International Journal of Food Science and Nutrition
ISSN: 2455-4898, Impact Factor: RJIF 5.14
www.foodsciencejournal.com
Volume 2; Issue 1; January 2017; Page No. 188-195

Utilization of green tea (*Camellia Sinensis*) extract for the production of antioxidant rich functional drinking yoghurt

N Thomas, MAJ Wansapala

Food Science & Technology Dept., Faculty of Applied Sciences. Sri Jayewardenepura Univ., Sri Lanka

Abstract
Aqueous extract was prepared from dried green tea leaves, and its antioxidant activities, DPPH radical scavenging and total phenolic content (TPC) was determined. Folin-Ciocalteu's reagent was used to determine the total phenolic content spectrophotometrically and Gallic acid was used as the calibrant. The yield was 162 mg GAE/g extract. This was obtained at extraction temperature 100 °C and extraction time 40 minutes. Drinking yoghurt was prepared with standardized & homogenized pasteurized milk fortified with 5%, 10% and 15% of the green tea extract after the incubation period of the yoghurt. The physico-chemical, microbial and sensory analysis was determined to ascertain the quality attributes of the product. A sensory analysis was carried out to determine the best ratio of yoghurt mix and green tea extract. The best ratio selected was the drinking yoghurt which consists of 15% (v/v %) GTE. The antioxidant activity of the drinking yoghurt was determined for three weeks. The highest antioxidant activity was obtained just after the green tea extract was incorporated into the drinking yoghurt and the lowest activity was recorded after 21 days. The antioxidant activity decreased at a higher rate in the first week compared to the second and third week. Addition of GTE had no significant effects on the sensory attributes (appearance, overall flavor, after taste, aroma and overall acceptability) as compared to the control sample. The results of physico-chemical analysis obtained showed increase in values for pH (4.37-4.40), soluble solids (13.9-14.7). There were also remarkable increase in the proximate values for moisture (79.7-82.99) and ash (0.34-0.42). A reverse trend was observed for acidity (0.741-0.738), total solids (20.30-17.01), protein (6.12-5.98) and fat (2.50-2.20) values in GTE enriched drinking yoghurts. The microbial analysis showed no presence of E. coli bacteria. Yeast and the mold growth was less in the green tea drinking yoghurt. Yeast count was recorded for the control sample after 21 days and in the selected sample after 26 days.

Keywords: green tea extract; antioxidant activity; total polyphenol content; drinking yoghurt; physico-chemical properties

1. Introduction

According to the FUFOSE (concentrated action Functional food science in Europe') consensus document a food can be regarded as " FUNCTIONAL" , if it is satisfactorily demonstrated to beneficially affect one or more target functions in the body, beyond adequate nutritional effects. This will lead to improved health state, well-being and/or reduction of risks of disease.

Examples of functional foods include foods that contain specific minerals, vitamins, fatty acids or dietary fibers. Foods with added biologically active substances such as phytochemicals and those that can support beneficial microbial cultures of interest also fall into this category [29].

Any consumer can buy functional food from a normal market. It is not pills or capsules it is a normal part of food pattern. Functional food can be a normal natural food, where one or more characteristics have been enhanced or some adverse health effects are minimized by adding beneficial compounds.

Functional dairy products are available with the required dairy-dose; this had been one reason for popularity of dairy products in the past few years. Consumers are more concerned about their personal health, due to this reason a market for functional food products was created. In future we will probably see more products targeting special consumer groups like gender specific, to improve physical and mental health and treat specific diseases etc.

So far functional dairy products have been developed. Fermented dairy products such as cheese, curd, yoghurt are some of the traditionally developed products. Yoghurt, as a fermented diary product is regarded as a probiotic carrier, is nutritionally rich in available protein, calcium, milk fat, potassium, magnesium, vitamin B2, B6 and vitamin B12 [20]. It has nutritional benefits beyond those of milk, because people who are moderately lactose intolerant can enjoy yoghurt without ill effects, as most of the lactose in the milk precursor has been converted to lactic acid by the bacterial culture [47]. Yoghurt also has medical uses because of the probiotic characteristics, in helping out on a variety of gastro intestinal conditions and in preventing antibiotic associated diarrhea [20]. Yoghurt is believed to promote good gum health, facilitates the absorption of calcium, thus preventing osteoporosis, possibly because of the probiotic effect of lactic acids presents in yoghurt [48].

Phytochemicals are a large group of plant-derived compounds, responsible in preventing diseases. Phytochemicals are rich in fruits, vegetables, beans, cereals, and plant based beverages such as tea, wine. Antioxidants are naturally occurring plant substances, which protect the body from damage caused by harmful molecules called free radicals. These compounds can delay, inhibit, or prevent oxidation of oxidizable materials by scavenging free radicals and diminishing oxidative stress. Antioxidants improve immune function and perhaps lower the risk for infection, cardiovascular disease, and cancer. Antioxidants exist as vitamins, minerals, polyphenols etc. Therefore a diet rich in antioxidants can be very beneficial. Antioxidants are also used as a food additive to prevent the deterioration of food. These are added to food products like oil, bread, cookies, biscuits and dairy products.

The extracts from green tea leaf were found to possess strong antioxidant activity, the antioxidant mechanisms of green tea leaf extracts may be attributed to their free radical- scavenging ability. In addition, phenolic compounds appear to be responsible for the antioxidant activity of green tea extract. On these results green tea extract from leaf can be used for a variety of beneficial chemo-preventive effects [18].

