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Development of value-added butter through the addition of green tea (*Camellia sinensis* L.) extract

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Abstract

The present work highlights the potential production of value-added butter with enhanced nutritional and microbiological properties through the addition of 2 - 10% (w/w) green tea (*Camellia sinensis* L.) extract. The results revealed no significant difference in the moisture content (13 - 14% w/w) of all the butter samples. However, an increase in the amount of green tea extract resulted in a significant increase ($p < 0.05$) of the ash content (0.00 - 1.00%) and redness (a^* value, 4.92 - 6.93), while both the lightness (L^* value, 150.65 - 145.74) and yellowness (b^* value, 54.45 - 50.30) of the butters significantly decreased ($p < 0.05$). Furthermore, the green tea butters (GTBs) exhibited significantly ($p < 0.05$) higher antioxidant properties in terms of total phenolic content (0.07 - 0.10 *vs.* 0.01 GAE% w/w db) and DPPH activity (7.27 - 13.94% *vs.* not detected) as compared to the control butter. After six weeks of storage, in relation to the control butter, the GTBs recorded significantly lower ($p < 0.05$) peroxide value (2.13 vs. 0.88 mEq/kg), total plate count (1.11×10^4 *vs.* 2.42×10^3 CFU/g), and yeast and mould count (2.02×10^3 *vs.* 6.05×102 CFU/g), but produced a significantly higher ($p < 0.05$) amount of acid value (0.56 vs. 1.36 mg KOH/g fat). The incorporation of up to 6% (w/w) green tea extract did not compromise the sensory acceptance of the GTBs. The overall result indicated that green tea extract can be used as a natural food additive, antioxidant, and preservative in butter.

Introduction

In a general sense, butter is well-known as a type of dairy product made of sweet cream, acidulated cream, or sour cream, and widely consumed by individuals all around the world. It is extensively used in the preparation of various dishes and manufacturing of butter oil as well as cooking oil, and has become a preferred choice to be directly consumed with bread for breakfast (Çakmakçı *et al.,* 2014; Dvořák *et al.,* 2016). The essential fatty acids as well as certain vitamins and minerals found in butter has made it an important source of energy (van Ruth *et al.,* 2008; Kahyaoğlu and Çakmakçı, 2016).

Despite these advantages, the high-water content and water activity in butter leads to its susceptibility to microbiological spoilage and hydrolytic rancidity (Fındık and Andiç, 2017). Hence, butter needs to be stored at a chilling temperature for preservation purposes. Butter is also widely known to exhibit poor nutritional properties due to its high level of saturated fatty acids. Therefore, a considerable amount of studies was conducted to improve the nutritional properties of butter due to its continuous consumption throughout the globe. Research by Mahmoudi *et al.* (2019) reported improved antimicrobial, antioxidant, and organoleptic properties of cow milk butter due to the addition of *Ziziphora clinopodioides* essential oil. Emami *et al.* (2014) added hazelnut powder in increasing the essential fatty acids and antioxidant properties of butter. Similarly, a study by Hailemariam and Emire (2013) reported enhanced nutritional properties and microbial stability of butter added with crude thyme extract containing natural antioxidant.

Based on these findings, the properties of butter are highly expected to be improved through the addition of compounds with nutritional benefits. In particular, the compound of interest in the present work is green tea (*Camellia sinensis* L.) which contains up to 30% (dry weight) polyphenols responsible for its distinctive aroma, colour, and taste (Namal Senanayake, 2013). Flavanol monomers known as catechins are considered as the most abundant group in green tea, which is majorly responsible for the tea's acclaimed health benefits (Ahmed and Stepp, 2013; Namal Senanayake, 2013; Hu *et al.,* 2018). As emphasised by Reto *et al.* (2014), green tea has cancer-preventive properties, while Reygaert (2017) reported that green tea

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contains a variety of potential health benefits. More importantly, green tea has been utilised to treat cardiovascular heart diseases, oral cavity, Parkinson's, Alzheimer's, diabetes, inflammatory bowel, and skin disorders (Sinija and Mishra, 2008; Singh *et al.,* 2014; Nunes *et al.,* 2015). The addition of green tea extract had also improved the nutritional properties of food and beverage products, particularly in pan bread (Ning *et al.,* 2017), soymilk (Nadifah and Sari, 2016), fresh meat products (Namal Senanayake, 2013), and biscuits (Mildner-Szkudlarz *et al.,* 2009).

