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Enhancing the sensory attributes and antioxidant properties of snus by mixing it with tea

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Abstract

In the present work, we investigated the chemical and volatile compositions of three tea-containing snus samples, after which their acceptability on the aromatic and taste coordination was evaluated by a professional panel. Results showed that the tea-containing snus samples exhibited better acceptability on the aroma and taste coordination profiles. Dahongpao tea (DT)-containing snus (DT-snus) exhibited the best acceptability of aromatic coordination, whereas the most favourable taste coordination was exhibited by Keemun black tea (KBT)-containing snus (KBT-snus). The antioxidant activity determined by the DPPH and ABTS assays revealed that Lu'an Guapian tea (LGT)-containing snus (LGT-snus) exhibited the highest free-radical scavenging ability. LGT-snus was also found to have the highest content of total polyphenols, amino acids, and caffeine. The highest levels of total flavonoids and soluble sugars were found in DT-snus and KBT-snus, respectively. There were 88, 68, and 74 volatiles found in DT-snus, LGT-snus, and KBT-snus, respectively, among which, nitrogenous compounds constituted the major category. High levels of nicotine, megastigmatrienone, neophytadiene, nicotyrine, and cotinine, which are the major volatiles in snus, were detected in the tea-containing snus samples. The mixing of tea introduced the flavour profiles of the volatiles present in the original tea into the tea-containing snus samples. Benzaldehyde, β-ionone, hexanoic acid, 3-(Z)-hexenyl ester, pyrazines, and nerolidol from LGT; furfural, benzeneethanol, nerolidol, linalool, and cedrol from DT; and nonanal, geraniol, *cis*-jasmone, benzenemethanol, and methyl salicylate from KBT were found in high concentrations in the corresponding tea-containing snus samples.

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Introduction

Tobacco (family Solanaceae, genus *Nicotiana*) is native to tropical America (Takebe and Otsuki, 1969), and has been cultivated in warm areas worldwide. Harvested tobacco leaves are cured prior to use. Dried/cured tobacco leaves are further processed into various products intended for smoking, including cigarette, cigar, and pipe tobacco (Rickert *et al.,* 2007). Cigarettes are the most consumed tobacco-containing products in the world. Cigarettes contain high levels of nicotine, and the smoke generated upon their combustion has multiple harmful elements, including tobacco tar, benzopyrene, and heavy metals (Yoshida and Tuder, 2007). These cigarette smoke compounds significantly increase the risk of several diseases in humans such as cardiovascular and respiratory (Morris *et al.,* 2015; Alexander *et al.,* 2015). The components from cigarette smoke also play a crucial role in increasing the risk of cancers (Stämpfli and Anderson, 2009). The World Health Organization reported that, annually, approximately seven million people succumb to diseases caused by smoking cigarettes. Cigarettes have been named the greatest preventable cause of death globally (Morris *et al.,* 2015).

Snus is a moist tobacco product obtained by mixing tobacco-leaf powder, water, and salt (Idris *et al.,* 1998). It has been considered an alternative to cigarettes since it is consumed through chewing, without the release of smoke and its related harmful components (Foulds *et al.,* 2003); therefore, snus can significantly reduce the incidence of lung cancer (Luo *et al.,* 2007). However, similar to cigarettes, snus is rich in nicotine, and the consumption of this preparation could increase the incidence of oral, pharyngeal, and pancreatic cancers, among others (Rodu, 2007).

Tea and tea-based products are the top three most consumed non-alcoholic beverages worldwide. Tea contains numerous bioactive components, including catechins, polysaccharides, alkaloids,

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vitamins, amino acids, and trace elements (Graham, 1992). These bioactive phytochemicals play important roles in benefiting human health (Yang and Landau, 2000). For instance, the consumption of tea and tea-based products can significantly lower the incidence of cancers and prevent the occurrence of chronic diseases, such as diabetes, obesity, and cardiovascular diseases (Tijburg *et al.,* 1997; Kao *et al.,* 2006). It has been reported that tea polyphenols can neutralise certain compounds in tobacco, which are known to be toxic, thereby mitigating their adverse effects after tobacco consumption (Mukhtar and Ahmad, 2000). In the present work, three tea-containing snus products were prepared, including snus supplemented with Dahongpao tea (DT), Lu'an Guapian tea (LGT), and Keemun black tea (KBT), and the effects of these tea products on the alteration of the sensory attributes and antioxidant activity of snus were investigated. The findings from the present work could provide useful information on the development of snus products.

Materials and methods

Tea-containing snus samples

Three tea-containing snus samples were prepared in the present work, including Dahongpao tea-containing snus (DT-snus), Lu'an Guapian tea-containing snus (LGT-snus), and Keemun black tea-containing snus (KBT-snus). These snus products are manufactured by the Shanghai New Tobacco Products Research Institute Co., Ltd. (Shanghai, China).

Chemicals and reagents

Anhydrous glucose, anthrone reagent, concentrated sulphuric acid, ethanol, ethyl acetate, ferrous sulphate, sodium potassium tartrate, disodium hydrogen phosphate dodecahydrate, and dipotassium hydrogen phosphate were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Methanol, acetonitrile, and acetic acid were of HPLC grade, and purchased from Tedia Co., Ltd. (Fairfield, OH, USA). 1,1-Diphenyl-2-picryl-hydrazyl (DPPH) and 2,2'-azino-bis(3-ethylenzothiazoline-6-suphonic acid (ABTS) were purchased from Sigma Aldrich Chemical Co., Ltd. (St. Louis, MO, USA). *n*-Alkanes C5-C20 were purchased from J&K Chemical Ltd. (Beijing, China).