Many attempts have been made and researches are carried out to use herbal additives to promote taste and medicinal values to improve the health of consumers. In this research green tea extract is taken as an additive to give a good aroma, taste and increase the antioxidant properties of drinking yoghurt.

The objective of the current study was to Green Tea extract in the manufacture of drinking yoghurt. Antioxidant and organoleptic properties were determined. Sensory acceptability of drinking yoghurt was assessed. Physiochemical properties of the final product in relation to shelf life and the proximate analysis of the final product were carried out.

2. Materials and methods

2.1 Materials

Standardized & homogenized pasteurized milk (cow milk source), skimmed milk powder (Spray dried, imported from New Zealand), sugar, gelatin, yoghurt culture (*Lactobacillus bulgaricus* and *Streptococcus Thermophilus*), dried green tea leaves (from Glassaugh Estate, Nanuoya). Gallic acid, Folin-Ciocalteu's phenol reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH) were purchased from Analytical Instruments (Pvt.) Ltd(Elvitigala Mawatha, Colombo 8,Sri Lanka) .All the other chemicals used including the solvents, were of analytical grade..

2.2 Methods

Preparation of Green tea extracts from dried Green tea leaves

Water extraction of green tea leaves was carried out as the method described by Ye *et al.,* 2013 and Sun *et al.,* 2016

Dried Green tea leaves were ground by grinder. The ground tea samples were extracted with 1:15 (weight/volume) deionized water at 100 °C for 40 min. The extracts were then centrifuged at 3176 rpm for15 min, and the resulting insoluble residues were extracted again as described above. The supernatants were combined and were kept at 4 °C, namely green tea stock. The clear supernatants were used in the making of drinking yoghurt.

Manufacture of drinking yoghurt fortified with GTE

The yoghurt mix was heated to 105 °C for 1 min and cooled to (44 °C). Then 3% traditional starter culture was added and the mixtures were incubated at 42 °C until the gel structure was formed. The gel was stirred and the aqueous GTE was added at the ratios of 5%, 10% and 15% (v/v %). Then the final product was stored at refrigerator (4± 2 °C).

Total polyphenol content in the green tea extract

Total phenolic content was determined according to ISO 14502-1 method by using Folin-Ciocalteu's reagent. 0.5 milliliter of the sample extract was transferred into one-mark 100 ml volumetric flask and diluted up to the mark with distilled water and mixed. Exactly 1.0ml of diluted extract or one millilitre of gallic acid standard solutions (10, 20, 30, 40, 50, 60, 70 ppm) were transferred into separate test tubes that represent the samples and then 5ml of 10% Folin-Ciocalteu's phenol Solution , 4ml of 7.5% Sodium carbonate solution were added respectively to the sample test tubes. (7.5% Sodium carbonate solution should be added within 3min to 8min period after adding 10% Folin-Ciocalteu's Reagent). The mixture was vortexed vigorously and was left to stand at room temperature for 1 hour. A blank sample was also prepared as same as above but replacing 1.0ml of diluted tea extract with 1.0 ml of distilled water. Then the absorbance was measured with a spectrum UV–Vis Spectrophotometer at 765 nm. Results were expressed as mg of Gallic acid equivalent per gram of sample (mg GAE/g). All the samples were analyzed in triplicates.

Antioxidant activity of green tea extracts

Radical scavenging activity (RSA %) assay. Free radical scavenging activity (RSA) of the samples was measured using the method of Brand-Williams et al. (1995). A volume of 0.5 mL of different concentrations of sample/standard was mixed with 2.5 mL of 0.05ppm DPPH. This was allowed in the dark for 30 minutes and absorbance was measured at 517 nm. Gallic acid (0.001-0.01 mg mL^{-1}) was used as the standard. A control was prepared by mixing 0.5 mL of distilled water and 2.50 mL of 0.05mg dm^{-3} DPPH solution. The ability to scavenge the DPPH radical was found by using the following equation.

$$RSA\% = \frac{A\ Control - A\ Sample}{A\ Control} \times 100$$

Antioxidant activity of drinking yoghurt fortified with GTE

A concentration series of yoghurt samples were prepared. The solutions were vortexed for one minute and were centrifuged (3200 rpm for 5 minutes) to obtain a clear solution. A concentration series of yoghurt samples were prepared once a week for three weeks to measure the antioxidant activity. A volume of 0.5 mL of different concentrations of sample was mixed with 2.5 mL of 0.05ppm DPPH. This was allowed in the dark for 30 minutes and absorbance was measured at 517 nm. Gallic acid (0.001-0.01 mg mL^{-1}) was used as the standard. A control was prepared by mixing 0.5 mL of distilled water and 2.50 mL of 0.05mg dm^{-3} DPPH solution. The ability to scavenge the DPPH radical was found by using the following equation.

$$RSA\% = \frac{A\ Control - A\ Sample}{A\ Control} \times 100$$

Physico-Chemical Analysis

The moisture content and the ash content were determined according to the AOAC 2005 method. Protein content was determined using the kjeldhal method. Fat percentage was determined using the Gerber method.

Shelf life evaluation

Shelf life studies were carried out for the final samples of green tea drinking yoghurt. Samples were stored below 4 °C and organoleptic properties were tested time to time for three weeks period. The pH, titratable acidity (AOAC 1990) and the microbiological analysis was carried out time to time for two weeks period.