In light of these benefits, the development of butter mixed with green tea extract bears great potential as a new value-added product. Accordingly, this has led to the main objective of the present work which was to develop butter added with different amount of green tea extract (GTB). The GTB samples were evaluated in terms of their physicochemical, antioxidant, and microbiological properties, and sensory acceptability.

Materials and methods

Materials

The green tea leaves were purchased from Gourmet market in Pathum Wan, Bangkok, Thailand. Whipping cream was purchased from Tesco in Sepang, Malaysia, containing 35.1% (w/w) milk fat of which 24.6% (w/w) was saturated fat, as listed on the label. All chemicals used were of analytical grade.

Preparation of green tea extract

The green tea extract was prepared based on the method carried out by Demir *et al.* (2015) with slight modifications. The green tea leaves were ground using a blender (Panasonic, Malaysia). Two grams of the ground leaves were boiled in 200 mL distilled water at 95°C for 10 min. Next, the leaves were removed using a filter cloth, and the extract was prepared based on the amount of butter required.

Processing of green tea-butter

The GTB samples were independently produced in two batches. The processing steps involved in the production of GTB were conducted according to Shi (2015) with slight modifications. The whipping cream (1 L) was churned in a cake mixer (KitchenAid) from low to high speed until the butter grains were separated from the buttermilk. Next, the buttermilk was drained and washed for five times with distilled water, while the butter grains were divided into six portions. The green tea extract was further added into each portion based on the following concentrations: 0% (control, GTB-0), 2% (GTB-2), 4% (GTB-4), 6% (GTB-6), 8% (GTB-8), and 10% (GTB-10) (w/w). The butters were kneaded by hand to remove the moisture content and packed in aluminium foil before being stored at 4°C for six weeks. Determination of peroxide value (PV), acid value (AV), and antimicrobial properties were carried out every week throughout the storage period.

Determination of moisture content

The moisture content of the butter samples was determined according to Shreve *et al.* (2006). Two grams of each sample was dried in an oven at 105°C for 3 h. The moisture content was calculated using Eq. (1):

$$\text{Moisture content}\,(\%) = \frac{W_1 - W_2}{W_1} \times 100 \qquad \text{(Eq. 1)}$$

where, W_1 = weight (g) of the sample before drying, and W_2 = weight (g) of the sample after drying.

Determination of ash content

The determination of the ash content of the butter samples was performed based on AOAC Official Methods 942.05 (AOAC, 2005).

Determination of colour profile

The colour of the butter samples was measured using a HunterLab UltraScan PRO Spectrophotometer. Accordingly, the results were expressed in CIE L^* a^* b^* colour space, whereby L^* value represents whiteness (value 100) or blackness (value 0), the a^* value represents the red (+) to green (-), and b^* value represents the yellow (+) to blue (-) colour range.

Determination of total phenolic content

The total phenolic content (TPC) of all butter samples was determined following a method developed by Fuentes *et al.* (2012) with slight modifications. The butter sample (2.5 g) was mixed with 3 mL of methanol/water (80:20, v/v) for 2 min using a vortex. The mixture was then separated by centrifugation at 3,500 rpm for 10 min at 4°C. Next, the supernatant was transferred into a 50 mL volumetric flask, and the same procedure was applied to the residue for another two times. Next, all the supernatant was pooled, and 0.2 mL of the supernatant was added into the test tube and diluted to 2.5 mL with distilled water, followed by the addition of 0.25 mL of Folin-Ciocalteu reagent. Following this, 0.5 mL of sodium carbonate solution (5% w/v) was added to the reaction mixture after 3 min, and the mixture was further diluted with distilled water to an amount of 5 mL. The mixture was incubated in the dark at room temperature for 90 min. The absorbance of this mixture was measured at 765 nm using a spectrophotometer. A calibration curve was constructed using the

gallic acid standard at various concentrations, and the TPC was calculated using Eq. (2):

$$\text{Total phenolic content (GAE\%w/w dry basis)} = \frac{(A_s - b) \times V_s \times DF \times 100}{M \times W_s \times 10{,}000 \times \%DM} \quad \text{(Eq. 2)}$$

where, A_s = absorbance at 765 nm, b = *y*-intercept of calibration curve, V_s = extraction volume, DF = dilution factor, M = slope of calibration curve, W_s = weight of sample (g), and %DM = % dry matter of sample (100 - %MC).