Sensory evaluation of tea-containing snus samples

A taste panel comprising 10 professional panellists (four men and six women) evaluated the sensory parameters of these tea-containing snus samples, focusing on the overall aroma and taste coordination. These panellists were food science graduate students and faculty members, who were 20 - 40 years old and had the basic knowledge and experience of sensory evaluation. Additionally, these panellists also indulged in tobacco smoking and tea drinking. The taste panel was carried out in a professional panel room maintained at an average temperature of 25°C and relative humidity of 75%. For aroma evaluation, the three tea-containing snus samples were given to these panellists in random order. The panellists were asked to indicate with "√" if the overall aroma of the tea-containing snus was acceptable, and with "X" if they did not accept the aroma of the tea-containing snus. Between samples, a cup filled with roasted coffee was provided to the panellists to neutralise their olfactory sense. During the period of taste coordination among the panellists, the tea-containing snus samples (3.0 g) were extracted using 150 mL of water at 38°C for 10 min. The tea-containing snus extract was filtered, and the infusions were brought to the panellists in a random order for taste evaluation. A "√" was indicated if the panellists found the taste of the infusion acceptable, whereas the sample was marked "X" if unacceptable. A 5-min break was given to the panellists along with a cup of water and two crackers for palate refreshment.

Total polyphenol content

The total polyphenol in tea-containing snus was measured according to ISO 14502-1 (Chen *et al.,* 2011). Briefly, the tea-containing snus sample (0.2 g) was mixed with 5 mL of methanol:water (7:3, v/v). The mixture was stirred at 70°C for 10 min. After being cooled to room temperature (25°C), the mixture was centrifuged at 6,000 rpm for 10 min, and the extract was collected. The extract (1.0 mL) was diluted to 100 mL using deionised water, and 1 mL of the diluted extract was mixed with 5 mL of Folin-Coicalteu:water (1:9, v/v). Subsequently, the resultant mixture was mixed with 4 mL of 7.5% (w/v) sodium carbonate. The resulting mixture was kept at room temperature for 60 min in the dark. Lastly, the absorbance of the mixture was measured at 765 nm using a spectrophotometer (Shanghai Yuanxi Instrument Co., Ltd. Shanghai, China). Water was used as the reference, and each measurement was performed in triplicate. The concentration of the total polyphenols in samples was calculated using a standard curve of gallic acid over a concentration range of 10 - 50 µg/mL ($y = 94.34x - 0.86$; $R^2 = 0.9998$), where x = absorbance of the mixture, and y = concentration of the total

polyphenols in samples.

Free amino acid content

The total amino acid content in tea-containing snus samples was determined according to Bian *et al.* (2013) with slight modifications. Briefly, the tea-containing snus extract was prepared by mixing 6 g of tea-containing snus with 20 mL of boiling water for 10 min. The resultant extract (1 mL) was mixed in a 25-mL volumetric flask, with 0.5 mL of 0.067 M phosphate buffer (pH 8.04) and 0.5% ninhydrin containing 0.8 mg/mL tin (II) chloride dehydrate. Next, the resultant mixture was incubated at 100°C in a water bath for 15 min, and immediately cooled to room temperature under a stream of cold water. Subsequently, the volumes of the mixtures were adjusted to 25 mL in the volumetric flask using water, and kept at room temperature for 10 min. Finally, the absorbance of the mixture was measured at 570 nm using a spectrophotometer. Water was used as a blank, and each measurement was performed in triplicate. The concentration of free amino acids in samples was calculated from a standard curve of theanine over a concentration range of 40 - 320 μg/mL (y = 227.27x + 26.09; R^2 = 0.9957), where x = absorbance of the mixture, and y = concentration of the free amino acid in the sample.

Soluble sugar content

The soluble sugar content in tea-containing snus samples was measured using the anthrone colorimetry method (Dubois *et al.,* 1956). Briefly, the tea-containing snus (0.1 g) was mixed with 6 mL of ethanol:water (4:1, v/v) in a 10-mL centrifuge tube. The mixture was incubated in a water bath at 80°C for 30 min. Next, it was centrifuged at 3,000 rpm for 5 min, and the supernatant was collected. The solid pellet was mixed with 6 mL of ethanol:water (4:1, v/v), and incubated in the same water bath at 80°C for 30 min. The mixture was re-centrifuged at 3,000 rpm for 5 min, and the supernatant was collected. To the pellet, 6 mL of ethanol:water (4:1, v/v) was added, and the mixture was incubated in the same water bath at 80°C for 30 min, after which it was centrifuged. The supernatants were combined, and the total volume was made up to 50 mL using ethanol:water (4:1, v/v). Then, 1 mL of the supernatant was mixed with 1.5 mL of water and 6.5 mL of anthrone reagent. The resultant mixture was stirred well and incubated at room temperature for 15 min. The absorbance of the mixture was measured at 620 nm using a spectrophotometer. Water was used as the blank. Each measurement was performed in triplicate. The concentration of soluble sugar in samples was calculated based on a standard curve using glucose at concentrations ranging from 25 to 200 μg/mL (y = 312.5x - 11.43; R^2 = 0.9957), where x = absorbance of the mixture, and y = concentration of the soluble sugar in samples.

Total flavonoid content

The total flavonoid content in the tea-containing snus samples was analysed using the aluminium chloride colorimetric assay (Chang *et al.,* 2002). The extract was prepared by mixing 6 g of tea-containing snus with 20 mL of boiling water for 10 min. The extract (0.5 mL) was mixed with 1.5 mL of 95% ethanol, 0.1 mL of 10% aluminium chloride, 0.1 mL of 1 M potassium acetate, and 2.8 mL of water. The resultant mixture was allowed to rest at room temperature for 30 min, and then the absorbance of the samples was determined at 415 nm using a spectrophotometer. Aluminium chloride was replaced with a similar volume of water, which constituted the blank. Quercetin was used as the reference standard for the quantitation of total flavonoid content. Each analysis was performed in triplicate.

