

Food and Applied Bioscience Journal





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Bitter-masking property of pea eggplant (Solanum torvum Sw.) fruit extract

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Abstract

Bitterness is an unpleasant attribute which influences consumer food choice. There is an attempt to eliminate the bitterness of food by several techniques. Pea eggplant (Solanum torvum Sw.) fruit has been consumed to reduce the bitter taste of some herbs but there is limited scientific research on this masking characteristic. Therefore, the effect of pea eggplant fruit extract on bitterness reduction of caffeine 654was determined in this study. The results indicated that the bitterness intensity of caffeine solution was significantly decreased (P≤0.05) immediately after tasting the water extract of pea eggplant fruit and the bitterness reduction efficiency in 5 mM and 10 mM of caffeine solutions were 56.1% and 36.3%, respectively. However, the complex formation of caffeine and any components in the extract was not observed by HPLC analysis. The application of pea eggplant fruit extract was further evaluated in various bitter foods including black tea, pomelo juice, and boiled bitter melon. The bitterness aftertaste of 2% (w/v) KCl aqueous solution was also assessed. The sensory results revealed that the extract could reduce bitterness intensities of all samples (P≤0.05) and the efficacy of bitterness reduction ranged from 25.8% to 32.3%.

Keywords: Flavor, tastant, pea eggplant (*Solanum torvum Sw.*), bitterness reduction, masking agent

1. Introduction

Taste is vitally important in food choice and acceptance. For example, human normally like sweet taste, while bitter food is frequently unpalatable and tends to be rejected from consumer. Bitterness was linked to the basic emotion of disgust (Schienle et al., 2015). It was also reported as an indicator of some food toxicities (Glendinning, 1994) for example, spoiled meat (Jones, 1969) and some poisonous berries (Schwab, 2017). Therefore, the manufacturers need to handle unpleasant taste in their food product. Although the bitter substances are necessary to be removed by using several techniques such as ion exchange (Sohi et al., 2004) or adsorption technique (Avenew et al., 2009), some have been reported as phytonutrients. Various bitter phenolic compounds, triterpenes, and organosulfur compounds are bioactive components and contain pharmacological properties. For example, 730 mg quercetin supplementation daily for 28 days could reduce arterial pressure in stage 1 hypertensive patients (Edwards et al., 2007). The incidence of colonic adenocarcinoma in rats was decreased after receiving 0.05% (w/w) limonin diet (Morishita et al., 1998). Sinigrin, an aliphatic glucosinolate found in Brassicaceae plant, could inhibit the proliferation of rat liver tumor cells (Tanaka et al., 2000). In addition, potassium chloride (KCl) is often used as sodium chloride (NaCl) substitute to reduce sodium intake in low-sodium food, but it has a limitation of use because of bitterness aftertaste. Thus, it is necessary to reduce the bitter taste without removing the bitterness bioactive compound such as using masking technique. There are many publications regarding bitterness suppression method which can be categorized into three actions. Coating is the most popular technique used to mask the bitter taste. The bitter substance can be formed the complex or can be encapsulated by wall materials such as hydrophobic polymers, hydrophilic polymers, lipids, or sweeteners to reduce the release of tastant in the oral. For example, cyclodextrin was used to form the complex with caffeine in the solution (Binello et al., 2004). The bitterness of dichlofenac sodium and paracetamol was masked by cationic copolymer (Maungthaingam et al., 2018) and precirol using fluidized hot melt granulation process (Masic et al., 2012), resplectively. The second technique is associated with saliva which is a carrier between tastant and taste receptor. High saliva viscosity decreases the tastant diffusion coefficient according to the Noyes-Whitney dissolution model which resulted in the reduction of the tastant dissolution rate (Banakar, 1992). Using taste suppressant is another technique to mask the bitterness by association with taste receptor (Sagar et al., 2012). For instance, NaCl solution reduced the bitterness of L-tryptophan and L-phenylalanine (Keast et al., 2001), quinine-hydrochloride (QHCl) (Keast, et al., 2002) as well as limonin and naringin in pomelo albedo (Pichaiyongvongdee and Rattanapun, 2015 Sweeteners, such as aspartame and sucralose, could also decrease the bitter taste of pharmaceutical drugs (Suzuki et al., 2004).

In addition, valerian (*Valeriana officinalis*) root extract could be used to mask perceived bitter taste of cocoa-based foodstuff (Gregory, 2016). Similarly, there is Thai indigenous knowledge using pea eggplant (*Solanum torvum Sw.*) fruit to minimize the bitterness perception of food. The bitterness intensity of guduchi (Tinospora cordifolia) is decreased after consuming that fruit. The addition of pea eggplant fruit in cassia curry could also reduce the bitterness of cassia leaves (Santasombat, 1999). However, there is no scientific proof on this property of pea

eggplant fruit. Therefore, the objectives of this study were 1) to study the effect of pea eggplant fruit extract on bitterness reduction of caffeine and 2) to study the application of pea eggplant fruit extract on bitterness reduction in various bitter foods and food ingredient.