Determination of antioxidant activity

The antioxidant activity of all butter samples was determined following the method of Ferhat *et al.* (2017). Briefly, 1 g of butter sample was weighed into a centrifuge tube and then added with 1 mL of methanol/water (80/20, v/v). The mixture was mixed for 10 min using a vortex mixer, and later centrifuged at 3,800 rpm for 15 min at 4°C. Next, the supernatant was separated into a beaker. The same procedure was applied to the residue for another two times. Next, the supernatant was pooled, and approximately 50 µL of the supernatant was added with 1.95 mL DPPH prepared in methanol (0.025 mg/mL). The absorbance was then measured at 517 nm using a spectrophotometer after 30 min. Methanol (80%) was used as a control that was subjected to the similar procedure of the sample, except that the control sample was only added with distilled water.

Determination of peroxide value

The PV of the butter samples was determined following the method developed by Koczon *et al.* (2008). Briefly, 20 g of butter sample was dissolved in 100 mL of chloroform/acetic acid (2:3, v/v) solution, followed by the addition of saturated potassium iodine solution. Next, the mixture was agitated and kept in darkness for 5 min. Distilled water was then added, while the released iodine was titrated with 0.001 M sodium thiosulphate using 1% starch solution as an indicator. A blank solution was prepared without the addition of butter. The butter samples were then analysed for PV at week 0, 1, 2, 3, 4, 5, and 6. The PV was expressed in mmol of active oxygen per 1 kg of fat, and calculated using Eq. (3):

$$PV = \frac{(V - V_0) \times 1000 \times c}{m} \quad \text{(Eq. 2)}$$

where, V (mL) = volume of $Na_2S_2O_3$ solution used for the titration of GTB samples, V_0 (mL) = volume of $Na_2S_2O_3$ solution used for the blank sample, c (1 mmol/L) = $Na_2S_2O_3$ solution concentration, and m (g) = sample mass.

Determination of acid value

The AV of the butter samples was determined following the AOCS Official Methods CD 3d-63 (AOCS, 2009), and analysed at week 0, 1, 2, 3, 4, 5, and 6.

Determination of antimicrobial properties

The method described by Vidanagamagea *et al.* (2016) was followed. Briefly, 1 g of butter sample was transferred into a culture tube and dissolved with 9 mL of peptone water. Serial dilution of this was also performed using peptone water. Plate count agar was used as the bacterial culture medium from which the total plate count was obtained following incubation at 36°C for 48 h. Potato dextrose agar was used as the fungal culture medium from which the yeast and mould count was obtained following incubation at 25°C for 5 d.

Determination of sensory acceptability

The sensory acceptance of all butter samples was determined following the method described by Fernandes *et al.* (2007) with slight modifications. The attributes evaluated in the present work included the butters' appearance, colour, odour, taste, and overall acceptance. The sensory test was carried out by 30 untrained panellists using a 9-point hedonic scale whereby 9 = extremely like, and 1 = extremely dislike.

Statistical analysis

All experiments were performed in triplicates, and the data obtained were expressed as mean ± SD. One-way analysis of variance (ANOVA) was performed using SPSS® 18.0 software. The significant differences (confidence level = 95%) were evaluated based on Duncan's test.

Results and discussion

Moisture content, ash content, and colour profile

The moisture content ranging from 13 - 14% (w/w) in all the butter samples did not show any significant difference, which agree with the standard requirement of a butter (moisture content ≤ 16%) as recommended by Codex Alimentarius (2010). On the other hand, a significant increase ($p < 0.05$) from 0.00 - 1.00% (w/w) was detected in the ash content due to the increased amount of green tea extract; however, none of the values exceeded 1.00% (w/w).