Caffeine content

An Agilent 1260 HPLC was used to determine the caffeine content in the tea-containing snus samples (Agilent Technologies, Santa Clara, CA, USA). The sample extracts were prepared by mixing 6 g of the tea-containing snus with 20 mL boiling water for 10 min. A Synergi 4u Fusion-RP 80A C18 column (4.6 mm × 250 mm, 5 μm; Phenomenex, Terrance, CA, USA) was used, and the flow rate was set to 1 mL/min. The mobile phase consisted of 1% (v/v) acetic acid in water (A) and acetonitrile (B). The gradient program was as follows: 0 - 7 min, 10 - 30% B; 7 - 10 min, 30% B; 10 - 12 min, 30 - 60% B; 12 - 15 min, 60 - 10% B; 15 - 20 min, 10% B. The column temperature was maintained at 40°C during the gradient elution, and the eluted samples were monitored at a wavelength of 280 nm using an ultraviolet detector. Caffeine was used as an external standard for the quantitation of caffeine in the extracted samples.

Antioxidant capacity of tea-containing snus samples by DPPH assay

The antioxidant activity of the tea-containing snus samples was analysed by the DPPH assay according to Wojdyło *et al.* (2007) with slight modifications. Briefly, the tea-containing snus (0.06 g) was mixed with 10 mL of boiling water in a 10-mL centrifuge tube. The mixture was incubated in a

water bath at 100°C for 45 min. After cooling to room temperature, the mixture was centrifuged at 3,000 rpm for 10 min. The supernatant was transferred to a new tube, and the volume was adjusted to 10 mL with water. This constituted the extract sample. 1,1-Diphenyl-2-picrylhydrazyl (DPPH) was dissolved in ethanol to yield a 20-µM concentration just before use. DPPH solution (5 mL) was mixed with 1 mL of tea-containing snus extract. The resultant mixture was stirred well and incubated in the dark for 30 min at room temperature. The absorbance of the mixture (Ai) was recorded at 517 nm using spectrophotometry. A mixture containing 1 mL of the tea-containing snus extract and 5 mL of anhydrous ethanol served as the negative control, and its absorbance (Aj) was measured at 517 nm after incubation at similar conditions. Anhydrous ethanol was used as a blank and its absorbance (A_0) was recorded at 517 nm. Each measurement was carried out in triplicate. The DPPH free-radical scavenging activity of each tea-containing snus sample was calculated using Eq. 1:

DPPH Free-Radical Scavenging Rate = (A_0 + Aj - Ai) / A0 × 100%

(Eq. 1)

Antioxidant capacity of tea-containing snus samples using ABTS assay

The antioxidant activity of the tea-containing snus samples was analysed by the 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) assay according to Wojdyło *et al.* (2007) with slight modifications. The sample preparation for the assay was similar to that used for the DPPH assay. ABTS was dissolved in water to yield a 7-µM ABTS stock solution. The ABTS radical cation was generated by mixing the ABTS stock solution with 2.45 µM potassium persulfate in the dark at room temperature and storing for 16 h. For the analysis, the ABTS radical cation solution was diluted using distilled water to an absorbance of 0.70 ± 0.02 at 734 nm, which was recorded using a spectrophotometer. Next, the diluted ABTS radical cation solution (3 mL) was mixed with 30 µL of the tea-containing snus extract. The mixture was kept at room temperature for 6 min, and the absorbance of the mixture (A_t) was recorded at 734 nm using a spectrophotometer. The absorbance of the reference standard (A_0) containing 30 µL of water and 3 mL of the diluted ABTS radical cation solution was recorded at 734 nm. Each measurement was conducted in triplicate. The ABTS free-radical scavenging activity was calculated using Eq. 2:

ABTS Free-Radical Scavenging Rate = (A_t - A_0) / A_t × 100%

(Eq. 2)

Volatile compounds

Volatile compounds in the tea-containing snus samples were extracted using headspace solid-phase microextraction (HS-SPME) according to Ye *et al.* (2012) with minor modifications. Briefly, the tea-containing snus (6 g) was mixed with 4 g of potassium chloride and 20 mL boiling water in a vial containing a magnetic stirrer. The vial was immediately capped and incubated at 70°C for 5 min. After equilibration, a 65-µm polydimethylsiloxane (PDMS)/divinylbenzene (DVB) fibre (Supelco, Bellefonte, PA, USA) was inserted into the headspace of the vial, and was allowed to adsorb the volatile compounds at 70°C for 50 min under similar conditions of agitation. Then, the SPME fibre was removed from the vial headspace, and immediately inserted into the injector of the gas chromatograph to desorb the volatile compounds for 5 min at 250°C.

An Agilent 7870A Gas Chromatography coupled with an Agilent 5975 Mass Spectrometer (Agilent Technologies, Santa Clara, CA, USA) was used to analyse the volatile compounds in the tea-containing snus extracts. An HP-5MS capillary column (30 m × 0.25 µm × 0.25 mm film thickness; Agilent Technologies, Santa Clara, CA, USA) was used to separate the volatile compounds under a carrier gas (helium) at a flow rate of 1 mL/min. The oven temperature gradient was set as follows: maintained at 50°C for 3 min, increased to 80°C at a rate of 5°C/min, held at 80°C for 2 min, then increased to 170°C at a rate of 3°C/min, held at 170°C for 2 min, and finally increased to 250°C at a rate of 5°C/min, and held at 250°C for 5 min. The temperature of the mass spectrometer interface was set to 230°C with a 70-eV electron impact mode. A selective ion mode with a mass scan range of 35 - 350 AMU was used. The retention indices were determined using a C6-C25 *n*-alkane series (Supelco, Bellefonte, PA, USA) under similar chromatographic conditions. The volatile compounds were identified by comparing their mass spectra with the retention indices and the NIST11 library. The relative concentration percentage of each volatile compound was calculated using the following equation: (peak area of each volatile) / (total peak area) × 100%.

Statistical analysis

Data were expressed as mean ± standard deviation of triplicate tests. Analysis of variance (ANOVA) was used to determine significant

differences among the means using the Duncan test at a significant level of 0.05. SPSS 20.0 (IBM, Chicago, IL, USA) was used for all statistical analyses.

