DEVELOPMENT AND CHARACTERIZATION OF PHYSICO-CHEMICAL AND FUNCTIONAL PROPERTIES OF GREEN TEA YOGHURT

Aparna Agarwal1, Manya Gupta2, Anjana Kumari1, Abhishek Gupta2

Address(es): 1Department of Food and Nutrition & Food Technology, Lady Irwin College, Delhi University, Sikandra road, 110001, New Delhi. 2Nestlé R & D, Sector-8, Gurugram, 122050, Haryana, India.

*Corresponding author: aprana.agarwal@nic.in
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ABSTRACT
The present study aimed at developing a low-fat, low-sugar green tea yoghurt. The final acidičities of the green tea and plain yoghurts were similar, but the lactic acid content in green tea yoghurt (5.8*10^6 cfu/g) was twice of that in the plain yoghurt (2.7*10^6 cfu/g). This suggested that the tea extract buffered pH lowering during fermentation. Sensorially, the green tea yoghurt was at par acceptable with the plain yoghurt. The total phenolic content of green tea yoghurt was significantly higher (397.9 µg GAE/mL) than that of the plain yoghurt (81.0 µg GAE/mL). These polyphenols have proven health benefits like gut health, weight loss, and bone health.

INTRODUCTION

The Indian snacking market is booming and is expected to grow annually at a CAGR of 18% (Bhattacharya 2019). This is due to increased emphasis on long-term wellness and health management and evolving consumer habits around traditional meals and snacking. The big dedicated meals have been replaced by snacks at frequent occasions. These snacks are expected to not only deliver pleasure, but also fulfil the physical and mental performance needs. Amongst healthy snacks, yoghurts have gained high consumer acceptance owing to their easy digestibility and superior nutritional profile. They contain proteins of high biological value and are an excellent source of B-vitamins and minerals (calcium, phosphorus, magnesium, and zinc) (Mckinley et al. 2005). Yoghurts are commonly added with fruit, vegetable, or plant extracts (e.g. caffeine, guarana, green tea extract, coenzyme Q10, ginseng, aloe vera, cranberry, pomegranate pulp, flaxseed powder, and plant fibres) to enhance its nutritional benefits (Ngajebauer-Lejko et al. 2014; Kumar et al. 2018). Tea infusion, in particular, is a promising ingredient since tea is widely consumed around the world. It has several functional benefits and has low pH of 4.2, which makes it easily compatible with yoghurts. Some reported health benefits of tea consumption are antioarcigenic properties (Krahwinkel et al. 2000; Otake et al. 1991), improvement in bone density (Hegarty et al. 2000), weight loss (Dulko et al. 1999), antibacterial effect against pathogens like Escherichia coli, Streptococcus salivarius, Clostridia, and Streptococcus mutans (Jeong et al. 2018), growth promotion of bifidobacteria and lactobacilli (Goto et al. 1998; Savard et al. 2011) and anti-stress/relaxation (Zhang et al. 2002). These benefits are mostly associated with the high polyphenol content of tea. Green tea is one of the most concentrated sources of polyphenols (30-45% of its dry weight) (Vu et al. 2017; Sharma et al. 2007). Tea polyphenols are astringent and bitter and hence pose challenges in its application in yoghurts. Therefore, in many studies (Chatterjee et al. 2018; Muniandy et al. 2016; Amirdivani and Baba 2013), low levels of up to 2% (w/v) of tea infusion has been evaluated in the yoghurts. Most of these studies have been carried out in regions with high per capita green tea consumption, like Turkey (Cakmakci et al. 2019), Egypt (El-Zinay et al. 2017), Europe (Gilbowski et al. 2019), China (Liu 2018), Malaysia and Iran (Amirdivani and Baba 2013). As a result, the acceptability of the green tea infused yoghurt by the consumers has been reasonably high. However, for other regions like India where black tea is predominantly consumed, it is therefore important to investigate the consumer acceptance of the green tea yoghurt. So far, only Chatterjee et al. 2018 has studied the development and sensorial acceptance of the green tea yoghurt amongst Indian consumers. Chatterjee et al. 2018 developed green tea yoghurt containing 2% green tea, 3% honey and 9% chocolate syrup. The acceptability of this green tea yoghurt was higher than the plain yoghurt, but the fermentation process was 14-16 hours long.