2. Materials and Methods

2.1 Materials

Agricultural products used in this study were harvested at commercial maturity during January to February 2018. Pea eggplant fruit was obtained from the local supermarket in Bangkok, Thailand. It was ground and extracted with drinking water in a 1:10 (w/v) ratio by shaking at 200 rpm (New Brunswick Scientific, Edison, NJ, USA) for 30 min at room temperature. The mixture was filtered through a cotton cloth to collect a pea eggplant fruit extract and kept in refrigerator until analysis.

Black tea (3horsestea Co., Ltd., Bangkok, Thailand) and bitter melon were purchased from the local supermarket in Bangkok, Thailand. Pomelo was obtained from the orchard in Samut Songkhram province, Thailand. The food–grade of caffeine and potassium chloride (KCl) were supplied from Sigma–Aldrich (St. Louis, MO, USA) and Srichand United Dispensary Co., Ltd. (Bangkok, Thailand), respectively.

2.2 Methods

2.2.1 Property of pea eggplant fruit extract on bitterness reduction of caffeine

2.2.1.1 Sensory evaluation by rating test.

Fifty panelists (18 males and 32 females, between 20 to 40 years old) were selected based on their good health, time availability and willingness to participate. They were semi-trained to familiarize with scale and sensory test procedure according to the literature (Meilgaard et al., 2007). The participants were asked to evaluate two tested samples including 1) 5 mM caffeine aqueous solution and 2) this caffeine solution immediately after tasting pea eggplant fruit extract. These two tested samples were randomly served in a balanced monadic sequential order. The participants were requested to clean their palate with green apple a drinking water before testing each tested sample. Ten milliliters of caffeine aqueous solution and the extract were presented at room temperature in 2-oz plastic cup covered with aluminum foil. The caffeine solution was labelled with three digits code, while the extract was masked as pea eggplant fruit extract. The panelists rated the bitterness intensity of caffeine solution using a category scale (0 = none, 2 = slight, 4 = moderate, 6 = strong, and 8 = extreme). A paired-sample *t*-test was analyzed by using SPSS (version 16, IBM, North Castle, NY, USA). and the percentage of bitterness reduction was calculated according to the equation (1).

$$\% Reduction = \frac{I_C - I_{CA}}{I_C} \times 100$$
 (1)

when: %Reduction = percentage of bitterness reduction

I_C = bitterness intensity of caffeine solution

= bitterness intensity of caffeine solution immediately after

tasting pea eggplant fruit extract

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The bitterness reduction of 10 mM caffeine immediately after tasting pea eggplant fruit extract was also evaluated in the same manner.

2.2.1.2 Sensory evaluation by 3-alternative forced choice (3-AFC) test.

This sensory analysis was conducted to test the difference between the various concentrations of caffeine solutions which obtained from the quantitative chemical analysis.

Chemical analysis. High performance liquid chromatography (HPLC) was applied to check the free caffeine content in the mixture of caffeine aqueous solution and pea eggplant fruit extract. Four milliliters of caffeine solution (100 µg/mL) was added with 0, 1, 2 or 4 mL of pea eggplant fruit extract to obtain 1:0 (as control sample), 1:0.25, 1:0.5 or 1:1 (v/v) ratio, respectively. Four milliliters of pea eggplant fruit extract without caffeine solution was also used as a blank. The final volume (10 mL) was adjusted by deionized water and then passed through the 0.45-μm membrane-filter (Minisart® RC 15; Sartorius, Hannover, Germany). One microliter of each sample was injected into a HPLC (Waters 600, Milford, MA, USA) equipped with C18 reversed-phase column (250 nm × 4.6 mm × 0.25 μm) (Agilent Technologies, Middlesex County, MA, USA) by means of an autosampler. Caffeine was eluted under isocratic condition with 25% (v/v) methanol and 75% (v/v) water at 1 mL/min flow rate with a run time of 15 min. The absorbance of caffeine was monitored at 250 nm using a diode array detector (DAD) (Waters 2998, Milford, MA, USA). The free caffeine concentration was calculated from the calibration curve of standard caffeine and the independent two-sample *t*-test of three replications was performed to indicate the difference between each mixture and the control sample.

Sensory analysis. The caffeine aqueous solution was then prepared at the concentrations of 97, 96, and 95 $\mu g/mL$ which were equal to the free caffeine in the mixtures. Each tested solution was compared with the control sample (100 $\mu g/mL$) by 3–AFC test. The panelist was received three samples consisted of one control sample and two identical tested samples. Ten milliliters of each sample were prepared in 2–oz plastic cup covered with aluminum foil and labelled with different random three–digit code. The samples were served at room temperature in balanced random order along with white bread and drinking water as palate cleansers. Twenty panelists (7 males and 13 females, between 20 and 40 years old) were asked to choose the most bitter sample and the data was analyzed by binomial test.