Results and discussion

Aroma and taste coordination by panellists

Figure 1 shows the aroma and taste acceptability of the tea-containing snus samples judged by ten professional panellists. Five of ten panellists accepted the aromatic coordination of the snus sample without mixing the tea. The acceptability of the aroma of both LGT-snus and KBT-snus samples was found to be higher (six of ten panellists) than the snus without the tea. It was worth noting that most panellists preferred the aromatic coordination of the DT-snus. These findings indicated that the addition of tea into the snus indeed improved the aromatic coordination of the snus. Regarding the taste, only four panellists found the taste of the snus sample without the incorporation of tea to be acceptable (Figure 1). However, the incorporation of tea could effectively enhance the taste of the snus samples. For example, the LGT-snus was found to be acceptable by six panellists in terms of taste, whereas seven panellists showed their appreciation for the taste of the DT-snus sample. It should be noted that the KBT-snus sample was found to have the highest acceptability by eight panellists in terms of taste. These results indicated that the addition of tea to the snus resulted in an improvement of the taste coordination of the snus. It should also be noted that the panellists selected in the present work had long-term habits of cigarette-smoking and tea-consumption. Therefore, it was speculated that the incorporation of tea into snus could impart the sensory attributes of tea to the snus, thus effectively supplementing the complexity of sensory attributes of snus. Furthermore, it should also be noted that the use of different types of teas resulted in the alteration of the aroma and taste coordination acceptability of the snus samples, thus indicating that the phytochemicals and aromatic components of tea played an important role in altering the sensory attributes of the snus.

Antioxidant capacity of tea-containing snus samples

The oxidation of tobacco results in the formation of nitric oxide. High levels of nitric oxide in the body can cause hypotension and even a collapsed lung (Im *et al.,* 2003). Tea, as a natural antioxidant, can effectively scavenge free radicals and inhibit the formation of reactive oxygen species in the body (Pillai *et al.,* 1999). In the present work, we investigated if the incorporation of tea could improve the antioxidant ability of snus samples (Figure 2).

Results of the DPPH assay indicated that the antioxidants in the extracts could scavenge DPPH radicals resulting in a colour change of the samples from red to yellow (Kumaran, 2006). The DPPH radical scavenging rate of the snus sample without the added tea was found to be 24.90% (Figure 2A). The addition of tea to the snus samples significantly altered the DPPH free-radical scavenging rate. For

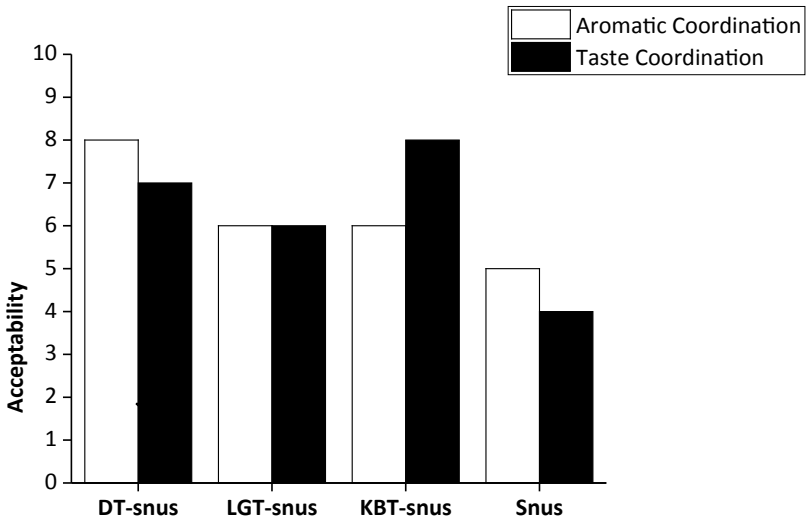

Figure 1. Acceptability of tea-containing snus samples as validated by a professional panel. Snus = snus without any incorporation of tea, DT-snus = Dahongpao-containing snus, LGT-snus = Lu'an-Guapian containing snus, KBT-snus = Keemun black tea-containing snus, and the number of "√" = the number of panellists who accepted the overall aroma/taste.

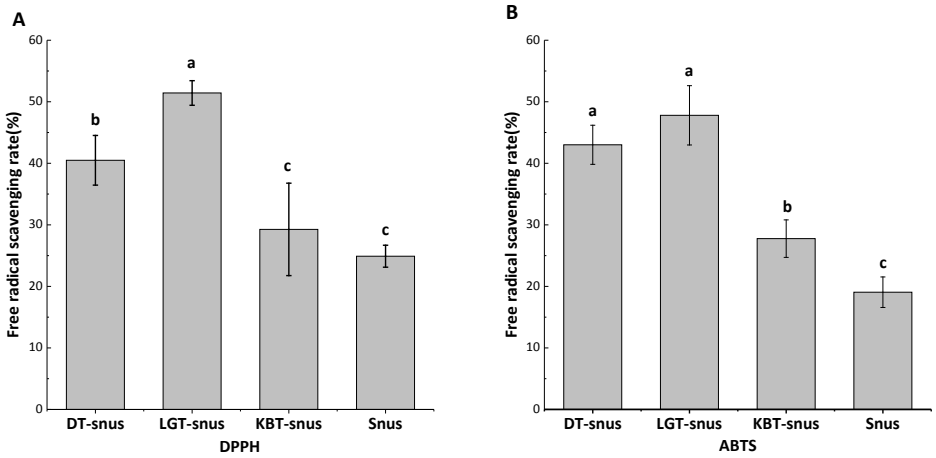

Figure 2. Antioxidant activity of tea-containing snus samples as analysed by the DPPH and ABTS assays. Error bars indicate standard deviation. Different lowercases indicate a significant difference ($p < 0.05$). Snus = snus without any incorporation of tea, DT-snus = Dahongpao-containing snus, LGT-snus = Lu'an-Guapian containing snus, and KBT-snus = Keemun black tea-containing snus.

instance, the KBT-snus sample exhibited an increased free-radical scavenging rate of 29.26%, whereas both DT-snus and LGT-snus samples exhibited about twice the scavenging rate that was obtained using snus samples without the added tea.