Microbiological Analysis

The determination of the microbial contamination in the yoghurts was performed by using Violet Red Bile Agar (VRBA) for the coliform count (ISO 6611:2004) and Yeast Extract Dextrose Chloramphenicol (YDC) Agar for the yeast and mould count (ISO 4832:2006 E). The colonies were counted using a

colony counter and the result was expressed as colony forming unit per ml. (cfu/ml).

Sensory Evaluation

Sensory evaluation of the drinking yoghurt samples were carried out by 30 panelists on a 9 point hedonic scale for different parameters such as appearance, aroma, overall flavor, after taste and overall acceptability. Each panelist was given with three coded samples with a score card and was asked to provide scores according to their preference. The temperature of samples when served was 5 °C in order to mimic realistic consumer behavior.

Statistical Analysis

Data collected from the sensory evaluation for the samples were analyzed using nonparametric Kruskal-Wallis test with 0.05 significant levels. Minitab 17 statistical software package was used for the analysis.

3. Results and Discussion

3.1 Sensory evaluation for the selection of best green tea extract amount for the drinking yoghurt

Three different amount of green tea extract were added to the drinking yoghurt mix while keeping all the other parameters constant

- Sample (248) – 95:5 (Yoghurt mix: Green tea extract)
- Sample (464) – 90:10 (Yoghurt mix: Green tea extract)
- Sample (315) - 85:15 (Yoghurt mix: Green tea extract)

Table 1: Average rank values of sensory attributes for incorporating green tea extract

Sample	Appearance	Aroma	Overall Flavour	After Taste	Overall Acceptability
248	46.1^{a}	35.3^{a}	40.5^{a}	46.6^{a}	35.8^{a}
315	48.3^{a}	56.3^{b}	52.6^{a}	46.0^{a}	55.3^{ab}
464	42.0^{a}	45.0^{ab}	43.4^{a}	43.9^{a}	45.4^{b}

When considering appearance, there was no significant difference between samples. According to the results shown in the table, the highest average rank in appearance was obtained by the sample 315. Therefore it was the best sample in appearance according to the results. There was a significant difference among the three samples (248,315,464) in aroma. According to paired wise comparison between samples using average rank values and the mean separation value, aroma attribute of sample 248 was significantly different from sample 315 as the average rank difference value is higher than the mean separation value. Also there was no significant difference between sample 464 with samples 248 and 315. When comparing the average rank values of the sensory attribute aroma sample 315 got the highest value. Therefore it was considered as the best sample which gave a good aroma.

When considering Overall Flavor, there was no significant difference between samples. According to the results obtained, the highest average rank in overall flavor was recorded to the sample 315. Therefore it was considered as the best sample which gave a good overall flavor.

When considering after taste, there was no significant difference between samples. According to the results shown in the table, the highest average rank in after taste was obtained by the sample 248. Therefore it was the best sample in giving a good after taste according to the results.

There was a significant difference among the three samples (248,315,464) in overall acceptability. There was a significant difference between sample 248 and 464. There was no significant difference between sample 315 with samples 248 and 464. When comparing the average rank values of the sensory attribute overall acceptability 315 (15% GTE) got the highest value. Therefore it was selected as the best sample.

3.2 Total phenolic Content

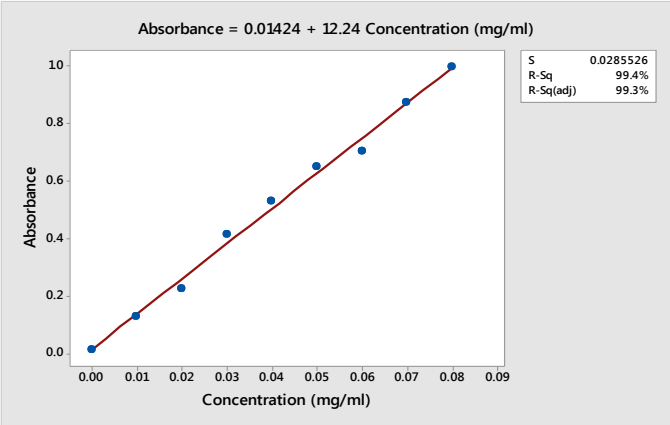

Fig 1: Absorbance of different concentration of Gallic acid standard

From the plot, TPC present in green tea extract was determined. Green tea extract showed a phenolic content of 162 mg GAE g^{-1}. Several studies have revealed that phenolic contents in plants are associated with their antioxidant activities, probably due to their redox properties, which allow them to act as reducing agents, hydrogen donors, and singlet oxygen quenchers and also may have a metallic chelating potential [49].

3.3 Antioxidant activity of green tea and drinking yoghurt fortified with GTE

DPPH radical scavenging activity

The IC_{50} values of the green tea extract and drinking yoghurt were found using Mini tab software and the results are summarized below.

