APPLICATION OF GREEN TEA EXTRACT TO BISCUIT CREAM

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Abstract
Tea, is well-known for its health benefits, and also plays an important role in domestic and foreign trade of Vietnam. In Vietnam, tea is used mainly as a beverage in various forms, while its utilisation in other fields is limited. In addition, only young tea leaves and buds are used for these purposes, leaving large amount of tea leaves unused. The aim of our current work was to examine the possibility to use freeze dried extract from “unused” tea leaves as ingredient for biscuit cream to improve oxidation stability of this product.

In the present work, the radical scavenging activity of the extract from older leaves of local tea was evaluated using DPPH assay. We measured the perception thresholds of some sensory attributes associated with green tea extract in samples of biscuit cream. We found that changes in brightness, astringency, sweetness, and bitterness were detected at level of 0.5%, 0.75%, 1% and 1% respectively. Finally, green tea extract was used as an ingredient for biscuit cream at three levels: 500ppm, 1000ppm and 1%. Samples were stored at 40°C and the oxidation stability of products with addition of BHT, green tea extracts, and without any antioxidant were evaluated by measuring the peroxide and TBA values. We found that green tea extracts showed good inhibition effect on lipid oxidation of the cream. The inhibition effect of green tea extract at level of 500ppm was equivalent to the effect of BHT at 200ppm. The higher the concentration of tea extract applied, the greater the effect.

Keywords: green tea, biscuit, antioxidant, sensory analysis, lipid oxidation.

1. INTRODUCTION
Green tea is a popular drink in Vietnam as well as in many parts of the world. Its consumption has increased in many countries since the discovery of its health benefits. It has been well documented that green tea possesses antioxidant, antimicrobial, anticarcinogen effect and others. These effects are supposed to be linked to bioactive substances present in tea such as catechins, methylxanthine. Among these substances, epigallocatechin galate (EGCG) is very important because of its high concentration and high activity (Gramza & Korczak, 2005; Shahidi & Nuczk, 2003; Wang et al., 2000; Hara, 2001). The chemical characterisation and bioactivity of green tea depends on many factors, such as geographical location, cultivar species, season, age of the leaves, climate and horticultural practices (Fernandez, Pablos, Martin, & González, 2002; Lin, Tsai, Tsai, & Lin, 2003). In most studies, green tea was from Chinese, Japanese or Indian origin, and tea of Vietnamese origin was rarely studied, this is particularly the case for tea from central Vietnam.
Vietnam is one of the biggest tea producers of the world. Tea here is made mainly from the buds and young leaves. Local people use older leaves to make drink as well, but using older leaves for other purpose is unpopular in the country, including for extending food shelf-life. Biscuit is a favourite food which is usually stored for extended time before consumption. During the storage, we observe oxidation of the lipid component of the biscuit, particularly from the cream which has a large proportion of shortening. This slow oxidation process deteriorates the nutritional and sensory value of the product, making it unacceptable for consumers. In order to delay this process, synthetic antioxidants such as BHA, BHT have been used, but the safety of these compounds is questionable and there is a trend to restrict their use.

This paper presents a study on the possibility to use the extract from older tea leaves for preventing oxidation of biscuit cream.

2. MATERIALS AND METHODS

2.1 Chemicals:
DPPH (2,2-diphenyl 1-picryl hydrazyl) from Sigma, Singapore; TBA (Thiobarbituric acid) from Merck, Germany; Methanol in DPPH assay from Merck, Germany; other chemicals of Chinese origin.

2.2 Preparation of green tea extract
Fresh green tea was collected from the tea field in the countryside near Danang City, Vietnam. Young tea leaves and the buds were left, and only the older leaves were used for experimental purposes. Tea enzymes were inactivated by exposing the tea leaves to steam for 45 seconds. The moisture of the tea measured after steaming was 63.97% (analysed by Ohaus MB35 Moisture Analyser).

The green tea was extracted with water as follows: steamed tea leaves were cut into to 2-3 mm width bands and soaked in water (20 ml/1 g dry mass). All the materials were contained in a 2-necked round bottom flask, connected with a condenser and a thermometer. The extraction took place in water bath at 90 °C for 30 minutes. The extract was then filtered and concentrated in vacuum oven at 60 °C, 200 mbar. The concentrated extract was stored at -20 °C before freeze-drying for 12 h, yielding brown yellow powder.

