Improving the Flavor of Soy Ice Cream by Adding Lemongrass or Pandan Leaf Extracts

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ABSTRACT

Lemongrass or pandan leaf extracts were used separately to improve the flavor of soy ice cream. Lemongrass or pandan leaf extracts with water in concentrations of 10:100, 15:100, and 20:100 w/w were examined using sensory evaluation for the best flavor acceptance. The best ratio was selected to spray dry using maltodextrin as the drying medium at various concentrations (2, 4, 6, 8 and 10% w/w). A ranking preference test was used to determine the flavor of the soy ice cream. Threshold testing was used to determine the sensorial quality of soy isolate protein mixed with both herb extracts, and soy ice cream mixed with both herb extracts. The flavor compound was analyzed by using headspace, solid-phase microextraction, gas chromatography, mass spectrophotometry (HS-SPME-GC-MS). The best concentration of fresh lemongrass or pandan leaf extracts for improving the sensorial flavor of soy ice cream was 10:100 (w/w). The optimum quantity of maltodextrin for spray drying was 2% (w/w). The threshold values of fresh and powdered lemongrass extracts that could mask the beany flavor in soy protein isolate solution and soy ice cream were lower than those of fresh and powdered pandan leaf extracts. The beany flavor in soy protein isolate solution and soy ice cream was mainly composed of hexanal, pentanal, benzaldehyde, 2-pentyl-furan and 1-octen-3-ol. The flavor compounds in pandan leaf extract were 2-acetyl-1-pyroline and 3-methyl-2(5H)-furanone, while those of lemongrass extract were β-myrcene, α-pinene, 3-carene, neral, geranial and geraniol. From the results of HS-SPME-GC-MS, the beany flavor was masked by these extracts. This indicated that lemongrass and pandan leaf extracts could mask the beany flavor and improve the sensorial quality of soy ice cream.

Keywords: Ice cream, Lemongrass, Pandan leaf, Beany flavor, Headspace, Solid-phase microextraction, Gas-chromatography, Mass spectrophotometry

INTRODUCTION

Ice cream is a popular dairy-based frozen product. Generally, regular ice cream consists of milk proteins (whey and casein), sugar (lactose), fat, emulsifier, stabilizer and a flavoring agent. However, milk ice cream causes milk allergies and lactose intolerance in certain groups of consumers. Soy protein-based ice cream is an alternative, and has been found to help relieve people from such problems. The importance and role of soybean proteins in replacing milk proteins has been widely reported (Kinsella, 1979; Winarno and Muchtadi, 1984; Hua et al., 2005). Soybeans are widely recognized as a good source of protein and other nutrients such as vitamins, minerals, fibers, isoflavones, antioxidants and essential fatty acids (Messina and Bames, 1991). Several epidemiological studies have found an association between the consumption of soybeans and health improvement relating to a reduced risk of breast and prostate cancers, cardiovascular disease and atherosclerosis (Wu et al., 1996; Anderson, Smith and Washnock, 1999; Yamakoshi et al., 2000). In addition, soy proteins provide several functionalities in food, including retaining water, binding and emulsifying (Arres et al., 1991), which are beneficial to ice cream production. However, the beany flavor of soybeans has been the major obstacle to consumer acceptance. The beany flavor is a combination of green, grassy, painty, rancid, astringent and bitter flavors (Kobayashi et al., 1995; Wilson, 1996; Liu, 1997; Torres-Penaranda and Reitmeier, 2001). Instrumental volatile analysis reveals that the beany or grassy off-flavors comprised the greater amounts of hexanal, 1-hexanol, *trans*-2-nonenal, 1-octen-3-ol, *trans*-2, *trans*-4-decadienal and dimethyl trisulfide (Cadwallader, 2004). Several studies have investigated improving soymilk flavor by using heat treatments, soaking with ethyl alcohol (Ashraf, 1980), adding ingredients (Chien and Snyder, 1983), adjusting pH (Rehman et al., 2007a) and adding cyclodextrin (Suratma et al., 2004). However, these methods might affect the acceptable quality of the foods and increase production cost. Using herbal extracts to hide the beany flavor offers a potential cost-effective alternative. The food industry uses both lemongrass and the pandan leaf as flavor-improving agents.