Similar to the results of the DPPH assay, the stable ABTS radicals could interact with antioxidants, which resulted in a colour loss of the prepared aqueous samples (Kumaran, 2006). It has been accepted that the antioxidant property of a compound can be correlated with the extent of quenching of the ABTS radicals (Erel, 2004). We found that the snus sample without the tea exhibited an ABTS radical quenching rate of 19.05% (Figure 2B). The tea-containing snus samples exhibited significantly higher quenching of ABTS free radicals. For example, the ABTS free-radical scavenging rate of the KBT-snus sample was found to be 27.76%. Both DT-snus and LGT-snus samples were found to have ABTS quenching rates higher than 43%.

It is worth noting that the tea-containing snus samples exhibited significant differences in antioxidant activities in terms of their DPPH and ABTS free-radical scavenging abilities. These differences were mainly attributed to the difference in the antioxidant properties of the tea samples. DT and KBT are produced by fermentation, during which, polyphenol oxidases accelerate the oxidation and hydrolysis of polyphenols in the tea, thus causing a significant reduction in the levels of this enzyme. LGT, on the other hand, is a non-fermented green tea. It is likely that heat treatment effectively denatures the polyphenol oxidases in LGT, thereby leading to high levels of polyphenols in the tea. This could explain the high antioxidant capacity of the LGT-snus sample.

Chemical composition of tea-containing snus samples

Table 1 shows the chemical composition of the tea-containing snus samples. Polyphenols are the

Table 1. Polyphenolic, free amino acids, caffeine, total flavonoid, and soluble sugar contents in tea-containing snus samples.

Sample	Content (mg/g)				
	Polyphenol	Amino acid	Caffeine	Total flavonoid	Soluble sugar
DT-snus	35.78 ± 1.55^{b}	12.78 ± 1.56^{b}	9.45 ± 1.44^{b}	18.27 ± 1.82^{a}	24.18 ± 1.99^{c}
LGT-snus	45.16 ± 2.43^{a}	13.37 ± 1.27^{a}	13.51 ± 1.12^{a}	15.64 ± 1.84^{b}	27.64 ± 2.72^{b}
KBT-snus	34.83 ± 1.02^{b}	12.13 ± 1.16^{b}	12.02 ± 0.88^{a}	12.49 ± 0.58^{c}	32.25 ± 0.87^{a}

Data are mean ± standard deviation of triplicate tests ($n = 3$). Different lowercase superscripts in each row indicate a significant difference ($p < 0.05$). DT-snus = Dahongpao-containing snus, LGT-snus = Lu'an-Guapian containing snus, and KBT-snus = Keemun black tea-containing snus.

primary bioactive compounds responsible for the antioxidant properties of these samples (Scalbert *et al.,* 2005). The anticancer properties of polyphenols have been well documented, and it has been proven that these compounds can prevent several chronic diseases in humans (Barbosa, 2007). In the present work, the polyphenol content of the KBT-snus and DT-snus samples was significantly lower than that in the LGT-snus. This finding is consistent with the antioxidant activity of the tea-containing snus samples (Figure 2). KBT and DT are prepared using a fermentation process during which the polyphenols are easily oxidised by polyphenol oxidases (Tanaka and Kouno, 2003). On the other hand, LGT is thermally treated to preserve its colour, and the heat treatment significantly retards the activity of polyphenol oxidases in the oxidation of tea polyphenols. Flavonoids play an important role in imparting bitterness and astringency to tea-containing snus samples (Chaturvedula and Prakash, 2011). As expected, these bioactive compounds also conferred antioxidant properties to the tea-containing snus. DT-snus and KBT-snus were found to contain the highest and lowest level of total flavonoids, respectively.

Amino acids are the major components of tea-containing snus, which contribute to the umami taste. The amino acid levels in tea are mainly dependent on the origin of the tea and the period of storage (Kausar *et al.,* 2013). It has been shown that extending the storage period results in a significant loss in the total amino acid content of the tea (Baltes and Mevissen, 1988; Kausar *et al.,* 2013). In the present work, the highest amino acid content was found in the LGT-snus sample, followed by the DT-snus sample (Table 1). The KBT-snus sample exhibited the lowest amino acid content. Soluble sugars contribute to the sweetness of the tea-containing snus, and are also associated with the formation of volatile compounds (Baltes and Mevissen, 1988). The soluble sugar content in KBT-snus was significantly higher than that in the other tea-containing snus samples, thus indicating the hydrolysis of the polysaccharides in the tea to soluble sugars during fermentation (dos Reis *et al.,* 2014). The bioactive compound, caffeine, is a central nervous system stimulant, which provides bitterness and astringency to the tea-containing snus (Chaturvedula and Prakash, 2011). The caffeine content of tea is associated with its freshness. For example, fresh tea leaves are known to contain a high level of caffeine, whereas storage over prolonged periods tends to reduce the caffeine content in tea (Ananingsih *et al.,* 2013). In the present work, the LGT-snus and DT-snus samples were found to have the highest and lowest levels of caffeine, respectively.

Composition of volatiles in tea-containing snus samples

A total of 135 volatile compounds were identified using gas chromatography coupled with mass spectrometry; 88, 68, and 74 volatile compounds were detected in DT-snus, LGT-snus, and KBT-snus samples, respectively. Based on their chemical nature, the volatile compounds were further grouped into nitrogenous compounds, lactones, acids, hydrocarbons, esters, aldehydes, alcohols, and others.