This was due to the use of mesophilic cultures and probably the green tea polyphenols in the yoghurt formulation. Such long fermentation times are industrially inefficient because of high energy utilization. Also, the risk of cross-contamination with yeasts, moulds, spoilage bacteria, and even pathogens may increase. Therefore, from the perspective of microbiological safety and industrial feasibility, a faster fermentation process (e.g. up to max. 4-5 hours) is desired. Next, it is important to understand how the fermentation process gets impacted because of the addition of the green tea extract to milk.

The objective of our study was to develop the green tea yoghurt, study the physiochemical characteristics, and sensorial acceptance of green tea yoghurt amongst Indian consumers.

MATERIAL AND METHODS

Ingredients
Homogenized and pasteurized toned milk (3% fat, 8.5% SNF, 3% protein, Amul Taaza), milk powder (18% fat, 17% protein, Nestlé EveryDay), lemon and honey flavoured green tea (Twinings™ of London), sugar, pectin, and sun green colour containing tartrazine and brilliant blue FCF were purchased from local retail stores (Delhi, India). Freeze dried DVS Yoflex Express 1.0/30X30U containing Streptococcus salivarius subsp. thermophilus and Lactobacillus delbrueckii subsp. bulgaricus was kindly provided by DuPont India Pvt Ltd. (Gurgaon, India).

Preparation of green tea yoghurt
As per the Food Safety and Standards Authority of India (FSSAI, 2011), green tea yoghurt will be categorized as proprietary foods. In this category, there is no recommendation suggested for the composition of green tea yoghurts. Hence, as a guideline, the FSSAI standards for partly skimmed yoghurt (fat content of at least 0.5% (w/v) but not more than 3.0% (w/v) and a minimum of 3.0% protein (w/v) were followed. To comply with these requirements, 0.96% (w/v) and 0.97% (w/v) of milk powder was added to milk for the green tea and plain yoghurt formulations, respectively. The dry ingredients, i.e. sugar (2% w/v), milk powder, and pectin (0.2% w/v) were mixed together. This dry mix was added to the milk and continuously stirred using a mechanical agitator for 30 min at 60°C to ensure complete dissolution. The sun green colour Fast green FCF (0.025% w/v) and green tea leaves (0.5%, 1%, and 2% v/v) were added to the standardized milk and heated for 15-20 min at 80-90 °C to maximize the extraction. This corresponded to the strength of a normal cup of tea. Subsequently, the tea leaves were strained out using a sterile kitchen sieve and the standardized green tea milk was cooled to 43°C. The standardized green tea milk (200 mL) was inoculated with 0.15% (w/v) of starter.
culture, mixed well, and poured into pre-sterilized high-density polyethylene cups. The cups were sealed and incubated at 43°C for 3 hours. After 3 hours, pH was measured, and the yoghurts were stored at 4-5°C. The control yoghurt (without green tea extract) was prepared following the same process.

Sensory analysis

A panel of 10 members was trained for sensory evaluation following the procedures described by Folkenberg et al. 2006. A trained sensory panel of 10 members were asked to rank the yoghurts on different attributes on a scale of 1 to 9, with 1 being ‘dislike extremely’ and 9 being ‘like extremely’. The yoghurt samples were evaluated based on colour, appearance, firmness, texture, flavour, mouthfeel, and overall acceptability.