Lemongrass (*Cymbopogon citratus*) is common in drinks, a sweet ingredient in exotic foods and recognized as a medicinal herb. The volatile compounds in lemongrass that provide its desirable flavor are neral, geranial, limonene, citronellal, caryophyllene, 6-methyl hept-5-en-3-one, linalool and beta-myrcene (Ashurst, 1999; Kasali et al., 2001; Schaneberg, 2002). Laswai et al. (2009) suggested that the beany flavor in soymilk was suppressed by using lemongrass extract. Moreover, the extract also improved the taste of soymilk.

Pandan leaf (*Pandanus amaryllifolius*) is an herbaceous tropical plant with long green leaves that produces a desirable odor. The leaf has been commonly used as a flavoring ingredient for desserts, such as ice cream, and for some drinks (Che Man et al., 1999; Cheetangdee and Chaiseri, 2006). In Asia, the leaf has been used as an important ingredient providing a desirable flavor and green color in desserts for a long time. Laksanalamai and Ilangantilex (1993) identified the main volatile compound in pandan leaf as 2-acetyl-1-pyrroline (2AP). Laohakunjit and Noomhorm (2004) and Bhattacharjee et al. (2005) reported similar results. 2AP was recognized as the key compound contributing to the characteristic popcorn-like aroma in jasmine and basmati rice (Buttery and Ling, 1982). It was one of the best flavors to mask the beany flavor in soybean products.

As the beany flavor is an important factor affecting soy ice cream, the objective of this work was to use lemongrass or pandan leaf extracts to improve soy ice cream flavor. The determination of the flavor improvement was carried out by sensory evaluation, while the quantity of flavor compounds was investigated by using headspace, solid-phase microextraction, gas chromatography, mass spectrophotometry (HS-SPME-GC-MS).

MATERIALS AND METHODS

Raw materials

Lemongrass and pandan leaves were obtained from local markets in Nakhon Ratchasima Province, Thailand. They were selected for their uniformity without signs of physical damage, disease and insect attack, and were then cleaned using tap water and air-dried before extraction. Maltodextrin (DE10) was purchased from Shandong Xiwang Starch Co., LTD (China). Beta-cyclodextrin was obtained from Rama Foods Co., LTD (Bangkok, Thailand). Soy protein isolate was purchased from Solae Co., LTD (United States) while glucose syrup (DE42) was obtained from Corn Products Amardass Co., LTD (Thailand). Fulfil©400 as a stabilizer was purchased from SKW Biosystems Co., LTD (France), while soybean oil as a fat source in ice cream was obtained from Thai Vegetable Oil Co., LTD (Thailand).

Preparation of fresh and powdered extracts from lemongrass and pandan leaf

Extraction. The fresh extract of both herbs was prepared by blending freshly chopped lemongrass or pandan leaf with distilled water at room temperature in the ratios of 10:100, 15:100 and 20:100 (g sample per g water). The extracts were filtrated with nylon filter cloth and collected for sensory evaluation by using a hedonic test.

Spray drying. The extract was mixed with 1% (w/w) β-cyclodextrin and maltodextrin at the concentrations of 2, 4, 6, 8 or 10% (w/w). The mixtures were then spray dried at 135°C (inlet temperature) and 70°C (outlet temperature). The feed rate was fixed at 2 rpm and atomizer pressure was set at 1.5-2.0 psi (Saeli, 2006).