Table 1 presents the colour profiles of the GTB samples, while their images are shown in Figure 1. The colour of the green tea extract used in the present work was brown which caused the GTB-2 to GTB-10 to possess significantly different ($p < 0.05$) colour values from the control sample (GTB-0). In addition, the

increased amount of green tea extract led to a significant decrease ($p < 0.05$) in the lightness (L^* value) and yellowness (b^* value) of the butters, whereas the redness (a^* value) increased significantly ($p < 0.05$). Wang *et al.* (2007) similarly reported that the addition of 1.5 and 5 g/kg green tea extract (in flour form) resulted in significant changes ($p < 0.001$) in the colour of the bread. Meanwhile, research by Namal Senanayake (2013) revealed that green tea extract helps to improve the colour stability of fresh meat products.

Total phenolic content and antioxidant activity

According to Nadeem et al. (2013), phenolic antioxidants are able to act as scavengers by donating a proton, thus inhibiting the auto-oxidation process. The most effective radical scavengers in green tea are the catechins 3', 4', and 5'-trihydroxylated substitution patterns on the B-ring and/or hydroxyl groups at the C-3 position of the catechins. This offers a high degree of stability of catechin phenoxyl radical which is collaborated in electron delocalisation (Koech et al., 2013). The TPC and DPPH activities of GTB samples in the present work are shown in Table 1. The result revealed an extremely low TPC activity ranging from 0.01 to 0.1% (GAE% w/w db) in all samples. As suggested by Zeng *et al.* (2017), this finding may be related to the oxidation and degradation of tea polyphenols under hot conditions. However, the addition of 2% (w/w) green tea extract led to a significant increase ($p < 0.05$) of the TPC as compared to the control sample (not detected), and significant increase ($p < 0.05$) was further observed upon increasing amount of green tea extract. Al-Ghafari *et al.* (2016) reported a high DPPH activity (91%) of green tea. Hence, it can be concluded that the addition of green tea extract can produce butter with significant antioxidant properties. Ning *et al.* (2017) also demonstrated that the addition of green tea extract increased

Table 1. Colour profile, total phenolic content, and antioxidant activity of green tea butters (GTB) with different amounts of green tea extract.

Property	Green tea extract (% w/w)					
	0% (control)	2%	4%	6%	8%	10%
Colour profile						
L^* value	150.65 ± 0.57^{a}	148.42 ± 0.17^{b}	148.24 ± 0.41^{b}	146.80 ± 0.19^{c}	146.07 ± 0.75^{cd}	145.74 ± 0.21^{d}
a^* value	4.92 ± 0.13^{d}	5.86 ± 0.03^{c}	5.95 ± 0.05^{c}	6.40 ± 0.21^{b}	6.48 ± 0.45^{b}	6.93 ± 0.20^{a}
b^* value	54.45 ± 0.76^{a}	53.05 ± 0.02^{b}	52.42 ± 0.09^{c}	51.22 ± 0.08^{d}	50.71 ± 0.19^{de}	50.30 ± 0.36^{e}
Antioxidant properties						
TPC (GAE% w/w db)	0.01 ± 0.01^{d}	0.07 ± 0.00^{c}	0.08 ± 0.00^{bc}	0.08 ± 0.00^{bc}	0.09 ± 0.00^{ab}	0.10 ± 0.01^{a}
DPPH activity (%)	N.D.	7.27 ± 1.43^{c}	8.28 ± 0.86^{c}	10.81 ± 1.00^{b}	12.22 ± 0.71^{ab}	13.94 ± 0.57^{a}

Data are means ± SD ($n = 6$). Different letters in the same row indicate significant difference at $p < 0.05$. N.D. = not detected; TPC = total phenolic content