2.2.2 Application of pea eggplant fruit extract in various bitter foods and food ingredient

Black tea, pomelo juice, boiled bitter melon, and 2% (w/v) KCl aqueous solution were used to determine the bitterness reduction efficacy of pea eggplant fruit extract. Black tea leaves (20 g) were infused in 100 mL of hot water for 45 min. Peeled and seeded pomelo fruit was blended and pressed to obtain juice. Bitter melon was cut into 1 cm × 2 cm, boiled for 2 min and cooled down immediately. Ten milliliters of black tea, pomelo juice, and 2% (w/v) KCl aqueous solution and three grams of boiled bitter melon were served to panelists in the plastic cup labeled with a random three digits codes. The bitterness intensity of each sample compared with those of sample immediately after tasting pea eggplant fruit extract was rated

by the panel (18 males and 32 females, between 20 to 40 years old) as described in the section 2.2.1.1.

3. Results and Discussion

3.1 Property of pea eggplant fruit extract on bitterness reduction of caffeine

Caffeine was chosen as a representative of bitter substance in this experiment. The panelists were asked to compare the bitterness intensities of caffeine aqueous solution and this caffeine solution immediately after tasting pea eggplant fruit extract. The results indicated that the bitterness intensities of 5 mM and 10 mM caffeine solutions were significantly decreased (P≤0.05) after tasting the extract (Table 1). The bitterness reduction efficiency of pea eggplant fruit extract in 5 mM caffeine solution was higher than those in 10 mM caffeine solution. The capacity of pea eggplant fruit extract to decrease the bitterness of caffeine solution was up to 56.1%, while Ley et al. (2005) reported 40% bitterness reduction of 500 ppm (2.6 mM) caffeine solution by monosodium salt of homoeriodictyol extract from Yerba Santa (Eriodictyon californicum). The bitterness reduction property of pea eggplant fruit extract in this study might be because of some active compounds in the fruit. Since eggplant family was rich in pectin (Kazemi and Hosseini, 2019), this hydrocolloid was primary considered. The scientists (Banakar, 1992) reported that increase saliva viscosity could cause binding limitation between bitterness compound and taste receptor. In addition, Zeeb et al. (2018) reported that bitterness of pea protein, potato protein and whey protein solutions were decreased by addition of apple pectin. Ley et al. (2008) also observed slow release of bitter substance from high viscosity model. However, the panelists could not recognize any change of saliva viscosity after tasting the fruit extract. Thus, there were two possible actions of bitterness reduction including the interaction between active compounds and caffeine molecule or the binding of the compounds to the bitter receptors.

Table 1 Bitterness intensities of caffeine solution and the caffeine solution immediately after tasting pea eggplant fruit extract.

<u> </u>			
Cample	Bitterness	P-Waluo	Reduction
Sample	intensitya	1 – v arue	Reduction
5 mM caffeine solution	3.42 ± 1.11		
5 mM caffeine solution immediately after	1.50 ± 0.89	< 0.001	56.14%
tasting pea eggplant fruit extract	1.50 ± 0.69		
10 mM caffeine solution	4.08 ± 1.23		
10 mM caffeine solution immediately after	2.60 ± 1.43	< 0.001	36.27%
tasting pea eggplant fruit extract	∠.00 ± 1.43		

 $^{^{}a}$ Used a 9-point category scale: 0 = none, 2 = light, 4 = moderate, 6 = strong and 8 = extreme (n = 50).

HPLC was further applied to determine the free caffeine in the mixture of caffeine solution and pea eggplant fruit extract. The results showed a little small amount reduction of free caffeine in all ratio mixtures (Table 2). Since the extract (blank sample) did not contain the free caffeine (Fig 1a), decrease of free caffeine in

the mixture could imply 1) the complex formation of caffeine molecule and the active compounds in pea eggplant fruit or 2) the occurrence of chemical reaction. For example, Lin *et al.* (2019) reported the unknown peak in HPLC chromatogram which was the result of the reaction between amino group of pregabalin (the active pharmaceutical ingredient) and cyano group of acetonitrile (the mobile phase). However, there was no statistically significant difference (P>0.05) of free caffeine content between the mixture and the control (Table 2) and any new peak was not observed in the mixture chromatogram (Fig 1c). In addition, the panelists could not distinguish the difference of bitterness intensity between caffeine solution and the control sample by 3–AFC (Table 3). Therefore, the possibility of bitterness reduction property of pea eggplant fruit might occur at the taste receptor. Ley *et al.* (2005) speculated that binding of Yerba Santa flavanones to allosteric sites on receptors was a reason of bitterness reduction of caffeine. Hence, the bitterness–masking agent in pea eggplant fruit is currently being identified and the binding to taste receptor will be further investigated.