Preparation of soy ice cream

The ice cream mix was prepared with a portion of soy oil, soy protein isolate (SPI), sucrose, glucose syrup and Fulfil©400 as 10, 4.8, 12, 5, and 0.5% (w/w), respectively (Saeli, 2006). SPI was dissolved in water and then heated until the temperature reached 50°C. Sucrose, Fulfil©400 and glucose syrup were added into the mix with smooth agitation to dissolve all ingredients. The mix was then pasteurized at 80°C for 15 min and homogenized by 2-stage homogenizer (Kika T25 basic model, Germany) with 2,500 bar for the first stage and 500 bar for the second stage. The mix was then suddenly cooled and allowed to stand at 4°C for 24 hrs before freezing (Saeli, 2006). After freezing, the ice cream was stored at -20°C until analysis.

Sensory evaluation for the extracts

Hedonic test for fresh extract. A hedonic test with a 9-point scale (1=dislike extremely, 2=dislike very much, 3=dislike moderately, 4=dislike slightly, 5=neither like nor dislike, 6=like slightly, 7=like moderately, 8=like very much and 9=like extremely) assessed by 30 laboratory panels was used to select the ratio that had the best acceptance of odor, taste, color and overall quality (Herald et al., 2008).

Preference ranking test for powdered extract. The best result in the sensory evaluation of both herbs from fresh extract was chosen for powdering using a spray dryer. The preference-ranking test of PE evaluated by 30 laboratory panels was determined. All conditions of sensory evaluation were held at 25°C in the sensorial evaluation room.

Threshold test. Firstly, a proper concentration of fresh extract and PE was determined using a threshold test by adding various concentrations of the extracts into 4.8% (w/w) soy protein isolate (SPI) solution. Secondly, a proper concentration of both extracts was investigated by adding into ice cream. The sensorial quality was also determined using a threshold test. Twenty partially trained panelists were used to determine the sensorial quality via an ascending force choice test (AFCT). Thresholds were calculated as the geometric mean of the best-estimated threshold of each panelist (Meilgaard et al., 1999). The threshold of each extract was used as a reference concentration of the extract, which was added into soy protein isolate and ice cream for volatile compound analysis.

Volatile compound analysis

HS-SPME-GC-MS was used to determine the flavor compounds in soy protein isolate solution and ice cream with added fresh extract and PE. Five grams of the sample were weighed into a headspace vial and mixed with 1.25 g of potassium chloride. The mixed samples were put into SPME-GC-MS using fiber film composed of divinylbenzene / carboxen / polydimethylsiloxane (DVB/CAR/PDMS, Supelco Inc., Bellefonte, PA, USA) in order to absorb the volatile substances. The process was run at 60°C for 20 min. Then, the volatile compounds were transferred to GC-MS (CP-3800-MS-1200 L, Varian, Darmstadt, Germany) using DB-wax column (60 m x 0.3 mm x 0.25 μm) with injection port temperature of 200°C and split ratio of 1:10. Helium was used as carrier gas with a flow rate of 2.0 ml/min. The column temperature was set to 60°C for 1 min, and then the temperature was gradually increased to 250°C at a rate of 10°C/min. The samples were held in the column for 5 min. Desorption time for the SPME fiber injection was 20 min at 250°C. Retention times and their mass spectra were detected using probability-based matching software and were compared with the standard spectra from the National Institute of Standards and Technology (NIST) version 98 Library (Garzon et al., 2004; Manattanawee, 2008).

Statistical analysis

The fresh extract data were statistically analyzed following a Complete Randomized Design and their mean differences were tested by Duncan's multiple range test (DMRT). The data of PE were statistically analyzed by Friedman's rank test. Then, multiple comparison procedures were used for determination. SPSS program version 16.0 (SPSS, Illinois, USA) was used for all statistical analysis procedures.