2.3 Evaluation of free radical scavenging capacity of tea extract with DPPH assay
The free radical scavenging capacity of the tea extracts was determined and compared with that of BHT using the DPPH discoloration method (Lu & Chen, 2008). Four concentrations of the tea extract in methanol 100ppm, 300ppm, 500ppm, 700ppm, were prepared. Dilutions of the tea extracts or BHT in methanol (1 ml) were added to 1 ml of DPPH (0.1 mM in MeOH) and mixed thoroughly and allowed to stand for 40 min before absorbance was measured at 517 nm using a Biorad spectrophotometer. The mixture of DPPH solution (1 ml) and methanol (1
ml) were used as a negative control. Results were expressed as Inhibition Capacity (IC) which is calculated as follows:

\[ IC = \left( \frac{A_{DPPH} - A_{sample}}{A_{DPPH}} \right) \times 100\% \]

where \( A_{DPPH} \) represents the absorbance of the negative control sample and \( A_{sample} \) the absorbance of analysed sample.

2.4 **Preparation of biscuit cream:**
The formula of basic biscuit cream was as follows: shortening, 41.03 g; lecithin, 0.54 g; icing sugar, 50.74 g; milk powder, 7.13 g (Manley, 2000). These materials were provided by Quang Ngai Confectionary, Vietnam. The shortening was agitated intensively by hand-mixer, so that it became soft. Then the melted shortening was added with other ingredients and mixed thoroughly again. The product was smooth without any grittiness.

2.5 **Measurement of peroxide value**
According to AOAC 965.33

2.6 **Measurement of thiobarbituric value** (Alexander, 1996)
Ten gram of cream was added with 2.5 ml HCl 4N and 97.5 ml distilled water in 500 ml round bottom flask which was connected to a simple distillation unit. The first 50 ml of distillate were collected, from which 5 ml were transferred to a glass tube. After addition of 5 ml TBA solution (2mg/ml butanol) the content of the tube was boiled in a water bath for 2 h, quickly cooled in tap water to reach ambient temperature and the absorbance of orange colour of the solution was measured at 532 nm against the control sample which was prepared in the same way with exception that 10 ml of distilled water was used instead of the 10 g of cream for distillation.

2.7 **Sensory analysis:**
We assessed, using rated difference scales (Lawless & Heymann, 1998), the perception thresholds of three attributes: bitterness, astringency and sweetness. Six different samples were prepared; among them there were two control samples without tea addition (one coded and one used as reference) and four cream samples to which were added tea extract at levels: 0.25%, 0.50%, 0.75% and 1% with regard to cream weight. All samples were coded with randomised 3-digit numbers except the control used as reference. For each attribute, the difference between the coded sample and the reference was rated. The rating scale ranged from 0 (no difference) to 9 (extreme difference). The brightness liking of control and four samples with tea addition were also determined using a 10-point scale (0: dislike a lot and 9: like a lot). The attributes were evaluated in the following order: astringency, bitterness, sweetness and brightness.
The evaluation took place in separate booths equipped with red and white lamps, except for the analysis of brightness which was carried out under white light. The evaluation of other attributes took place under red bulbs to mask the brightness difference.

The panel consisted of 30 students from Department of Food Technology, Danang University of Technology, who knew well sensory evaluation techniques. In order to reach an accurate result, panellists were provided with bread and distilled water to clean their palates after every tasting.

2.8 Statistical analysis
Statistical assessment was carried out with the software system of Statgraphics Plus for Windows 4.0. The rated difference of each attribute at all levels were compared using LSD test with significant level of $\alpha = .05$.

3. RESULTS AND DISCUSSION

3.1 Radical scavenging activity of the tea extract
The antioxidant effect of green tea is well known. However, in most studies only commercial tea products or young tea leaves have been used, and therefore very few studies have focused on the antioxidant property of older leaves. In order to examine the anti-radical effect of older leaves of the local tea, we employed the DPPH assay mentioned above and determined the Inhibition Capacity (IC) of the tea extract at various concentrations and of the BHT at 200ppm. Results are shown in Table 1.

<table>
<thead>
<tr>
<th>Samples</th>
<th>BHT(200ppm)</th>
<th>Tea extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>IC (%)</td>
<td>95.3</td>
<td>96.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>95.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>94.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>93.2</td>
</tr>
<tr>
<td>700ppm</td>
<td>96.4</td>
<td>95.4</td>
</tr>
<tr>
<td>500ppm</td>
<td>96.4</td>
<td>95.4</td>
</tr>
<tr>
<td>300ppm</td>
<td>94.3</td>
<td></td>
</tr>
<tr>
<td>100ppm</td>
<td>93.2</td>
<td></td>
</tr>
</tbody>
</table>

The result showed that the tea powder possessed very good anti-radical activity at all concentrations applied. Higher concentration scavenged the free radical DPPH better. Tea powder at concentration 500ppm had nearly the same radical scavenging activity of the synthetic antioxidant BHT at 200ppm.