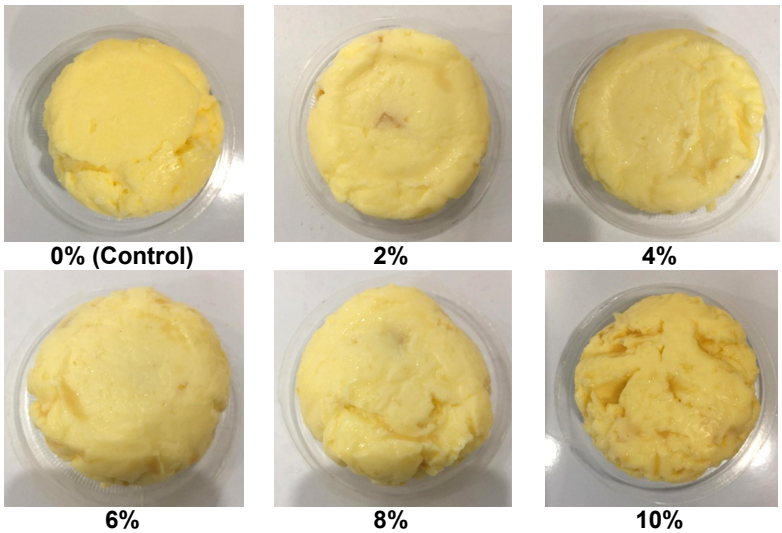

Figure 1. Butter samples containing different amounts (% w/w) of green tea extract.

the antioxidant activity in pan bread sample. On a similar note, Namita *et al.* (2012) stated that green tea contains certain minerals and vitamins that increase its antioxidant potential apart from thousands of bioactive ingredients which are beneficial to consumers' health.

Peroxide and acid values

Table 2 presents the peroxide value (PV) and acid value (AV) of the GTB samples during storage which act as quality indicators of fat and oil. Specifically, PV indicates a degree of rancidity and oxidation based on primary oxidation products including peroxides and hydroperoxides. Meanwhile, AV represents the acidity of fat and oil based on the quantity of free fatty acids derived from hydrolytic deterioration of triacylglycerols apart from the presence of other acidic compounds (Ekop *et al.,* 2007; Koczon *et al.,* 2008; Afoakwa *et al.,* 2014).

Based on Codex Alimentarius (2009), the acceptable PV of fat and oil must be lower than 5 mEq/kg. As shown in Table 2, all samples were within this value. Additionally, the increasing amount of green tea extract at every storage week led to a significant decrease ($p < 0.05$) of PV in all GTB samples. This finding was in line with the results on TPC and DPPH activity explained earlier, where the antioxidants in the green tea extract act as scavenger of oxygen free radicals and further decreased the rate of oxidation (Ekop *et al.,* 2007). In addition, these outcomes correspond with that of Park and Kim (2002) which reported a decrease in the PV of oil samples upon the addition of green tea extract. Mildner-Szkudlarz *et al.* (2009) also emphasised the effectiveness of green tea extract in inhibiting the decomposition of hydroperoxides in biscuits which contributed to a lower oxidation rate.

The results shown in Table 2 demonstrate that the PV in all GTB samples significantly increased ($p < 0.05$) along the storage period. As previously mentioned, the butter was prepared from a whipping cream containing 35.1% (w/w) milk fat which was composed of 10.5% (w/w) unsaturated fatty acids. According to Koczon *et al.* (2008), the unsaturated fatty acids most likely underwent oxidation along the storage period which resulted in the increase of PV. However, the oxidation rate significantly decreased ($p < 0.05$) as the amount of green tea extract increased, thus indicating the effectiveness of the extract as an antioxidant which delays the oxidation reaction. The same trend of PV was also observed in pan bread added with green tea powder (Ning *et al.,* 2017), butters fortified with hazelnut (Emami *et al.,* 2014), and thyme extract (Hailemariam and Emire, 2013) which reported significant antioxidant activities. Nadifah and Sari (2016) also highlighted green tea extract as a source of active antioxidant which may be

Table 2. Peroxide values and acid values of green tea butters (GTB) with different amounts of green tea extract during storage.