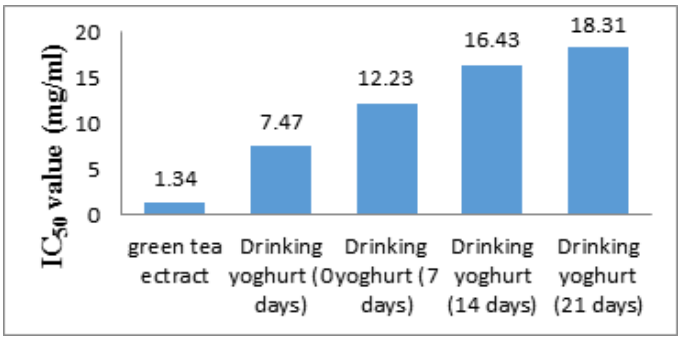

Fig 2: IC50 value of green tea and GTE enriched drinking yoghurt

According to the results obtained, green tea extract (IC_{50} = 1.34 mg ml^{-1}) and the drinking yoghurt (IC_{50} = 7.47-18.31 mg ml^{-1}) showed less activity than the standard (IC_{50} = 40 µg ml^{-1}). A decrease of activity with time in the drinking yoghurt was observed. The lowest activity was observed for the drinking yoghurt after 21 days (IC_{50} = 18.31 mg ml^{-1}) and the highest activity was observed within the first week of the drinking yoghurt's shelf life (IC_{50} = 7.47 mg ml^{-1}). The antioxidant activity is mainly due to the presence of polyphenols present in the drinking yoghurt, therefore a reduction in the activity is due to the loss of polyphenols in the yoghurt with time.

When preparing the samples the starter culture used consists of the bacterial strain *Lactobacillus bulgaricus* & *Streptococcus thermophiles* and the probiotic culture added consist of the strain *Bifidobacterium lactis*. The antioxidant activity of polyphenols in a green tea extract with probiotic bacteria has been studied. A decrease in the antioxidant activity has been observed in the green tea extract treated with bacteria and the antioxidant activity of untreated tea extract had not changed throughout the whole period of incubation [19]. Therefore the loss of polyphenols in the yoghurt is due to the presence of probiotics.

The antioxidant activity has decreased in the sample throughout its shelf life. In the drinking yoghurt within the first week the increase in the IC_{50} value is higher (increment of 38.9%) compared to the increment in the second and third week. Lowest increment in the IC_{50} value was observed within 14 to 21 days (increment of 10.2%). The antioxidant activity of the GTE enriched drinking yoghurt samples have reduced at a high a rate in the first week and at a lower rate in the second and third week. The reason is that the activity of *Lactobacillus bulgaricus* & *Streptococcus thermophiles* have decreased as the samples were stored under refrigerated conditions.

3.4 Proximate Composition

The proximate analysis results of control sample and green tea incorporated drinking yoghurt samples were obtained for three replicates and all results will be expressed as mean values ± standard deviation.

Table 2: Summarization of proximate analysis results of value added drinking yoghurt by incorporating green tea extract verses control

Constituents %	Green tea drinking yoghurt (85:15 ml)	Control
Fat	2.20±0.00	2.5±0.00
Protein	6.12±0.12	5.98±0.047
Ash	0.42±0.042	0.34±0.018
Moisture	82.99±0.07	79.7±0.2
Total solids	17.01±0.07	20.30±0.2
Total soluble solids	14.7±0.05	13.90±0.01

According to SLSI standards the minimum protein content in drinking yoghurt is 2.7%. In both the samples the protein content was higher than the required level. When the protein content is high it contributes to a hard texture. A high protein value was obtained for the two samples due to the usage of skimmed milk powder. Skimmed milk powder contains 34% of protein. Green tea fortified drinking yoghurt showed a less protein content compared to the plain drinking yoghurt.

The moisture content of the GTE enriched yoghurt sample was 82.99% and the plain drinking yoghurt was 79.7%. Plain drinking yoghurt had a low moisture content compared to the enriched drinking yoghurt. The moisture contents of the yoghurt samples fell within the range of most commercial yoghurts (80-86%).

According to SLSI standards the minimum fat content of drinking yoghurt is 2.2%. The fat content of the enriched drinking yoghurt was within the standard level. Fat content has been reported by other researchers to have positive influence on the physical and sensory characteristics [28] and negative impact on the shelf stability of yogurts [28].

According to the results obtained green tea incorporated drinking yoghurt contained a high amount of ash percentage compared to the plain drinking yoghurt. Mainly minerals contribute to the ash content. Tea contains around 5-7% minerals, mainly potassium (K), calcium (Ca), phosphorus (P), and magnesium (Mg), as well as small quantities of manganese (Mn), zinc (Zn) and copper (Cu). Therefore this can be the reason for green tea enriched drinking yoghurt to have a high ash percentage.

3.5 Shelf life evaluation

Microbial Count

Coliform count during the storage life

None of the samples showed a positive result for E.Coli/Coliforms during the storage period. According to SLS standards E.Coli count should not be more than 1 per gram.

Results obtained can be accepted with SLS Standards for yoghurts. Coliform count was negative in both samples due to hygienic preparations, handling and it was not subjected to cross contamination and precautions were taken to overcome microbial contamination

Yeast and mould count during the storage life

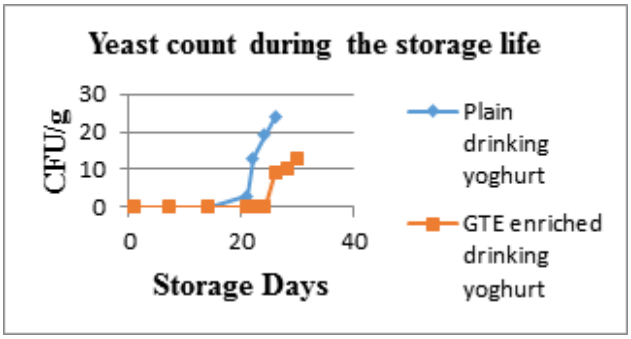

Fig 3: Graphical representation of Yeast count in final drinking yoghurt products

Yogurt is a selective environment for the growth of yeast and mold, because of its acidic nature. The spoilage of yogurt by yeast can be generally identified by the development of off flavors, textural losses due to gas production and swelling of the

product container (Kroger, 1976). Reasons for the growth of yeast and mold, could be poor hygienic practices contaminate due to unfiltered air, improper sealing and improper storage conditions. Therefore according to the Davis findings a yogurt produced with good manufacturing practices and correctly stored under refrigeration conditions (4ºC), the product shelf life will be 3 to 4 weeks.