Shelf life evaluation

All yoghurt samples were stored for 20 days at 4°C till visible spoilage was observed. During storage, changes in appearance, colour, and aroma in yoghurts were recorded. The yoghurts were also analysed for total solids, pH, ash, titratable acidity, protein, fat, antioxidant capacity, total phenolic content, and lactic acid bacteria (LAB) counts, yeast and mould, and coliform counts.

Total solids/Moisture content

The total solids content was determined according to the gravimetric method described in Indian standards 1479 (IS 1479 part II-1961). The moisture was calculated as 100 - % total solids content.

pH measurement

The pHs of milks and yoghurts were analysed using a digital pH meter (Metrohm, India). The pH meter was calibrated using standard buffer solutions of pH 4, 7, and 10.

Ash content

The ash content was measured using the gravimetric method (IS 1479 part II-1961).

Titratable acidity (% lactic acid)

The titratable acidity was determined using the standard method (IS 1166 (1986)).

Protein content

The protein content was analysed following the Kjeldahl method (IS 1479 part II-1961). The nitrogen to protein conversion factor used was 6.28.

Fat content

The fat content of milk was analysed using the Gerber method (IS 1224 part II-1977). And, for milk powder and yoghurts, the Rose Gottlieb method was used as described in IS 11721 (2013).

Antioxidant activity

The antioxidant activities of milk and yoghurt samples were analysed following the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical inhibition assay (Apostolidis et al. 2007; Amirdivani and Baba 2015).

Total phenolic content

The total phenolic content (TPC) was determined as described by Shetty et al. 2005. Briefly, the pH of the sample (15 g) was adjusted to 4.6 and then centrifuged (4000 rpm, 20°C). The supernatant obtained was filtered through 0.45 µm filter and taken for analysis. For the filtrate (1 mL), 1 mL of 95% ethanol and 5 mL of H2O2 was added. Then, Folin-Ciocalteu reagent (diluted 1:1 with distilled water) was added to the sample followed by thorough mixing using a vortex mixer. To the reaction mixture, 1 mL of Na2CO3 (5%) was added and left to stand at room temperature for 1 h. After 1 h of incubation, the absorbance was measured at 725 nm and converted to total phenolic content (µg of Gallic acid equivalents (GAE)/mL of the sample). The calibration curves were prepared using different concentrations of gallic acid (5-60 µg GAE/mL) in methanol.

Microbiological analyses

The total plate (IS 5402 (2012)), coliforms (IS 5401 part I (2002)), LAB (IS 12899-1989 (Reaffirmed 1999), and yeast and moulds (IS 5403:1999 (Reaffirmed 2005)) counts in yoghurts were determined according to the standard methods. Colony counts were expressed as colony forming units (cfu)/g.

Data analyses

All analyses were performed in duplicates. Averages and standard deviations were calculated for all data. Sensory evaluation scores were analysed using SPSS (version 24, SPSS Inc., Chicago, IL, USA). T-test and one-way analysis of variance (ANOVA) at 5% significance were used to identify significant differences between the means.

RESULTS AND DISCUSSION

Recipe optimization

Toned milk (3% fat, 3% protein), sugar, milk powder (18% fat, 17% protein), green tea extract, green color, pectin, and starter culture were used to formulate the green tea yoghurt (Table 1).

Table 1 Formulations of green tea and plain yoghurts

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Green tea yoghurt</th>
<th>Plain yoghurt</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Quantity (%)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Toned milk</td>
<td>95.60</td>
<td>96.99</td>
</tr>
<tr>
<td>Sugar</td>
<td>1.91</td>
<td>1.94</td>
</tr>
<tr>
<td>Milk powder</td>
<td>0.96</td>
<td>0.97</td>
</tr>
<tr>
<td>Green tea extract</td>
<td>0.96</td>
<td>0.00</td>
</tr>
<tr>
<td>Green colour</td>
<td>0.48</td>
<td>0.00</td>
</tr>
<tr>
<td>Pectin</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>100.00</td>
<td>100.00</td>
</tr>
</tbody>
</table>

As expected, the green tea and the plain yoghurts had similar fat (2.91% and 2.96%) and protein contents (3.16% and 3.00%) (Table 2).