Week	Green tea extract (% w/w)					
	0% (control)	2%	4%	6%	8%	10%
Peroxide value (mEq/kg)						
1	0.79 ± 0.00^{a}	0.69 ± 0.13^{ab}	0.59 ± 0.01^{ab}	0.50 ± 0.13^{bc}	0.30 ± 0.00^{cd}	0.10 ± 0.03^{d}
2	0.88 ± 0.14^{a}	0.79 ± 0.00^{ab}	0.68 ± 0.14^{ab}	0.59 ± 0.14^{b}	0.30 ± 0.17^{c}	0.19 ± 0.02^{c}
3	1.18 ± 0.00^{a}	0.90 ± 0.14^{b}	0.78 ± 0.01^{b}	0.69 ± 0.01^{b}	0.40 ± 0.10^{c}	0.30 ± 0.16^{c}
4	1.48 ± 0.14^{a}	1.08 ± 0.00^{b}	0.99 ± 0.15^{b}	0.90 ± 0.14^{bc}	0.68 ± 0.00^{cd}	0.59 ± 0.04^{d}
5	1.96 ± 0.14^{a}	1.64 ± 0.14^{b}	1.44 ± 0.00^{b}	1.15 ± 0.13^{c}	0.89 ± 0.14^{d}	0.78 ± 0.15^{d}
6	2.13 ± 0.14^{a}	1.94 ± 0.01^{ab}	1.68 ± 0.14^{b}	1.34 ± 0.01^{c}	1.09 ± 0.01^{cd}	0.88 ± 0.14^{d}
Acid value (mg KOH/g fat)						
1	0.33 ± 0.00^{b}	0.39 ± 0.08^{b}	0.39 ± 0.08^{b}	0.38 ± 0.07^{b}	0.39 ± 0.08^{b}	0.56 ± 0.01^{a}
2	0.39 ± 0.08^{b}	0.39 ± 0.08^{b}	0.44 ± 0.00^{b}	0.44 ± 0.00^{b}	0.50 ± 0.09^{ab}	0.61 ± 0.08^{a}
3	0.39 ± 0.08^{d}	0.44 ± 0.00^{cd}	0.45 ± 0.00^{cd}	0.56 ± 0.00^{bc}	0.60 ± 0.09^{ab}	0.71 ± 0.06^{a}
4	0.45 ± 0.08^{d}	0.49 ± 0.07^{d}	0.55 ± 0.00^{d}	0.66 ± 0.00^{c}	0.84 ± 0.08^{b}	1.00 ± 0.00^{a}
5	0.52 ± 0.05^{d}	0.60 ± 0.08^{d}	0.67 ± 0.01^{cd}	0.82 ± 0.09^{c}	1.05 ± 0.07^{b}	1.25 ± 0.10^{a}
6	0.56 ± 0.00^{d}	0.65 ± 0.01^{d}	0.72 ± 0.08^{d}	0.93 ± 0.09^{c}	1.15 ± 0.07^{b}	1.36 ± 0.10^{a}

Data are means ± SD (n = 6). Different letters in the same row indicate significant difference at $p < 0.05$.

used in food lipid stabilisation. On the other hand, Mahmoudi *et al.* (2019) reported a reduced PV of cow milk butter after 10 days of storage due to the antioxidant properties of *Ziziphora clinopodioides* essential oil.

In contrast with the favourable result of PV, the increased amount of green tea extract resulted in a significant increase ($p < 0.05$) of AV in all butter samples. Moreover, the AV of the GTB samples significantly increased ($p < 0.05$) along the storage period. The increase in the rate was found to be higher in GTB samples containing a higher amount of green tea extract. These findings disagree with the enhanced antioxidant properties and decreased PV of the GTB samples reported earlier. The existing study on the addition of hazelnut in butter formulation also demonstrated a significant increase in AV along the storage period with a higher rate in the fortified samples (Emami *et al.,* 2014). Additionally, Mahmoudi *et al.* (2019) reported significant changes in the AV of cow milk butter upon the addition of *Ziziphora clinopodioides* essential oil after 10 days of storage. As stated earlier, the moisture content of 13 - 14% (w/w) was found in the butter samples which were insignificantly different from each other. Nevertheless, the amount of added water increased from GTB-0 to GTB-10 because the green tea extract was prepared in aqueous form. A possible explanation for this matter may be that the added water remained as unbound water. Therefore, the rate of hydrolysis of glyceride linkage may have increased as the amount of green tea extract increased, thus subsequently producing higher amount of free fatty acids which resulted in increased AV. Furthermore, the hydrolysis may have occurred continuously along the storage period as long as the water is present which also resulted in the increase of AV.