RESULTS

Sensory evaluation

The selection of the best extract. The sensorial quality attributes of fresh lemongrass and pandan leaf extracts are shown in Table 1. For the fresh lemongrass extract (FLE), no difference in color was detected in any concentration. The odor, taste and overall acceptance scores of the fresh extract at the concentration of 20:100 (w/w) were significantly lower than those of both 10:100 and 15:100 (w/w) fresh extract ($p<0.05$). For the fresh pandan leaf extract (FPE), the lowest sensorial scores of all quality attributes were also found at the concentration of 20:100 (w/w). No significant difference in color and odor scores was detected in the fresh extract at the concentration of 10:100 and 15:100 (w/w) ($p>0.05$). The highest scores of taste and overall acceptance were found in the fresh extract at the concentration of 10:100 (w/w). These showed that the proper concentration of both FLE and FPE for further study was 10:100 (w/w). The physicochemical qualities of both FLE and FPE at the concentration of 10:100 (w/w) are presented in Table 2. FPE presented a green color while FLE displayed a yellow brown color. Typical aroma characteristics of each herb were detected. Total soluble solids content (TSS) of both fresh extract were low whereas water activity (a_w) and moisture content were about 0.99 and 98.9%, respectively. Due to the very low TSS content, the addition of a drying medium and flavor trapping agents such as maltodextrin and cyclodextrin was necessary.

The corresponding statistical analyses of ranking preference tests for lemongrass extract powder (PLE) and pandan leaf extract powder (PPE) are shown in Tables 3 to 5. Ranking preference scores of all PLE with different maltodextrin concentrations were not significantly different ($p>0.05$), but those of PPE were considerably different ($p<0.05$) (Table 3), since the calculated χ^2 from PLE and PPE was lower and higher, respectively, than that from the statistical table. The mean difference test using LSD confirmed that ranking preference scores of PLE were not different, but those from PPE were (Table 4). The preference rank sum of PE samples, especially PPE with 2% added maltodextrin provided the highest scores among the rest of the maltodextrin added samples (Table 5). Therefore, PE with 2% maltodextrin was selected to prepare for mixing into soy protein isolate solution and to mix in ice cream. The physical and chemical properties of both PLE and PPE mixed with 2% maltodextrin are shown in Table 6. The yields of PPE and PLE after processing were at 33.5% and 35.4%, respectively. The moisture content and aw of both powdered extracts were about 0.21-0.23% and 2.43-2.45%, respectively.

Table 1. Hedonic scores of fresh lemongrass extract (FLE) and pandan leaf extract (FPE) for each attribute.

Fresh lemongrass: Water (w/w)	Color	Odor	Taste	Overall acceptance
10:100	6.03^{ns}	6.47^{b}	5.90^{b}	6.07^{b}
15:100	6.27^{ns}	6.90^{b}	5.90^{b}	6.37^{b}
20:100	5.47^{ns}	5.67^{a}	4.57^{a}	4.90^{a}
Fresh pandan leaf: Water (w/w)	**Color**	**Odor**	**Taste**	**Overall acceptance**
10:100	6.63^{b}	6.87^{b}	3.87^{b}	5.97^{c}
15:100	6.53^{b}	5.93^{b}	2.33^{a}	4.43^{b}
20:100	5.57^{a}	4.60^{a}	1.80^{a}	3.37^{a}

Note: Different small letters in the same column in each extract are significantly different at $p < 0.05$. ns = not significant.

Table 2. Physical and chemical properties of fresh pandan leaf extract (FPE) and fresh lemongrass extract (FLE) prepared from the extraction with water at a ratio of 10:100 of herb to water.

Quality factors	FPE	FLE
Color	Green	Yellow-brown
Odor	Aroma of pandan and rank	Aroma of lemongrass
Total soluble solids (obrix)	1.21	1.45
Water activity (aw)	0.996	0.995
Moisture content (% w/w)	98.984	98.982

Table 3. Friedman's ranking test of powdered lemongrass extract (PLE) and powered pandan leaf extracts (PPE).

Parameter	PLE	PPE
Test Statistic (T)	5.73	77.84
Level of Significance (∝)	0.05	0.05
χ^2 with 3 degrees of freedom	9.49	9.49

Note: χ^2 = Chi-Square Distribution.

Table 4. Multiple comparison test using Fisher's LSD for rank sum of powdered extract.