Tea catechins are believed to be the main compounds responsible for the antioxidant activity of tea. It is also known that the content of tea catechins in older leaves is lower than in young leaves (Hara, 2001). However, the results of our measurements indicate that the extract from old leaves of the local tea could be a good source of antioxidants and could replace BHT to inhibit the oxidation of food lipid and because tea extract is natural, it could overcome the problem rising from concerns about synthetic antioxidants.

3.2 Evaluation of recognition threshold of astringency, bitterness, sweetness and brightness change in biscuit cream.
The tea extract had brown yellow colour along with a bitter and astringent taste and this could affect the sensory properties of biscuit cream. Therefore, it is necessary to measure the
recognition threshold of astringency, bitterness, sweetness, and brightness change of biscuit cream because these properties could guide how much tea extract can be applied without changing much the sensory properties of the product.

Five samples of cream were prepared and analysed according to sensory analysis method mentioned earlier. Mean values and standard evaluation were calculated for each attribute. LSD test at significance level 5% was employed using software Statgraphics Plus 4.0. Results were shown in table 2 and table 3.

Table 2: Score of rated difference between coded and reference samples.

<table>
<thead>
<tr>
<th>Attribute</th>
<th>Samples</th>
<th>0.5%</th>
<th>0.75%</th>
<th>1.00%</th>
<th>1.25%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Astringency</td>
<td>Control</td>
<td>0,03±0.18a</td>
<td>0,93±1.57ab</td>
<td>1,33±2.15b</td>
<td>1.40±2.11b</td>
</tr>
<tr>
<td>Bitterness</td>
<td>0,03±0.18d</td>
<td>0,83±1.58de</td>
<td>0,90±1.99de</td>
<td>1,17±2.09e</td>
<td>2,87±2.89f</td>
</tr>
<tr>
<td>Sweetness</td>
<td>0,01±0.31g</td>
<td>0,73±1,05gh</td>
<td>1,17±1,32gh</td>
<td>1,53±1,50h</td>
<td>1,83±1,42i</td>
</tr>
</tbody>
</table>

Scores were expressed as mean ± standard deviation. Index above the score showed significant difference at level 5% by LSD test. Scores with the same letter were not significantly different each other.

The results showed that addition of tea powder affected the sensory properties of the samples of biscuit cream. The brightness of product was less favoured even at the lowest level of tea extract application (0.5%) in comparison with the control. The LSD test showed significant difference in brightness liking at this level. When tea was added at a concentration of 1.25%, the average hedonic score was less than five, meaning that people do not like this colour of cream, this suggest that colorant may be added, in this case, in order to improve the colour appearance of the cream. The brightness change was due to the brown yellow colour of tea extract, this change was more evident at high concentration of the extract. Because of this change in appearance, the evaluation of other sensory attributes was carried out under colour mask (i.e., under red light).

Tea possesses a characteristic astringency and bitterness therefore we can expect that, the addition of the extract to the cream will create a change in the perception of astringency and bitterness of the cream. However, when the tea extract was added at a level of 0.5%, only few panellists could recognise astringency and bitterness, as indicated by the LSD test which showed no difference between tested and reference samples. At a level of 0.75%, astringent taste in the cream samples was detected by more panellists but still few panellists detected the bitterness of the products. The difference in astringency between samples with a 0.75% extract addition and the reference sample was statistically significant, while the difference in
bitterness was not significant. It could be conjectured that the sweetness of the product masked the bitterness better than the astringency. The bitterness of the samples was more evident at level of 1.00% when LSD test showed that the samples with a 1.00% tea addition were significantly different from the reference samples.

Sweetness is also a characteristic property of the biscuit cream because it contains a large amount of sugar. Results from Table 2 also showed that the addition of tea powder could change this characteristic. The LSD test showed that this change was significant only at the tea concentration above 1.00%.

Therefore, it is possible to conclude that the recognition threshold of astringency, bitterness, and sweetness of the tea extract in biscuit cream is 0.75%, 1% and 1% respectively, while brightness liking of the cream decreases at a level of 0.5%.