When considering about the yeast count in the final products, yeast count was recorded for the plain drinking yoghurt sample after 21 days (3 CFU/g) and there was an increase in the yeast count every day. After 28 days of storage life the number of yeast count became too numerous to count. In the GTE enriched drinking yoghurt sample yeast count was recorded after 26 days (9CFU/g). At the beginning acidity in the drinking yoghurt is less and with time acidity is increased and this is preferred by yeast. Due to this reason yeast count was recorded in the final days of storage life.

Growth rate of yeast is less in the GTE enriched drinking yoghurt sample compared to the plain drinking yoghurt. Green tea is a rich source of polyphenols. It consists of C, EC, ECG, CG, EGC. Tea catechins show antifungal properties [50]. Due to the antifungal effect of green tea catechins the growth of yeast cells are less in the green tea incorporated drinking yoghurt.

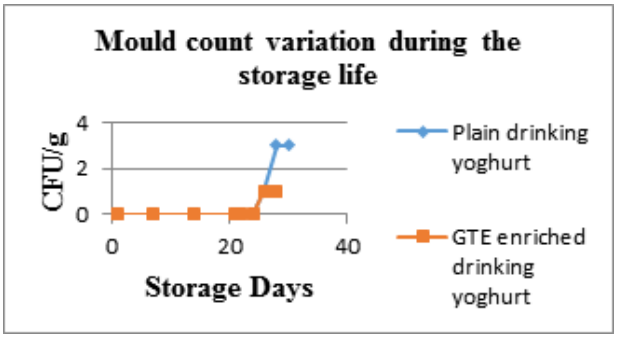

Fig 4: Graphical representation of Mould count in final drinking yoghurt products

Mould growth in both the samples was less compared to the growth of yeast cells. There was no mould count recorded for both the samples within the first three weeks. Mould grows more slowly than yeast contaminants in yoghurts.

Change in pH over the storage period

During the storage life of 21 days at 4 °C±1 pH values of both plain and GTE enriched drinking yoghurts were observed. During the storage period pH of both products gradually decreased. But the pH range during the period was within the acceptable range of yoghurt. Table shows the change of pH during 21 days of storage period.

Table 3: pH change in final drinking yoghurt

Time Intervals/Days	Plain drinking yoghurt/pH	GTE enriched drinking yoghurt/pH
0 days	4.37	4.40
3 days	4.35	4.38
6 days	4.34	4.37
9 days	4.32	4.35
12 days	4.31	4.34
15 days	4.26	4.30
18 days	4.24	4.29
21 days	4.24	4.27

As shown in the table the pH values of both control and drinking yoghurts were decreased gradually due to fermentation. During the 21 days of fermentation period pH value of the GTE enriched drinking yoghurt sample was between 4.40-4.27 and pH of the plain drinking yoghurt sample was between 4.37-4.24. Therefore both drinking yoghurt samples have shown a similar pattern of pH variation. At the end of 21 days the pH value of green tea drinking yoghurt was having a high value compared to the control sample. As reported by Akpan (2014) pH 4 was considered as the cutoff level of product deterioration. After that pH level, the acidity taste of the product will increase and it becomes unacceptable for consumers. Therefore both the samples had a pH value greater than 4 at the end of 21 days.

pH variation of drinking yoghurts during the storage period mainly depends on the temperature and the type of culture used for the yoghurt. If the temperature is not maintained at 4 °C±1 rapid pH changes can occur. When the temperature is higher than 4 °C the fermenting microbes grow very rapidly and this results in giving a low pH value to the drinking yoghurt. And at the same time if the drinking yoghurts were kept at a very low temperature just after incubation this will lead to a high pH value due to less action of fermenting microbes.

In both drinking yoghurt types the same culture was used which contain *Lactobacillus bulgaricus* & *Streptococcus thermophilus*. Both the samples were transferred from the incubator into the refrigerator at pH 4.37. After the addition of green tea extract into the drinking yoghurt pH increased to 4.40. Various microbes succeed one another as chemical environment of the product changes with time. The microbes are responsible in bringing out these changes. The stages of microbial growth are *Streptococcus*, then *Lactobacillus*, next yeast and moulds and finally *Bacillus*. *Streptococci* convert the milk sugar (Lactose) into lactic acid. The acidity of milk increases to the point where further Streptococci growth is inhibited. After that *Lactobacillus* begin to grow and convert the remaining lactose into lactic acid. The sour taste is due to the lactic acid present.

Change in titratable acidity over the storage period

During the cold storage of 21 days at 4 °C±1, acidity value was changed. During this period the acidity value of both products were gradually increased as shown in the table below.