PLE	B	C	D	E
A	11	-1	18	22
B		-11	7	11
C			19	23
D				4
PPE				
A	40*	67*	84*	109*
B		27*	44*	69*
C			17	42*
D				25*

Note: A, B, C, D and E were 2, 4, 6, 8 and 10 % (w/w) added maltodextrin, respectively. Significantly different mean if the LSD rank > 24.0

Table 5. Rank sum of powdered extracts (PEs) with different concentrations of maltodextrin.

Samples	Rank sum				
	A	B	C	D	E
PPE	150a	110b	83c	66c	41d
PLE	100ns	101ns	89ns	82ns	78ns

Note: A, B, C, D and E were 2, 4, 6, 8 and 10 % (w/w) added maltodextrin, respectively. ns = not significant.

Table 6. Physical and chemical properties of powdered pandan leaf extract (PPE) and powdered lemongrass extract (PLE) with 2% maltodextrin.

Quality factors	PPE	PLE
Total soluble solids (oBrix) before spray-drying	3.85	4.10
% Yield after spray-drying	33.49	35.44
Water activity (a_w) after spray-drying	0.21	0.23
Moisture content after spray-drying (% w/w)	2.45	2.43

Table 7. The threshold of extracts which masked the beany flavor in soy protein isolate or soy ice cream (mg/kg).

Sample	In soy protein isolate	In soy ice cream
FLE	224.75	339.30
PLE	82.00	327.91
FPE	265.65	147.60
PPE	895.40	458.15

Note: Different small letters in the same column are significantly different at $p < 0.05$. Different capital letters in the same row are significantly different at $p < 0.05$.

Threshold test. The threshold is defined as the lowest concentration at which a sensory response is detectable (Lawless and Heymann, 1999; Meilgaard et al., 2007). As the results shown in Table 7 confirm, the threshold values which could hide the beany flavor for FLE, PLE, FPE and PPE in SPI solution were 224.8, 82.0, 327.9 and 265.6 mg/kg, respectively, and the threshold values which suppressed the beany flavor for FLE, PLE, FPE and PPE in soy ice cream were 339.3, 147.6, 895.4 and 458.2 mg/kg, respectively. The data of the threshold study showed that PLE provided the lowest concentration that masked the beany flavor in the SPI solution and soy ice cream (Table 7).

Volatile compounds. The HS-SPME-GC-MS results showed that the major compounds detected in SPI were pentanal, hexanal, 2-pentyl-furan, 1-octen-3-ol and benzaldehyde (Figure1(a) and Figure 2(a)). The major compounds contributing to the characteristic flavor of lemongrass were β-myrcene, α-pinene, 3-carene, neral, geranial and geraniol (Figure 1(d), 1(e) and Figure 2(d), 2(e)). The major compounds contributing to the characteristic flavor of pandan leaf were 2-acetyl-1-pyroline (2AP) and 3-methyl-2(5H)-furanone (Figure 1(b), 1(c) and Figure 2(b), 2(c)).

DISCUSSION

The highest hedonic scores of taste and overall acceptance for both herbal extracts were at 10:100 (w/w) herb to water. However, the hedonic scores in FLE were higher than those of PLE. Jather (2010) and Sasidharan et al. (2011) reported that pandan leaf extract provided an astringent taste, which consisted of high alkaloids, glycosides and tannin content, resulting in lower acceptance at high concentrations. They also found that these compounds in lemongrass extract existed in lower quantities than those in pandan leaf extract.

This showed that FLE, with its small amount, was the best for hiding the beany flavor in this study, while the fresh extract from pandan leaf (FPE) was the worst choice to hide the beany flavor in SPI solution, but better than fresh pandan leaf extract (FPE) in ice cream. Higher concentrations of all extracts must be used with ice cream compared to SPI solution, since ice cream is a highly complex food matrix, containing proteins, fat, sugar, air, minerals, etc., which might interfere with and absorb the flavor from the extracts (Hatchwell, 1996). This resulted in a smaller release of flavors for panelists to detect. The flavor of products like ice cream depend on a fine balance between the release and the retention of volatile compounds, which, in turn, is controlled by the reversibility of binding of the volatile compounds to non-volatile food ingredients under defined conditions (Gkionakis, Anthony-Taylor, Ahmad and Heliopoulos, 2006). This might be the reason why addition of extracts in ice cream need to be at higher concentrations than in SPI solution.