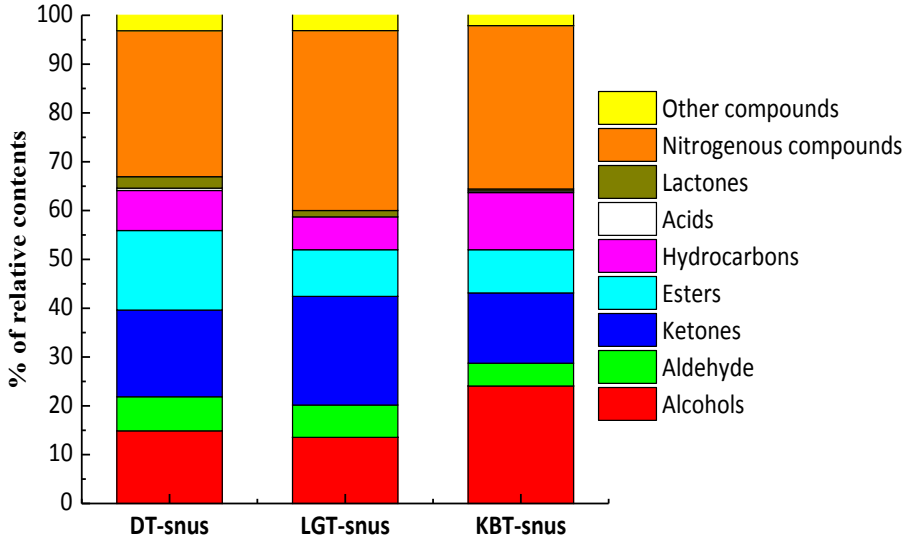

Figure 3. Relative concentration percentage of each volatile category in the three tea-containing snus samples. DT-snus = Dahongpao-containing snus, LGT-snus = Lu'an-Guapian containing snus, and KBT-snus = Keemun black tea-containing snus.

Table 2. Retention time (RT), retention index (RI), and relative concentration percentage of individual volatile compounds in tea-containing snus samples.

RT (min)	RI	Volatile	Relative concentration percentage (%)		
			DT-snus	LGT-snus	KBT-snus
Alcohol					
14.88	1069	Cyclooctyl alcohol	n.d.	n.d.	0.13
15.01	1072	*trans*-Linalool oxide (furanoid)	n.d.	n.d.	0.51
15.74	1081	*cis*-Linalool oxide	n.d.	n.d.	0.70
13.42	1032	Benzenemethanol	0.64	0.98	1.58
15.07	1078	1-Octanol	n.d.	0.98	0.78
15.03	1080	Furfuryl alcohol	0.87	n.d.	n.d.
15.25	1070	*p*-Cresol	0.13	n.d.	n.d.
19.61	1163	Epoxylinalol	n.d.	n.d.	0.90
20.09	1182	*p*-Menth-1-en-4-ol	n.d.	n.d.	0.38
16.39	1098	Linalool	0.19	1.86	3.26
20.78	1189	α-Terpineol	n.d.	n.d.	0.67
16.55	1106	Hotrienol	0.44	1.01	0.69
16.92	1109	Benzeneethanol	1.21	1.72	1.70
22.14	1228	*cis*-Geraniol	n.d.	n.d.	0.23
20.42	1192	Creosol	n.d.	0.21	n.d.
20.78	1192	L-alpha-Terpineol	n.d.	0.40	n.d.
19.45	1151	Phenol, 2,5-dimethyl-	0.09	n.d.	n.d.
23.38	1255	Geraniol	0.99	2.14	3.36
34.39	1502	Phenol, 2,4-bis(1,1-dimethylethyl)-	n.d.	n.d.	6.54
36.54	1565	Nerolidol	6.33	3.56	0.74
39.74	1645	δ-cadinol	n.d.	n.d.	0.61
31.9	1449	*trans*-Isoeugenol	0.60	n.d.	n.d.
40.05	1653	α-Cadinol	n.d.	0.17	0.44
34.09	1497	Butylated Hydroxytoluene	0.35	0.46	n.d.
38.3	1596	Cedrol	2.80	0.24	0.40
50.41	1949	Isophytol	0.09	n.d.	n.d.
54.34	2114	Phytol	0.12	1.16	0.74

Aldehyde					
6.46	818	Furfural	0.28	n.d.	n.d.
8.66	896	Heptanal	n.d.	n.d.	0.29
10.66	959	Benzaldehyde	1.84	2.33	1.08
12.12	1000	Octanal	n.d.	n.d.	0.24
12.46	1015	2,4-Heptadienal, (E,E)-	1.77	0.20	n.d.
13.82	1043	Benzeneacetaldehyde	0.21	n.d.	0.28
17.17	1116	α-Cyclocitral	n.d.	0.18	n.d.
16.64	1101	Nonanal	0.15	1.73	2.23
17.63	1016	1H-Pyrrole-2-carboxaldehyde, 1-methyl-	0.18	n.d.	n.d.
20.94	1201	Safranal	0.24	0.88	0.19
21.36	1203	Decanal	0.12	0.41	0.26
21.77	1210	2,4-Nonadienal, (E,E)-	0.16	n.d.	n.d.
21.86	1219	β-Cyclocitral	0.47	n.d.	n.d.
24.14	1270	α-Citral	0.39	0.38	0.19
26.44	1314	2,4-Decadienal, (E,E)-	0.74	n.d.	n.d.
28.72	1355	2-Octenal, 2-butyl-	0.45	0.49	n.d.
Ketone					
14.76	1062	Acetophenone	n.d.	0.41	0.26
18.45	1139	2-Cyclohexene-1,4-dione	0.06	0.25	n.d.
20.29	1183	Ethanone, 1-(4-methylphenyl)-	0.91	n.d.	n.d.
31.27	1433	β-Ionone, dihydro-	n.d.	0.19	n.d.
29.62	1396	*cis*-Jasmone	0.82	2.33	5.15
30.85	1426	α-Ionone	1.95	2.08	n.d.
31.99	1449	*trans*-Geranylacetone	2.20	2.06	1.44
33.21	1477	β-Ionone	8.97	10.4	6.23
33.34	1485	β-Ionon-5,6-epoxide	1.62	2.52	0.31
39.02	1472	Megastigmatrienone	0.30	1.34	0.78
47.2	1847	2-Pentadecanone, 6,10,14-trimethyl-	0.92	0.69	0.42
Ester					