Table 4: Acidity variation in final drinking yoghurt products

Time Intervals/Days	Plain drinking yoghurt/acidity (±SD)	GTE enriched drinking yoghurt/acidity (±SD)
3 days	0.741(±0.0056)	0.738(±0.045)
6 days	0.772(±0.0237)	0.766(±0.015)
9 days	0.806(±0.0029)	0.792(±0.0022)
12 days	0.844(±0.0015)	0.834(±0.004)
15 days	0.881(±0.0885)	0.872(±0.000)
18 days	0.906(±0.000)	0.904(±0.0104)
21 days	0.954(±0.002)	0.923(±0.000)

According to SLSI Standards the acidity value of yoghurts should be within 0.8 to 1.25% range. (SLS: part 2; 1989) In these products the acidity value was within the required range. In the plain drinking yoghurt sample the acidity value was recorded as 0.741% and it was gradually increased up to 0.954%. And in green tea drinking yoghurt it was changed from 0.738% to 0.923%. Therefore it shows that Bifidiobacterium and Lactobacillus spp. are less severely affected by tea phenolics. They continue making lactic acid even in the presence of green

tea phenolics. Initially the acidity value recorded for both samples were lower but with time it has come to the acceptable range.

During the fermentation process lactic acid is produced due to the activity of *Lactobacillus bulgaricus* & *Streptococcus thermophiles*. The titratable acidity is expressed as the percentage of lactic acid. Changes in acidity during the storage period are dependent on storage temperature and the culture used. Results recorded show that both the samples had an almost similar acidity variation within the storage life.

4. Conclusion

Based on the results of the sensory evaluation carried out in this research the appropriate and preferred amount of GTE to be incorporated into the drinking yoghurt was discovered.

Green tea extract is a rich source of polyphenols. TPC recorded in the GTE was 162 mg GAE $g^{-1.}$

The highest antioxidant activity of the drinking yoghurt sample was observed in the first week, with time the antioxidant activity reduced in the sample. The antioxidant activity reduced at a high rate in the first week and after that the activity was reduced at a lower rate. The substantial amounts of TPC in GTEs were well correlated and dominantly responsible for the antioxidant activity.

According to the results of the shelf life evaluation yeast and mold growth was less in the green tea incorporated drinking yoghurt compared to normal drinking yoghurt due to the antifungal properties of GTE and none of the products recorded Coliform count. The GTE incorporated drinking yoghurt can be safely stored up to a period of 26 days at 4 °C, without deterioration in the microbiological qualities.

Initially the pH values of the green tea drinking yoghurt and the control drinking yoghurt samples were 4.37 and 4.34. Acidity and the pH were recorded within the acceptable limits throughout the storage period of 21 days. Therefore a period of 21 days can be considered as the shelf life of the product. As the green tea incorporated drinking yoghurt and the control sample showed a similar pH and acidity variation it shows that Bifidiobacterium and Lactobacillus spp. are less severely affected by tea phenolics.

The GTE incorporated drinking yoghurt may provide health benefits ascribed to the presence of polyphenols.

However further researches are required to find out the behavior of the fermenting microbes when green tea extract is added additionally to the yoghurts and to do a comparison of antioxidant activity of yoghurt by using different extraction methods to extract green tea polyphenols.