3.3 Effect of green tea extract on the oxidation stability of biscuit cream

Biscuit cream contains large amount of lipid that could readily oxidize during storage. To examine if the extract from old leaves of local tea could replace BHT to protect biscuit cream from oxidation an experiment was carried out as follows.

We prepared six lots of biscuit cream with the formulation mentioned earlier: one was a control sample without any addition; one was treated with 200ppm BHT; tea extract was added to the other samples, with tea extracts having the following concentrations: 500ppm, 1000ppm and 1%. Thirty grams of cream from each lot was weighted into 100 ml beaker. Samples were prepared for six measurements in duplicates (12 beakers). The beakers were stored in the oven at 40°C during 16 days. Every three days, the samples were taken for analysis of peroxide value and TBA value; mean values were calculated. Results are shown in Figures 1 and 2 which display the relationship between measured values and storage time.

Peroxides are primary by-products of oxidation process of lipids and their concentration in lipids is characterised by their peroxide value. It could be noted from Figure 1 that this parameter increased continuously during the storage of all samples at elevated temperature (40°C). Addition of the synthetic antioxidant BHT or tea extract lowered the peroxide value. The analysis of BHT samples and samples with 500ppm tea extract indicated that the BHT at 200ppm and the tea extract at 500ppm had the same inhibiting effect on the formation of peroxides. The results also showed that higher concentration of tea extract decreased PV levels more. We also found that application of the tea extract at a concentration level of 1% was the most effective. This suggested that tea extracts did not show prooxidant effect at high level, in contrast with what is observed for vitamin E which has prooxidant effect at high concentration (Pokorny, 2001).

Similarly, the increase of TBA Value (TBAV) during storage was observed from Figure 2. TBAV reflected the concentration of malonaldehyde in samples. It is a typical secondary product of lipid oxidation. The courses of TBAV changes during the storage showed that all extract
treatments could delay the formation of malonaldehyde and that this effect could be rank ordered as: 1% > 1000ppm > 500ppm ≈ BHT.

![Figure 1](image1.png)

**Figure 1:** Changes of peroxide value during storage.

![Figure 2](image2.png)

**Figure 2:** Changes of thiobarbituric acid value during storage.

Results from the analysis of PV and TBAV in cream samples during the storage showed that the extract from old leaves of local tea could delay the oxidation of biscuit cream. Green tea extracts at concentration 500ppm were as effective as BHT at 200ppm. This concurs with the conclusions from the DPPH assay. The antioxidant effect of the tea extract could be explained by the radical scavenging activity, but, the chelating ability of catechins could also contribute to this effect (Pokorny, 2001; Hara, 2001).
In a similar study on antioxidant property of tea extract, Wanasundara and Shahidi (1998) reported that an aqueous ethanol extract of green tea showed prooxidant effect in edible oil possibly due to catalytic effect of chlorophyll. In our work, we used water as solvent, so the content of chlorophyll is negligible and the extract exhibits strong antioxidant property.

Our study suggests that application of green tea to biscuit cream does not have a prooxidant effect and this could be an advantage if the extract is needed to be applied at high concentration (e.g. in production of functional food). As an aside, some authors also suggested to use some spices in prevention of lipid oxidation in biscuits (Reddy, Vrooj, & Kumar, 2005).

In summary, the extract from old leaves of the local tea near Danang, Vietnam has been proven to be a potential natural antioxidant that could replace BHT in application to biscuit cream. Its recognition threshold for astringency, bitterness, sweetness change and brightness change in biscuit cream is 0.75%, 1%, 1% and 0.5% (with regard to cream weight) respectively.

4. CONCLUSIONS

Using natural antioxidants to replace synthetic ones is a current trend in food industry. However, only few natural antioxidants and few applications have been successful. This work evaluated the possibility to use older leaves of local tea in central Vietnam to inhibit oxidation of lipids in biscuit cream. In order to do so, antiradical activity of the extract was evaluated and the effect of the tea extract on sensory properties of the cream was also studied.

The results from the sensory and chemical analysis provided useful information on the threshold value of green tea extract in biscuit cream and on the oxidation stability of the cream fortified with tea extract. Below the threshold value, the sensory quality of green tea extract fortified cream is not significantly compromised. Also the cream is proven to be more stable against oxidation.

So it seems that green tea extract fortified biscuit could be a functional food product with additional health benefits. This provides a good guide for those biscuit manufacturers who want to pursue the production of functional biscuits with green tea extract fortification.

REFERENCE