Table 2 Physico-chemical and microbiological characterization and antioxidant capacities of green tea and plain yoghurts

<table>
<thead>
<tr>
<th>Physico-chemical characterization</th>
<th>Green tea yoghurt</th>
<th>Plain yoghurt</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total solids (%)</td>
<td>15.75</td>
<td>14.20</td>
</tr>
<tr>
<td>Fat (%)</td>
<td>2.91</td>
<td>2.96</td>
</tr>
<tr>
<td>Protein (%)</td>
<td>3.16</td>
<td>3.00</td>
</tr>
<tr>
<td>Ash (%)</td>
<td>1.24</td>
<td>0.97</td>
</tr>
<tr>
<td>pH</td>
<td>4.65</td>
<td>3.38</td>
</tr>
<tr>
<td>Acidity (% lactic acid)</td>
<td>0.80</td>
<td>0.81</td>
</tr>
<tr>
<td>Microbiological analyses</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LAB (cfu/g)</td>
<td>5.8 x 10^7</td>
<td>not detected</td>
</tr>
<tr>
<td>Yeast and Mould (cfu/g)</td>
<td>not detected</td>
<td>not detected</td>
</tr>
<tr>
<td>Coliforms (cfu/g)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antioxidant analyses</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total phenolics (µg GAE/mL)</td>
<td>397.9</td>
<td>81.0</td>
</tr>
<tr>
<td>Antioxidant activity (% inhibition of DPPH oxidation)</td>
<td>68.8</td>
<td>49.7</td>
</tr>
</tbody>
</table>

* Average values are shown in the table. Coefficient of variation % (Average/Standard deviation *100) < 10% for all measurements.

The yoghurts were made partly skimmed because consumers typically associate consumption of high fat products to elevated risks of hypertension, heart diseases, and strokes (Bus and Worsley 2003). Sensorially, on the other hand, consumers correlated positively the high fat content to rich mouthfeel and enhanced creaminess, viscosity, and body and texture of the products (Bus and Worsley 2003). In the absence of fat, the partly skimmed or low-fat yoghurts may exhibit sensory defects, like poor texture, low viscosity, increased syneresis, and dry and chalky mouthfeel (Lee and Lucey 2009; Yam et al. 1997; Cakmakci et al. 2019). Also, in this study, 0.5%, 1.0%, and 2.0% (v/v) of green tea extracts have been evaluated in the formulation (Fig. 1). From these formulations, the green tea yoghurt containing 1% (v/v) extract had slightly higher acceptance than the yoghurts containing 0.5% and 2.0% (v/v) extracts (Fig. 1).
The acceptability of the green tea yoghurt can be improved further by using bitterness masking natural flavours and increasing the sugar content to 4-5% without compromising the nutritional/health benefits.

**Fermentation profile**

Based on the supplier (Danisco™) specifications, a thermophilic LB/ST starter culture was selected. The fermentation of the green tea yoghurt (pH 4.65) was much slower than that of the plain yoghurt (pH 3.38) (Fig. 3).

Similar slowing down of the fermentation process due to addition of green tea extract has been reported earlier. The yoghurts containing 0%, 0.4%, 0.8%, and 1.6% (v/v) of green tea infusion had fermentation times of 4.11, 5.10, 7.30, and 8.33 hours, respectively (Ochanda et al. 2015). However, in that study, the fermentation profiles were not shown. It is interesting to observe that the fermentation process of green tea yoghurt (pH 4.65) was slower than the plain yoghurt (pH 3.38) (Table 2), but the LAB count in green tea yoghurt (5.8*10^7 cfu/g) was more than double of that measured in the plain yoghurt (2.6*10^7 cfu/g). Also, both the yoghurts had similar acidities of ~0.81% lactic acid (Table 2). This suggests that the compounds present in the green tea extract exerted buffering action against the lowering of pH in the green tea yoghurt. Similar effects have been reported in previous research (Jeong et al. 2018). In contrast to slowing down, there are studies showing promotion (Amirdiviani and Baba 2015; Alwaezer et al. 2020) and studies showing no effect of green tea extract addition (Jaziri et al. 2009) on the fermentation process. Hence, there is a need to understand the impact of green tea extract on the starter culture and the fermentation process.