18.24	1145	Methyl nicotinate	0.07	0.26	0.17
20.39	1187	Butanoic acid, 3-hexenyl ester, (Z)-	0.16	n.d.	0.22
20.63	1193	Methyl salicylate	3.21	2.31	2.09
27.09	1335	Benzoic acid, 2-methoxy-, methyl ester	n.d.	n.d.	n.d.
27.29	1317	Methyl anthranilate	n.d.	n.d.	n.d.
22.46	1235	*cis*-3-Hexenyl isovalerate	0.21	n.d.	0.27
23.51	1256	Acetic acid, 2-phenylethyl ester	0.53	n.d.	n.d.
26.55	1322	Methyl geranate	0.54	n.d.	n.d.
29.19	1369	Hexanoic acid, 3-hexenyl ester, (Z)-	3.88	4.81	5.75
29.43	1381	Hexanoic acid, hexyl ester	2.30	0.55	n.d.
29.57	1381	Hexanoic acid, 2-hexenyl ester, (E)-	1.70	n.d.	n.d.
36.9	1568	*cis*-3-Hexenyl benzoate	n.d.	0.89	0.25
31.62	1447	β-Phenylethyl butyrate	0.96	n.d.	n.d.
37.25	1580	Benzoic acid, hexyl ester	1.24	n.d.	n.d.
39.63	1638	Methyl jasmonate	0.66	n.d.	n.d.
43.95	1762	Cedryl acetate	0.11	n.d.	n.d.
46.64	1812	Isopropyl myristate	0.03	0.19	n.d.
49.87	1927	Hexadecanoic acid, methyl ester	0.44	0.38	n.d.
50.56	1922	Dibutyl phthalate	0.09	0.18	0.19
53.99	2079	Linoleic acid, methyl ester	0.05	n.d.	n.d.
54.15	2086	9-Octadecenoic acid (Z)-, methyl ester	0.11	n.d.	n.d.
Hydrocarbon					
8.29	893	Styrene	n.d.	0.43	0.49
11.58	975	β-Pinene	n.d.	n.d.	0.46
13.16	1028	Limonene	n.d.	0.23	0.31
13.93	1026	β-Ocimene	n.d.	n.d.	0.33
12.96	1020	*p*-cymene	0.19	n.d.	n.d.
17.74	1130	2,6-Dimethyl-1,3,5,7-octatetraene, E,E-	0.08	0.25	0.11
26.16	1298	1-Oxaspiro[4.5]dec-6-ene,2,6,10,10-tetramethyl-	n.d.	0.94	0.10
27.58	1351	α-Cubebene	n.d.	0.27	1.08
33.97	1499	α -Muurolene	n.d.	n.d.	0.41

31.77	1447	Acenaphthylene	n.d.	0.39	n.d.
34.54	1485	Naphthalene,1,2,4a,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-	n.d.	n.d.	0.45
34.76	1485	Naphthalene, 1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, (1S-*cis*)-	n.d.	n.d.	4.36
32.17	1446	*cis*-β-Farnesene	n.d.	0.40	0.25
33.97	1490	β-Guaiene	n.d.	0.28	n.d.
34.3	1505	α-Farnesene	n.d.	0.33	n.d.
37.84	1588	Cetene	n.d.	n.d.	0.13
30.52	1404	α-Cedrene	1.03	n.d.	0.16
30.67	1415	Caryophyllene	0.33	0.20	0.17
32.19	1458	*cis*-β-Farnesene	1.58	n.d.	n.d.
32.32	1455	Alloaromadendrene	0.26	n.d.	n.d.
33.86	1494	α-Selinene	0.39	n.d.	n.d.
34.42	1509	β-Bisabolene	0.75	n.d.	n.d.
35.47	1536	α-Cadinene	0.19	n.d.	n.d.
35.69	1523	α-Calacorene	0.44	0.34	n.d.
47.64	1836	Neophytadiene	2.63	1.90	1.62
Acid					
15.43	1071	Heptanoic acid	0.07	n.d.	0.33
24.5	1272	Nonanoic acid	0.27	n.d.	n.d.
50.84	1970	*n*-Hexadecanoic acid	0.15	n.d.	n.d.
Lactone					
35.12	1520	Dihydroactinidiolide	1.98	1.31	0.21
31.31	1432	Coumarin	0.37	n.d.	0.19
Nitrogenous compound					
9.01	915	Pyrazine, 2,5-dimethyl-	n.d.	0.27	n.d.
12.05	994	Pyrazine, 2-ethyl-6-methyl-	n.d.	0.29	n.d.
14.75	1060	Ethanone, 1-(1H-pyrrol-2-yl)-	0.48	n.d.	n.d.
18.16	1135	Benzyl nitrile	2.64	n.d.	n.d.
18.84	1150	Pyrazine, 3,5-diethyl-2-methyl-	0.05	2.16	n.d.
26.1	1310	Pyrazine, 2,5-dimethyl-3-(3-methylbutyl)-	n.d.	1.75	n.d.

