ CFU/mL and the yeast and mould count in all treatments did not exceed the allowable limits during the storage period. Due to the acidic nature of yoghurt, it is a good media for the growth of yeast and mould. Reasons for the development of yeast and mould in samples could be the poor hygienic practices, improper storage conditions and sealing. Yeast and mould counts were higher in the samples with higher GTE level. Normally fungal growth occurs in raw green tea with the storage. Therefore, fungus spores could be added into drinking yoghurt with GTE. This could be the reason for higher yeast and mould counts in GTE added samples.

Sensory evaluation

Fig 1 shows the estimated mean rank sums of sensory attributes namely colour, aroma, texture, flavour and overall acceptability of different GTE levels incorporated drinking yoghurt samples. Good quality yoghurt has pleasant milky to slight sour taste with natural yoghurt flavour (Agarwal and Prasad, 2013). Flavour and texture of yoghurt is important for its marketable quality (Patil *et al.* 2015). According to Fig 1, the yoghurt samples showed significant differences ($p < 0.05$) in all sensory attributes except texture. The lowest sensory values for colour, aroma, flavour and overall acceptability were recorded from the control (0% GTE) drinking yoghurt while recording the highest value for texture. Except for texture, the highest sensory values were recorded from 25% GTE incorporated drinking yoghurt. Therefore, 25% GTE incorporated drinking yoghurt was the most preferred.

When considering the colour, there was a significant difference ($p < 0.05$) among samples. The highest average rank was recorded from 25% GTE incorporated drinking yoghurt while the lowest was recorded from 0% GTE

Table 2: Changes in pH, titrable acidity and yeast and mould counts of green tea incorporated drinking yoghurt over the storage period.

Storage period	Treatments					SE	P value
	T1 (25%)	T2 (20%)	T3 (15%)	T4 (10%)	T5 (0%)		
pH							
Day 01	4.91^{a}	4.90^{ab}	4.89^{b}	4.90^{ab}	4.78^{c}	0.00	0.0001
Day 04	4.83^{ab}	4.81^{a}	4.84^{b}	4.84^{bc}	4.70^{d}	0.01	0.0001
Day 08	4.74^{a}	4.75^{a}	4.75^{a}	4.74^{a}	4.58^{b}	0.01	0.0001
Day 12	4.57^{a}	4.57^{a}	4.57^{a}	4.57^{a}	4.49^{b}	0.01	0.0001
Day 15	4.54^{a}	4.53^{ab}	4.53^{ab}	4.51^{b}	4.42^{c}	0.01	0.0001
Titrable acidity							
Day 01	0.62^{a}	0.64^{b}	0.65^{bc}	0.67^{d}	0.70^{e}	0.00	0.0001
Day 04	0.72^{a}	0.73^{ab}	0.73^{ab}	0.72^{a}	0.75^{b}	0.01	0.0182
Day 08	0.75^{a}	0.76^{ab}	0.77^{bc}	0.79^{cd}	0.80^{d}	0.01	0.0001
Day 12	0.79^{a}	0.81^{b}	0.82^{b}	0.83^{bc}	0.85^{c}	0.00	0.0001
Day 15	0.83^{a}	0.85^{b}	0.86^{bc}	0.87^{bc}	0.87^{c}	0.00	0.0001
Yeast and mould counts (CFU/mL)							
Day 01	60	12	60	0	4		
Day 08	100	16	100	40	10		
Day 15	140	40	120	60	60		

^{a,b,c,d,e}Differing superscripts within a column indicate means that were significantly different (P<0.05).

incorporated drinking yoghurt. This can be due to the unique yellowish-brown colour of the GTE incorporated drinking yoghurt. Flavonols, flavones, isoflavones and anthocyanins are the chemical compounds that are responsible for the colour of tea infusion (Chaturvedula and Prakash, 2011) and, by adding green tea extract, that colour was imparted to the drinking yoghurt.

There was a significant difference ($p < 0.05$) among samples for aroma. The highest average rank was recorded from 25% GTE incorporated drinking yoghurt while the lowest was recorded from 0% GTE incorporated drinking yoghurt. According to the results, the consumer preference was higher for the aroma of drinking yoghurt that comes with the aroma of green tea rather than the plain drinking yoghurt. The aroma is one of the critical aspects of food quality which can determine consumer acceptance or rejection. It is a complex sensation that occurs due to the interaction of the volatile compounds of food with the olfactory receptors. There are many biochemical compounds which are responsible for the aroma of green tea such as linalool, phenylacetaldehyde, benzaldehyde and phenyl ethanol (Chaturvedula and Prakash, 2011). The most responsible aromatic compounds for the drinking yoghurt aroma are acetaldehyde, acetone, acetoin and diacetyl (Routray and Mishra, 2011). The combination of chemicals from both drinking yoghurt and GTE had given a pleasant aroma to the final product.

There was no significant difference ($p > 0.05$) among samples for texture. That means the incorporation of green tea extract does not affect the texture of drinking yoghurts. This could be due to the reduction of the amount of water added with respect to the amount of GTE added. Therefore, all the treatments had the same amount of liquid (either water or GTE) added. The highest average rank was recorded from 0% GTE incorporated drinking yoghurt while the lowest was recorded from 15% GTE incorporated drinking yoghurt.