The HS-SPME-GC-MS results showed that the major compounds detected in SPI were pentanal, hexanal, 2-pentyl-furan, 1-octen-3-ol and benzaldehyde (Figure1(a) and 2(a)). This was similar to Friedeck et al. (2003). Suratman et al.

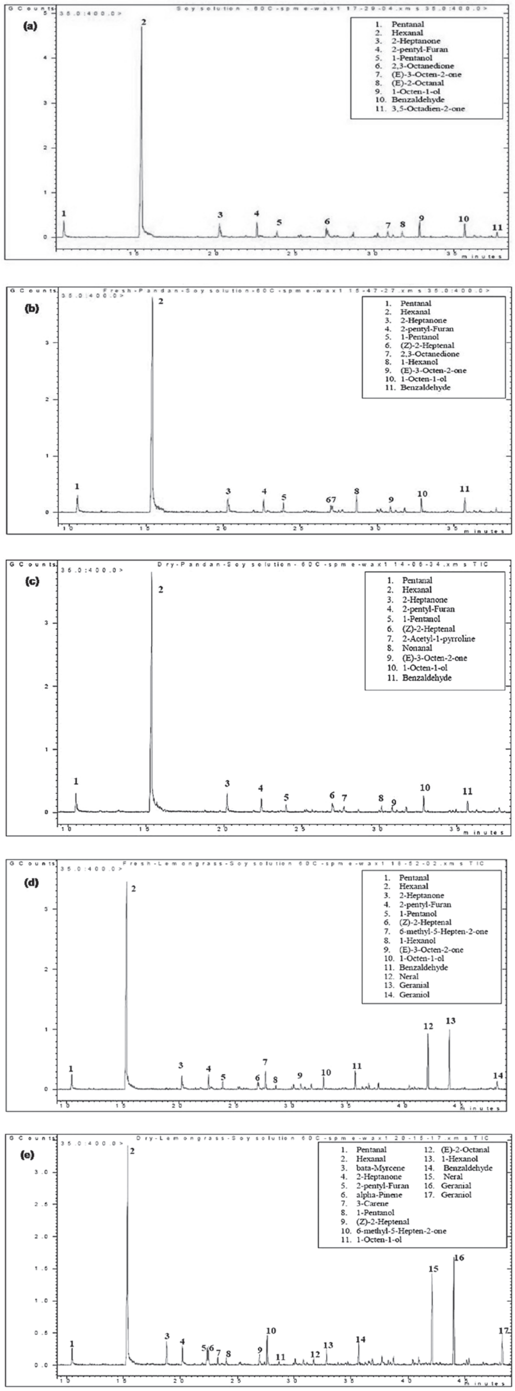

Figure 1. Volatile compounds from the threshold test in soy protein isolate solution effected by the addition of: nothing (a); fresh pandan extract (b); powdered pandan extract (c); fresh lemongrass extract (d); and powdered lemongrass extract (e).