28.2	1292	Indole	7.91	14.23	10.33
32.72	1360	Nicotine	16.65	17.74	18.20
33.3	1488	Nicotyrine	2.14	0.11	4.07
41.68	1716	Cotinine	0.03	0.28	0.10
Other compound					
7.6	866	Benzene, 1,3-dimethyl-	0.15	n.d.	n.d.
8.89	904	Ethanone, 1-(2-furanyl)-	0.04	n.d.	n.d.
12.46	997	Benzene, 1-chloro-4-methyl-	n.d.	n.d.	0.08
11.71	995	Mesitylene	0.38	n.d.	n.d.
12.75	1016	Benzene, 1,2,3-trimethyl-	0.35	n.d.	n.d.
15.9	1080	Benzene, 1-methyl-4-(1-methylethenyl)-	0.14	n.d.	n.d.
20.18	1181	Naphthalene	n.d.	0.47	0.42
18.6	1144	Benzene, 1,2,3,4-tetramethyl-	0.10	n.d.	n.d.
25.89	1306	Naphthalene, 1-methyl-	n.d.	0.24	0.55
30.59	1402	Naphthalene, 2,7-dimethyl-	n.d.	n.d.	0.50
28.95	1377	Biphenyl	n.d.	0.41	n.d.
25.9	1290	Naphthalene, 2-methyl-	0.14	0.30	0.17
30.57	1416	Naphthalene, 1,3-dimethyl-	n.d.	0.12	n.d.
27.93	1355	1H-Indene,2,3-dihydro-1,1,4,5-tetramethyl-	0.54	n.d.	n.d.
34.92	1521	Naphthalene, 1,2,3,4-tetrahydro-1,6-dimethyl-4-(1-methylethyl)-, (1S-*cis*)-	0.31	0.78	1.27
35.31	1528	Naphthalene, 1,2,3,4,4a,7-hexahydro-1,6-dimethyl-4-(1-methylethyl)-	n.d.	0.25	0.50
30.03	1408	Naphthalene, 1,6-dimethyl-	0.79	n.d.	n.d.
40.7	1674	Naphthalene, 4-isopropyl-1,6-dimethyl-	0.36	n.d.	n.d.
42.52	1716	2,6-Diisopropylnaphthalene	0.20	n.d.	n.d.

Data are mean from triplicate tests (n = 3). n.d.: not detected.

Figure 3 shows the level of each volatile category in the tea-containing snus samples. Nitrogenous compounds appeared to be the major volatile component in tea-containing snus samples, and the relative concentration percentage of the total nitrogenous compounds represented more than 25% of the total volatile content in these samples. It should be noted that acids (< 1%) constituted the lowest relative concentration percentage of the total volatile concentration. Ketones and alcohols were present in a relatively high concentration percentage (above 10%), whereas aldehydes, lactones, and other compounds comprised less than 5% of the total content. Among these tea-containing snus samples, DT-snus had the highest concentration percentage of esters, whereas LGT-snus showed the highest percentage of nitrogenous compounds and ketones. KBT-snus was found to be abundant in alcohols and hydrocarbons.

Among the individual volatile compounds (Table 2), those with a relative concentration higher than 5% were found to include phenol-2,4-bis(1,1-dimethylethyl), nerolidol, *cis*-jasmone, β-ionone, hexanoic acid, 3-(Z)-hexenyl ester, indole, nicotine,

megastigmatrienone, neophytadiene, nicotyrine, and cotinine in the tea-containing snus samples. It should be noted that nicotine, megastigmatrienone, neophytadiene, nicotyrine, and cotinine are the major volatile compounds in tobacco, of which nicotine constitutes more than 15% of the total volatile content (Fujimori *et al.,* 1976; Miyake and Shibamoto,1995). The incorporation of the tea, indeed, introduced the specific volatile compounds from that specific tea into the snus sample. For instance, the LGT-snus sample was found to contain several volatile compounds that are present in LGT. Benzaldehyde was the major volatile compound found in LGT-snus. This volatile imparted a nutty and caramel-like aroma to the LGT-snus. Additionally, β-ionone, heptanoic acid, and 3-(Z)-hexenyl ester played a crucial role in giving a floral note to the LGT. These volatiles were also found in a relatively high concentration in the LGT-snus. Pyrazines and nerolidol in LGT are responsible for the toasty and woody aroma, respectively (Zhu *et al.,* 2017). The LGT-snus sample exhibited high levels of these volatiles. Furfural imparts a creamy flavour profile, whereas benzeneethanol, nerolidol, and linalool are the primary volatiles responsible for the floral aroma in DT. These volatiles constituted a high proportion of DT-snus. The aroma reported in DT is that of rosin, which is mainly attributed to cedrol (Zhu *et al.,* 2015). The incorporation of DT into the snus gave a rosin-like flavour to the snus owing to its high levels in the tea. The KBT-snus sample was found to have a high concentration of nonanal, geraniol, *cis*-jasmone, benzenemethanol, and methyl salicylate (Table 2). It has been reported that nonanal and geraniol contribute citrus and rose notes, respectively (Schuh and Schieberle, 2006), whereas both *cis*-jasmone and benzenemethanol are associated with a floral aroma (Schuh and Schieberle, 2006). Methyl salicylate was the primary volatile that gave a mint aroma to the KBT (Takeo, 1983).

Conclusion

The incorporation of tea in snus effectively improved the acceptability of the aroma and taste coordination of snus, which was confirmed by a panel of professional evaluators. The antioxidant activity of the tea-containing snus samples was significantly enhanced, and was confirmed using the DPPH and ABTS assays. LGT-snus was found to have the highest level of total polyphenols, total amino acids, and caffeine, whereas the highest level of total flavonoids was found in DT-snus. KBT-snus exhibited the highest content of soluble sugars. Additionally, snus featured the presence of volatiles, including nicotine, megastigmatrienone, neophytadiene, nicotyrine, and cotinine, all of which were found to be the major volatile compounds in the tea-containing snus samples. Meanwhile, benzaldehyde, β-ionone, hexanoic acid, 3-(Z)-hexenyl ester, pyrazines, and nerolidol were the featured volatile compounds in LGT, which were also found in a high concentration percentage in the LGT-snus. The featured volatiles in DT, including furfural, benzeneethanol, nerolidol, linalool, and cedrol, were found to be present in significantly high levels in DT-snus. KBT-snus was found to have the volatiles reported in KBT, namely, nonanal, geraniol, *cis*-jasmone, benzenemethanol, and methyl salicylate. Therefore, by mixing tea and snus, the volatile compounds originally present in the tea could be introduced into the snus samples.

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