Antibacterial properties

Table 3 shows a significant decrease ($p < 0.05$) in total bacteria, and yeast and mould counts despite the significant increase ($p < 0.05$) in the AV upon higher amount of green tea extract. These results indicated that the added green tea extract could inhibit the growth of these microorganisms in butter. On the other hand, the bacterial count significantly increased ($p < 0.05$) in all samples during storage. The highest increment rate was observed in the control sample, whereas a decreased rate was recorded upon higher amount of green tea extract. As emphasised by Koech *et al.* (2013), the catechin components of green tea are responsible for the antibacterial activity, while the most important antibacterial agents include the epigallocatechin (EGC), epigallocatechin gallate (EGCG), and epicatechin gallate (ECG). According to Ponmurugan *et al.* (2016), green tea has antimicrobial activities

Table 3. Changes in total plate counts, and yeast and mould counts of green tea butters (GTB) with different amounts of green tea extract during storage.

Week	Green tea extract (% w/w)					
	0% (control)	2%	4%	6%	8%	10%
Total plate count (CFU/g)						
1	2.74×10^{3a}	2.33×10^{3b}	2.12×10^{3bc}	1.92×10^{3cd}	1.83×10^{3cd}	1.62×10^{3d}
2	4.50×10^{3a}	2.76×10^{3b}	2.32×10^{3bc}	2.19×10^{3bcd}	1.90×10^{3cd}	1.66×10^{3d}
3	5.90×10^{3a}	4.95×10^{3b}	2.81×10^{3c}	2.39×10^{3cd}	2.16×10^{3de}	1.89×10^{3e}
4	8.85×10^{3a}	6.90×10^{3b}	4.90×10^{3c}	2.74×10^{3d}	2.49×10^{3d}	2.05×10^{3d}
5	1.11×10^{4a}	8.65×10^{3b}	6.70×10^{3c}	4.30×10^{3d}	3.35×10^{3de}	2.42×10^{3e}
Yeast and mould count (CFU/g)						
1	7.90×10^{2a}	6.20×10^{2b}	5.35×10^{2b}	4.25×10^{2c}	3.05×10^{2d}	2.70×10^{2d}
2	8.85×10^{2a}	7.10×10^{2b}	6.15×10^{2c}	4.95×10^{2d}	3.80×10^{2e}	2.90×10^{2f}
3	1.02×10^{3a}	8.05×10^{2b}	7.30×10^{2b}	5.85×10^{2c}	4.65×10^{2cd}	3.70×10^{2d}
4	1.41×10^{3a}	9.90×10^{2b}	8.90×10^{2b}	7.35×10^{2c}	5.85×10^{2d}	4.25×10^{2e}
5	2.02×10^{3a}	1.22×10^{3b}	9.80×10^{2c}	8.70×10^{2c}	7.70×10^{2cd}	6.05×10^{2d}

Data are means ± SD ($n = 6$). Different letters in the row indicate significant difference at $p < 0.05$. CFU/g = colony forming units per gram.

against various pathogenic microorganisms including *Staphylococcus aureus, Vibrio parahemolyticus, Clostridium perfringens, Bacillus cereus,* and *Pleisomonas shigelloides*. An important mechanism of antimicrobial activity in green tea refers to its ability to prevent oxidation in cells and cell membranes (Fakheri *et al.,* 2015). This seems to suggest that high antimicrobial activity can correspond with the increased in both TPC and DPPH activities due to the increased amount of green tea extract in the butter samples (Table 1). According to Aafrin Thasleema (2013), the catechins and antioxidant compounds in green tea have equal effectiveness as an antioxidant. A study conducted by Nadifah and Sari (2016) revealed that the addition of green tea extract can inhibit the growth of bacteria and be utilised as a natural preservative in soymilk. Also, Mahmoudi *et al.* (2019) reported a decrease in the microbial count and total mould and yeast of cow milk butter added with *Ziziphora clinopodioides* essential oil. Therefore, it can be concluded from the overall findings that green tea extract is a suitable choice of natural preservative for foods and drinks.