**Antioxidant capacity of yoghurts**

The total phenolic contents of the green tea extract, plain yoghurt, and green tea yoghurt were 404, 81, and 398 µg GAE/mL, respectively (Table 2). Sigdel et al. (2012) reported similar total phenolic content (66.3 µg GAE/g) for plain yoghurt. On the other hand, as expected, significantly higher total phenolic contents of 479 µg GAE/mL and 319 µg GAE/mL has been reported in green tea yoghurts by Muniandy et al. 2016 and Shokery et al. 2017, respectively. There is a large variation in the reported total phenolic contents of the green tea extracts and the yoghurts between different studies. This variation is because of many reasons, like type and source of green tea, method of preparation of the extract, and dosages of green tea extract in the yoghurt. In the current study, 1% green tea extract corresponded to approx. 20 mg of green tea powder added to the yoghurt (0.96 grams of green tea used for extraction; Assuming 2% of solids extracted from the green tea infusion; thus, 20 mg of green tea powder/100 gram yoghurt). Even at this low level of supplementation, functional benefits of the green tea yoghurt may be expected. In a clinical study, 58 volunteers consumed Yoptimal™ (10^5 cfu/100 gram of BB-12 and LA-5 and 40 mg of green tea extract) over a period of 4 weeks. At the end of that study, a significant increase in the faecal bifidobacteria and L. acidophilus was observed (Savard et al. 2011). This...
shows that the yoghurt containing green tea powder supported the growth of beneficial bacteria and suppressed pathogenic bacteria like Enterococcus. Similar findings were reported by Goto et al. (1998), where administration of 300 mg of tea catechins in long-term care patients significantly increased the bifidobacteria and lactobacillus which decreases the pathogenic bacteria, like bacteroids, eubacteria, enterobacteria, and clostridia. In addition to gut health improvement, other health benefits like weight loss and cholesterol management may be expected from green tea yoghurts. To establish the full spectrum of health benefits, additional clinical studies need to be undertaken.

Changes during storage

During storage (4°C), minimal changes were observed in the moisture, ash, fat, and protein contents of the yoghurts. There was an 11% and 13% decrease in the total titratable acid and the respiration activities of the yoghurts, respectively. This could be due to the degradation of phenolic compounds during storage. No coliform and yeast and mould growth were observed in the green tea and plain yoghurts suggesting hygienic production of the yoghurts. Also, the lactic acid bacteria count did not change over storage. However, the titratable acidity of the yoghurts increased from 0.72 to 1.0% lactic acid and the pH decreased from 4.55 to 3.47. Due to this rapid post-acidification, the sensory acceptance of the green tea yoghurt decreased during the storage. A detailed overview of the factors causing post-acidification and its control is reviewed in literature by Deshwal et al. 2021. In our study, the fermentation process needs to be further optimized by selecting thermophilic starter cultures with slower rate of post-acidification.

CONCLUSION

A sensorially acceptable green tea yoghurt was developed but there is a need to study the influence of green tea extract composition on the starter culture. This will help in optimizing the fermentation process and enhance the nutritive value of yoghurts. Some health benefits of the green tea yoghurts are already established, but it is necessary to investigate if these benefits can be enhanced further by the addition of functional ingredients like prebiotics.

REFERENCES

Cleaning up your labels, Leverage the benefits of the industry’s most enduring ingredients.html
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