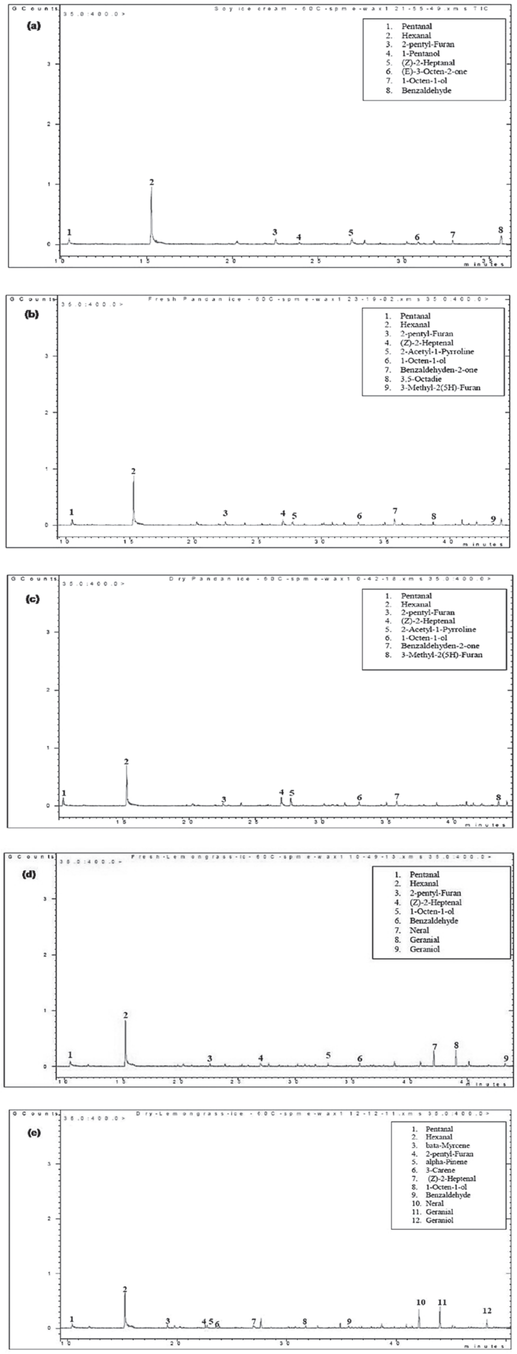

Figure 2. Volatile compounds from the threshold test in soy ice cream effected by the addition of: nothing (a); fresh pandan extract (b); powdered pandan extract (c); fresh lemongrass extract (d); and powdered lemongrass extract (e).

(2004) reported that hexanal was the major substance for enhancing the beany flavor in SPI solution. The aromatic characteristic of hexanal was described as green or grassy, rancid, painty, and with a bitter flavor (Nelson et al., 1976; Kobayashi et al., 1995; Torres-Penaranda, 2001). Moreover, the primary off-flavor precursor in soybeans that gives the beany flavor is linoleate. This compound can change to hexanal and linolenate, inducing more off-flavor in food products (Kobayashi et al., 1995). Addition of PEs into SPI solution or soy ice cream decreased hexanal content and increased β-myrcene, α-pinene, 3-carene, neral, geranial, geraniol, 2-acetyl-1-pyroline and 3-methyl-2(5H)-furanone, indicating that the extracts potentially suppressed the beany flavor in these samples. However, the interaction between the flavor compounds in PEs and macromolecules in food, including proteins, lipids and carbohydrates, could mask the beany flavor in samples containing PEs. This resulted in entrapping the beany flavor in food metrics via the formation of covalent, hydrogen, electrostatic and hydrophobic bonds that ultimately altered the proportion of flavor chemicals in food (Sucan, 2004).

In our study, PEs masked the beany flavor more than fresh extracts. Moreover, β-cyclodextrin and maltodextrin entrapped various small flavor compounds, leading to the elimination or modification of food flavor profiles in the samples (Kant et al., 2004; Suratman et al., 2004). Kim (2002) suggested that cyclodextrins could entrap many low-molecular weight compounds, such as soybean flavor. According to our results, the powder form of the herbal extract had more potential to suppress the beany flavor than the liquid form. This is probably due to the synergistic effect between the chemical compounds in the herbs and in the maltodextrin that was used in spray drying.

CONCLUSIONS

The beany flavor in soy protein isolate solution and soy ice cream could be masked by using lemongrass and pandan leaf extracts. This satisfied the sensorial acceptance and preference of panelists. Headspace solid-phase microextraction, gas chromatography, mass spectrophotometry was a useful and reliable technique to confirm the results from sensory evaluations of hiding the beany flavor by such extracts. However, based on statistics, a study of the relationship between the sensorial results and chemical analysis should be more thoroughly investigated in future studies.

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