There was a significant difference ($p < 0.05$) among samples for flavour. The highest average rank value was recorded from 25% GTE incorporated drinking yoghurt while the lowest was recorded from 0% GTE incorporated drinking yoghurt. Taste of food is mainly composed of five basic sensations; astringency, sweetness, sourness, bitterness and umami (Chaturvedula and Prakash, 2011). So the taste of the prepared drinking yoghurt samples is a balance of various taste sensations and various biochemical compounds from various ingredients which have been used for the production. Green tea polyphenols are responsible for the astringent taste and caffeine is responsible for the bitter taste (Chaturvedula and Prakash, 2011). The acidity of the drinking yoghurt is mainly responsible for the flavour of drinking yoghurt. Addition of different flavourings and incorporation of other food materials cause to mask the original flavour of drinking yoghurt. In this research also the original drinking yoghurt flavour was masked to some extent by green tea flavour. But most of the panellists preferred that new flavour. Therefore, 25% GTE incorporated sample was the best sample for flavour.

When considering the overall acceptability there was a significant difference ($p < 0.05$) among samples. The 25% GTE incorporated drinking yoghurt had the highest overall acceptability, while the control (0% GTE) had the lowest overall acceptability.

Cost-benefit analysis

Table 3 shows the cost of production (without the manufacturing cost) of drinking yoghurts produced from 1 L of milk. Due to the addition of GTE, the cost of all the green tea incorporated drinking yoghurts has increased compared to the control. The highest cost of production was recorded from the highest level of GTE (25%) incorporated drinking yoghurt. When comparing all the treatment, there was no huge difference among the cost of production.

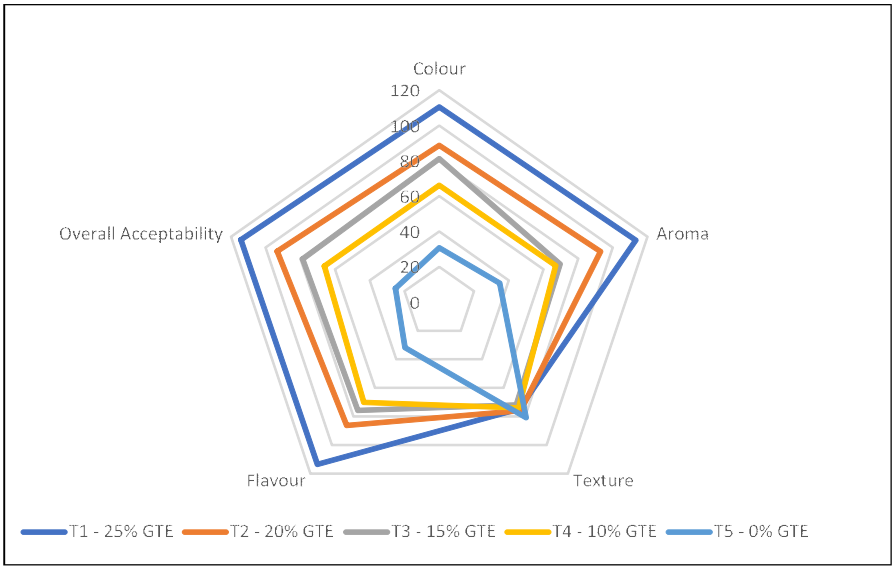


Fig 1: Pattern of variation of sensory properties in green tea incorporated drinking yoghurts.

Table 3: Cost of production of green tea incorporated drinking yoghurt.

Ingredient	Unit	Unit price (LKR)	Price per litre (LKR)				
			T1 (25%)	T2 (20%)	T3 (15%)	T4 (10%)	T5 (0%)
Full cream milk	Litre	178.00	106.80	106.80	106.80	106.80	106.80
Non-fat sterilized milk	Litre	240.00	96.00	96.00	96.00	96.00	96.00
Gelatine	Kg	4,000.00	12.40	12.40	12.40	12.40	12.40
Sugar	Kg	95.00	9.50	9.50	9.50	9.50	9.50
Skimmed milk powder	Kg	1,000.00	38.00	38.00	38.00	38.00	38.00
Culture	Kg	250.00	5.00	5.00	5.00	5.00	5.00
Packet	Nos	2.50	12.50	12.50	12.50	12.50	12.50
Green tea	Kg	600.00	3.000	2.40	1.80	1.20	-
Total cost for 1 L	-	-	283.20	282.60	282.00	281.40	280.20
Number of packets	-	-	5	5	5	5	5
Cost for 1 packet (180 mL)	-	-	56.64	56.52	56.40	56.28	56.04

CONCLUSION

Drinking yoghurts can be developed by incorporating 25% GTE with best organoleptic and nutritional properties. Drinking yoghurts incorporated with 25% GTE can be stored up to 15 days in refrigerated conditions (4°C), without addition of preservatives and no quality deterioration. The lowest cost of production was reported in control yoghurt than GTE incorporated drinking yoghurts.

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