Sensory evaluation

Sensory evaluation is a quantitative technique in numerical data collection which aims to establish lawful and specific relationships between product characteristics and human perception (Wang *et al.,* 2007). The mean scores of hedonic sensory evaluations for appearance, colour, odour, taste, and overall acceptability of GTB are presented in Table 4. The addition of green tea extract significantly affected ($p < 0.05$) the colour, odour, taste, and overall acceptability of the GTB samples. Nevertheless, no significant difference was found between the samples in terms of appearance. Colour is another important attribute that is considered by consumers in judging the acceptability of food products (Shakerardekani *et al.,* 2013). It was discovered that consumers acceptance of the colour of GTB samples significantly decreased ($p < 0.05$) with increasing amount of green tea extract. This may be caused by the brown colour of green tea extract which resulted in a definite effect on this parameter. Moreover, the increased amount of green tea extract led to a significant ($p < 0.05$) increase and decrease of consumers acceptability in terms of odour and taste attributes, respectively. Apart from that, the taste of GTB was affected by the bitterness of green tea extract which is highly correlated with phenol concentration (Lee, 2009). It should be noted that the bitter taste is a characteristic associated with the cultivation of tea (Ahmed and Stepp, 2013).

For overall acceptability, the GTB-2, GTB-4, and GTB-6 received significantly ($p < 0.05$) higher scores as compared to GTB-0. On the other hand, GTB-8 and GTB-10 recorded significantly ($p < 0.05$) lower scores as compared to GTB-0. Therefore, it can be concluded that the sensory properties of the GTB samples are dependent on the added amount of green tea extract.

Conclusion

The results of the present work revealed that the increase in the amount of green tea extract reduced both the lightness and yellowness of the GTB samples. The redness, however, increased due to the dark brown colour of the green tea extract. Meanwhile, the TPC and DPPH activities increased, while the PV decreased upon the addition of high amount of green tea extract, thus resulting in enhanced antioxidant activity of the GTB samples. Both PV and AV of all GTB samples increased during storage, but the highest increment rate was recorded in GTB-0 sample. Other than that, the green tea extract also inhibited the microbial growth in GTB samples which further indicated its potential as a natural preservative especially at a higher amount. Nevertheless, it is unfortunate that the GTB-8 and GTB-10 samples received lower sensory acceptability scores as compared to the other GTB samples. Therefore, it is recommended for future work to improve the

Table 4. Sensory acceptance scores of green tea butters (GTB) with different amounts of green tea extract.

Attribute	Green tea extract (% w/w)					
	0% (control)	2%	4%	6%	8%	10%
Appearance^{ns}	6.63 ± 0.93	6.93 ± 1.08	6.73 ± 1.20	6.63 ± 1.33	6.50 ± 1.50	6.70 ± 1.18
Colour	6.83 ± 1.46^{ab}	7.20 ± 1.06^{a}	7.03 ± 1.27^{ab}	6.87 ± 1.01^{ab}	6.73 ± 0.94^{b}	6.60 ± 0.86^{b}
Odour	5.50 ± 1.04^{c}	6.00 ± 0.91^{b}	6.17 ± 0.87^{ab}	6.27 ± 0.98^{ab}	6.40 ± 1.00^{ab}	6.57 ± 1.19^{a}
Taste	5.37 ± 0.96^{bc}	6.20 ± 0.89^{a}	5.80 ± 0.96^{ab}	5.53 ± 1.17^{bc}	5.37 ± 1.30^{bc}	5.13 ± 1.31^{c}
Overall	5.70 ± 0.99^{ab}	6.23 ± 1.14^{a}	6.17 ± 1.12^{a}	5.77 ± 0.90^{ab}	5.43 ± 1.33^{b}	5.30 ± 0.99^{b}

Data are means ± SD. Different letters in the same row indicate significant difference at $p < 0.05$. ns = not significant.

butter formulations for enhanced sensory acceptability. A higher number of sensory panellists of at least 50 is also recommended for better representation of the sensory acceptability of the products among consumers. Moreover, it is highly recommended to utilise the powder form of green tea extract instead of the liquid form for lower AV in the butter samples.

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