5. References

1. Bharadwaz A, Bhattacharjee C. 'Extraction of Polyphenols from Dried Tea Leaves', nternational Journal of Scientific & Engineering Research. 2012; 3(5):1-5.
2. Cardona F, Andrés-Lacueva, C, Tulipani S, Tinahones FJ, Queipo-Ortuño MI. 'Benefits of polyphenols on gut microbiota and implications in human health', The Journal of Nutritional Biochemistry. 2013; 24(8):1415-1422. doi: 10.1016/j.jnutbio.2013.05.001.
3. Chacko SM, Thambi PT, Kuttan R, Nishigaki I. 'Beneficial effects of green tea: A literature review', Chinese Medicine, 2010; 5(1):13. doi: 10.1186/1749-8546-5-13.
4. Dai J, Mumper RJ. 'Plant Phenolics: Extraction, analysis and their Antioxidant and Anticancer properties', *Molecules*, 2010; 15(10):7313–7352. doi: 10.3390/ molecules15107313.
5. De NX, HaNH, Hang TV, Quan PT, Tuyen TN. 'Microwave-Assisted Extraction Of Polyphenols From Fresh Tea Shoot, *Tạp Chí Phát Triển Kh&Cn, Tập*, 9, 2006
6. Duda-Chodak A, Tarko T, Satora P. and Sroka, P. 'Interaction of dietary compounds, especially polyphenols, with the intestinal microbiota: A review', 2015; 54(3).
7. El-Said MM, Haggag HF, Fakhr El-Din HM, Gad AS, Farahat AM. 'Antioxidant activities and physical properties of stirred yoghurt fortified with pomegranate peel extracts', *Annals of Agricultural Sciences*, 2014; 59(2):207-212. doi: 10.1016/j.aoas.2014.11.007.
8. Gadkari PV, Balaraman M. 'Catechins: Sources, extraction and encapsulation: A review', *Food and Bioproducts Processing*, 93, 2015; 122-138. doi: 10.1016/j.fbp. 2013.12.004.
9. Garma J, Kresser C, zhou C, Donna, Foster-Horwith L, Haines A, Salmi L, *et al.* (2014a) About. Available at: https://chriskresser.com/do-polyphenols-improve-your-gut-bacteria/ Accessed: 2016.
10. Hardin JR. 2015 Polyphenol PRebiotics archives - allergies & your *gut*. Available at: http://allergiesandyourgut.com/ polyphenol-prebiotics/ (Accessed: 10 December 2016).
11. Hes M, Korezak J, Michalowska AG. 'Purification Process Influence on Green Tea Extracts' Polyphenol Content and Antioxidant Activity', *Acta Sci. Pol., Technol. Aliment*, 2007; 6:41–48.
12. Karaaslan M, Ozden M, Vardin H. Turkoglu H. 'Phenolic fortification of yogurt using grape and callus extracts', LWT - Food Science and Technology, 2011; 44(4):1065-1072. doi: 10.1016/j.lwt.2010.12.009.
13. Kodama DH, Any E, De Souza S, Gonçalves Franco, M. and Genovese MI. 'Phenolic antioxidants in green tea, 2011
14. Landete J. 'RETRACTED ARTICLEUpdated knowledge about polyphenols: Functions, bioavailability, metabolism, and health', *Critical reviews in food science and nutrition.*, 2012; 52(10):936-48.
15. Landete JM. 'Dietary intake of natural Antioxidants: Vitamins and Polyphenols', *Critical Reviews in Food Science and Nutrition*, 2013; 53(7):706-721. doi: 10.1080/10408398.2011.555018.
16. Lee LS, Kim SH. Kim YB. 'Quantitative analysis of major constituents in green tea with different plucking periods and their Antioxidant activity', *Molecules*, 2014; 19(7):9173-9186. doi: 10.3390/molecules19079173.
17. Lewis MJ. Natural Product Screening: Anti-oxidant Screen for Extracts, 2012.
18. Lorenzo JM, Munekata PES. 'Phenolic compounds of green tea: Health benefits and technological application in food', Asian Pacific Journal of Tropical Biomedicine. 2016; 6(8):709-719. doi: 10.1016/j.apjtb.2016.06.010.
19. López de Lacey, A.M., Pérez-Santín, E., López-Caballero, M.E. and Montero, P. (2014a) 'Survival and metabolic activity of probiotic bacteria in green tea', *LWT - Food Science and Technology*, 55(1), pp. 314–322. doi: 10.1016/j.lwt.2013.08.021.
20. Lourens-Hattingh A, Viljoen BC. 'Yogurt as probiotic carrier food', International Dairy Journal. 2001; 11(1-2):1–17. doi: 10.1016/s0958-6946(01)00036-x. Major Components and health benefits of green tea (no date) Available at: http://www.itoen.co.jp/eng/allabout_greentea/

