The objective of our study was to replace sugar with green tea syrup. The acceptability of this green tea yoghurt was higher than the plain yoghurt, but the fermentation process was 14–16 hours long. However, for other regions like India where black tea is predominantly consumed, the green tea infused yoghurt by the consumers has been reasonably high.

Malaysia and Iran with high per capita green tea consumption. Some reported health benefits of tea consumption are astringent and bitter and hence pose challenges in its application in yoghurts. Green tea is a rich source of polyphenols in the yoghurt formulation. 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. Therefore, from the perspective of microbiological safety and industrial feasibility, a faster fermentation process (e.g. up to max. 4-5 hours) is desired. 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 The big dedicated meals have been replaced by snacks at frequent occasions.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 fulfill 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) (McKinnie 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 (Najgebauer-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 anticancerogenic 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; Savidar 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 Baha 2013), low levels of up to 2% (v/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-Ziney et al. 2017), Europe (Gilbowksi et al. 2019), China (Liu 2018), Malaysia and Iran (Amirdivani and Baha 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. 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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-1991). 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 was obtained after 0.45 µm filter and taken for analysis. To 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. 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 colour, 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>Quantity (%)</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>Total</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>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Microbiological analyses</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>LAB (cfu/g)</td>
<td>not detected</td>
</tr>
<tr>
<td>Yeast and Mould (cfu/g)</td>
<td>not detected</td>
</tr>
<tr>
<td>Coliforms (cfu/g)</td>
<td>not detected</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Antioxidant analyses</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Total polyphenols (µg GAE/mL)</td>
<td>397.9</td>
</tr>
<tr>
<td>Antioxidant activity (% inhibition of DPPH oxidation)</td>
<td>68.8</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 negatively with high fat products. It has been reported that consumers linked 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 2010). These defects may get enhanced even 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.
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).

**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 519 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 green tea corresponded to 10 mg green tea powder added to the yoghurt (0.96 grams of green tea used for extraction; assuming 2% solids extracted from the green tea infusion; thus, 20 mg of green tea powder/100 gram yogurt). 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™ (10^4 cfu/g) 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™ (10^4 cfu/g) yoghurt.

**Figure 1** Sensory scores of yoghurts containing 0.5% (■), 1.0% (□), and 2.0% (▲) green tea extracts

**Figure 2** Sensory evaluation scores of green tea (—) and plain (—) yoghurts

**Figure 3** (A) Fermentation profiles of green tea (—) and plain (—) yoghurts and (B) pH after 3 hours of inoculation
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, whereas decreased 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 coliform content and the antacid activity 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 nutrient 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 probiotics.

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