components_benefit.html (Accessed: 10 December 2016).
21. Manach C, Scalbert A, Morand C, Rémésy C, Jiménez L. 'Polyphenols: Food sources and bioavailability', The American Journal of Clinical Nutrition, 2004; 79(5):727-747.
22. Map AGS, Policy P, Disclaimer S. Forum EGCG, Catechins and Polyphenols - FAQ guide. Available at: http://www.amazing-green-tea.com/egcg.html (Accessed: 10 December 2016).
23. Mckinley MC. 'The nutrition and health benefits of yoghurt', International Journal of Dairy Technology, 2005; 58(1):1-12. doi: 10.1111/j.1471-0307.2005.00180.x.
24. Milk: Nutrition level, processed products and standards 2012 Available at: http://dl.nsf.ac.lk/bitstream/handle/1/14157/ER-34-(5-6)_19.pdf?sequence=2 (Accessed: 10 December 2016).
25. Mukhtar H. Ahmad N. 'Tea polyphenols: Prevention of cancer and optimizing health', The American Journal of Clinical Nutrition, 2000; 71(6):1698-1702.
26. Munasinghe MADD, Ruwanmali JKU, Rasika DMD. Weerathilaka, WADV. 'the evolution, processing, varieties and health benefits og yoghurt', International Journal of Scientific and Research Publications, 2014; 4(4):1-10.
27. Najgebauer-Lejko D, Sady M, Grega T, Walczycka M. 'The impact of tea supplementation on microflora, pH and antioxidant capacity of yoghurt', International Dairy Journal. 2011; 21(8):568-574. doi: 10.1016/j.idairyj.2011.03.003.
28. Ndife J. 'Production and quality assessment of functional Yoghurt enriched with coconut', International Journal of Nutrition and Food Sciences, 2014; 3(6):545. doi: 10.11648/j.ijnfs.20140306.19.
29. Nkhili E, Tomao V, El Hajji H, Boustani El ES, Chemat F. Dangles O. 'Microwave-assisted water extraction of green tea polyphenols', *Phytochemical Analysis*, 2009; 20(5):408-415. doi: 10.1002/pca.1141.
30. Pan X, Niu G, Liu H. 'Microwave-assisted extraction of tea polyphenols and tea caffeine from green tea leaves', Chemical Engineering and Processing: Process Intensification, 2003; 42(2):129-133. doi: 10.1016/s0255-2701(02)00037-5.
31. Pasrija D, Anandharamakrishnan C. 'Techniques for extraction of green tea Polyphenols: A review', *Food and Bioprocess Technology*, 2015; 8(5):935-950. doi: 10.1007/s11947-015-1479-y.
32. Pereira E, Barros L, Ferreira I. 'Relevance of the mention of Antioxidant properties in yogurt labels: In Vitro evaluation and chromatographic analysis', Antioxidants, 2013; 2(2):62-76. doi: 10.3390/antiox2020062.
33. Perva-Uzunalić A, Škerget M, Knez Ž, Weinreich B, Otto F, Grüner S. *et al.* 'Extraction of active ingredients from green tea (Camellia sinensis): Extraction efficiency of major catechins and caffeine', Food Chemistry, 2006; 96(4):597-605. doi: 10.1016/j.foodchem.2005.03.015.
34. Rusak G, Komes D, Likić S, Horžić D, Kovač M. Phenolic content and antioxidative capacity of green and white tea extracts depending on extraction conditions and the solvent used', Food Chemistry, 2008; 110(4):852-858. doi: 10.1016/j.foodchem.2008.02.072.
35. Schultz H, com, NUA. Great advances in food science and human health, 2016. Available at: http://www.nutraingredients-usa.com/Markets/Time-is-ripe-for-polyphenols-as-prebiotics-expert-says (Accessed: 10 December 2016).
36. Selvamuthukumaran M, Farahath K. 2013 'Evaluation of shelf stability of antioxidant rich seabuckthorn fruit yoghurt'. International Food Research Journal. 2006; 21(2):759-765.
37. Setyopratoma P. 'Extraction Of Phenolic Compounds From Green Tea Using Ethanol', Arpn Journal of Engineering and Applied Sciences, 9, 2014.
38. Skall Nielsen N, Debnath D, Jacobsen C. 'Oxidative stability of fish oil enriched drinking yoghurt', International Dairy Journal, 2007; 17(12):1478-1485. doi: 10.1016/j.idairyj.2007.04.011.
39. Spigno G, Faveri De, DM. 'Microwave-assisted extraction of tea phenols: A phenomenological study', Journal of Food Engineering, 2009; 93(2):210-217. doi: 10.1016/j.jfoodeng.2009.01.006.
40. Sun L, Warren FJ, Netzel G, Gidley MJ. '3 or 3′-Galloyl substitution plays an important role in association of catechins and theaflavins with porcine pancreatic α-amylase: The kinetics of inhibition of α-amylase by tea polyphenols', Journal of Functional Foods, 2016; 26:144-156. doi: 10.1016/j.jff.2016.07.012.
41. The antimicrobial activity of green tea extract against the major food born bacteria Journal of Advances in Health and Medical Sciences, 2016; 2(2). doi: 10.20474/jahms-2.2.2.
42. Vuong QV, Golding JB, Nguyen M, Roach PD. 'Extraction and isolation of catechins from tea', Journal of Separation Science, 2010; 33(21):3415-3428. doi: 10.1002/jssc.201000438.
43. Williamson G, Holst B. 'Dietary reference intake (DRI) value for dietary polyphenols: Are we heading in the right direction? British Journal of Nutrition, 2008; 99(3). doi: 10.1017/s0007114508006867.
44. Xi J, Shen D, Li Y, Zhangf R. 'Ultrahigh pressure extraction as a tool to improve the antioxidant activities of green tea extracts', Food Research International, 2011; 44(9):2783-2787. doi: 10.1016/j.foodres.2011.06.001.
45. Ye J, Fan F, Xu X, Liang Y. 'Interactions of black and green tea polyphenols with whole milk', Food Research International, 2013; 53(1):449-455. doi: 10.1016/j.foodres.2013.05.033.
46. Ziena HM, Abeer AM. Elhamid A. 'Production Of Functional Yogurt: Effect Of Natural Antioxidant From Guava (Psidium Guajava) Leaf Extract', J.Agric. & Env. Sci, 2009; 8(1):102-114.
47. Liong M-T. (ed.) Probiotics biology, genetics and health aspects. Berlin: Springer-Verlag Berlin and Heidelberg GmbH & Co. K. 2011.
48. Stephen Ezeonu, C. 'Quantification of Physicochemical components in Yoghurts from coconut, tiger nut and fresh cow milk', Advances in Biotechnology & Microbiology, 2016; 1(5). doi: 10.19080/aibm.2016.01.555573.
49. Adedapo AA, Jimoh, FO, Afolayan AJ, Masika PJ. 'Antioxidant activities and phenolic contents of the methanol extracts of the stems of Acokanthera oppositifolia and Adenia gummifera', *BMC* Complementary and Alternative Medicine, 2008; 8(1) doi: 10.1186/1472-6882-8-54.
50. Hirasawa M. 'Multiple effects of green tea catechin on the antifungal activity of antimycotics against Candida

albicans', Journal of Antimicrobial Chemotherapy. 2004; 53(2):225-229. doi: 10.1093/jac/dkh046.
51. http://www.jnutbio.com/article/S0955-2863(13)00094-6/abstract (Accessed: 10 December 2016).
52. file:///C:/Users/admin/Downloads/DPPH%20Peach%20and%20Pear%2024.08.2016%202.pdf (Accessed: 10 December 2016).
53. AOAC. Official Methods of Analysis, Association of Official Analytical Chemists, 16th edition, Washington D.C. 1999.
54. Ahmet A, Durmus S, Hakki K. Fehmi Y,. Physical, chemical, nutritional and organoleptic characteristics of fruit added yogurts. Journal of food technology, 2006; 4(1):44-49.
55. FDA. Yogurt. 21 CFR 131.200, Code of Federal Regulations. U. S. Dept. of Health and Human Services, Washington, 2013.
56. Specifications for Fermented Milk Products/part 2-Yoghurt/Sri Lanka Standard 824: part 2:1989,UDC 637.146.3