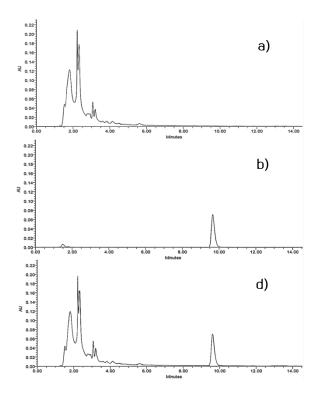


Fig 1 Chromatograms of a) the pea eggplant fruit extract b) the caffeine solution and c) the mixture of pea eggplant fruit extract and the caffeine solution at a 1:1 ratio

Table 2 Free caffeine contents in caffeine solutions with and without pea eggplant fruit extract quantified by HPLC.

Caffeine solution: pea eggplant fruit extract	Concentration of free caffeine (µg/mL)	P-Value ^a
caffeine solution (control sample)	100.26 ± 5.78	-
mixture at the 1:0.25 ratio	97.03 ± 7.93	0.571
mixture at the 1:0.5 ratio	96.73 ± 6.11	0.720
mixture at the 1:1 ratio	95.82 ± 2.87	1.192

^a Indicated the difference of the mixture from the control sample by t-test (n = 3).

Table 3 Three–alternative forced choice (3-AFC) response on bitterness of caffeine solution at different concentrations (n = 20).

Tested sample compared with control sample (100 µg/mL)	% Correct response (3–AFC)	P-Value
95 μg/mL	25	0.848
96 μg/mL	30	0.702
97 μg/mL	30	0.702

^a Obtained from HPLC analysis which corresponded to Table 2.

3.2 Application of pea eggplant fruit extract in various bitter foods and food ingredient

Black tea, pomelo juice, and bitter melon were chosen in this study as the representative of various chemical groups of bitter substances in food. The panelists were asked to rate the bitterness of food sample and the food sample immediately after tasting pea eggplant fruit extract. The results indicated that the bitterness intensities of all food samples were significantly decreased (P≤0.05) after tasting the extract (Fig 2a). Therefore, the pea eggplant fruit extract could apply to reduce the bitterness of various chemical groups. The key bitter compounds in pomelo juice are limonoids (triterpenes) such as limonin, nomilin, and naringin (Drewnowski and Gomez-Carneros, 2000; Dea et al., 2013). The bitterness of black tea causes by the flavonoids (phenolic compounds) such as quercetin, catechin, epicatechin, epicatechingallate, epigallocatechin, and epigallocatechingallate (Hodgson and Croft, 2010), while the bitterness of bitter melon is contributed by alkaloid momordicine (Fang and Ng, 2011). Moreover, the application of pea eggplant fruit extract on bitterness reduction was more diverse than using other masking agent. For example, glutamic acid could reduce the bitterness of caffeine, quinine, L-valine, and L-tryptophan solutions but was not effective on naringin and limonin (Warmke and Belitz, 1993). However, the bitterness reduction efficiency of pea eggplant fruit extract in food samples (Fig 2b) was lower than those in caffeine aqueous solution (Table 1). The complex of food matrix and the chemical structure of bitter substance might be the reasons. Guadagni et al. (1973) reported that the complex of orange juice affected the bitter perception of limonin. The threshold of limonin in aqueous solution was increased 3-fold when adding with neodiosmin, while this substance could increase the threshold of limonin only 1.8-fold in the orange juice (Guadagi et al., 1973). In addition, the pea eggplant fruit extract had ability to decrease the bitter aftertaste of 2% KCl. This salt is the most popular sodium salt substitute but there is a limitation usage because of its

aftertaste. Thus, using pea eggplant fruit extract might be the potential substance to mask the bitterness of KCl in low-sodium food.

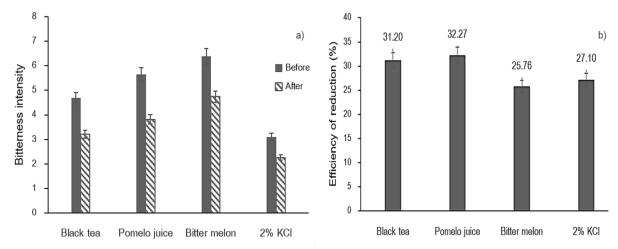


Fig 2 a) Bitterness intensity and b) efficiency of bitterness reduction in food samples after tasting of pea eggplant fruit extract *Significantly decreased (P≤0.05)

4. Conclusion

Pea eggplant fruit extract can reduce the bitterness of caffeine and it has higher efficiency in low concentration compared to the high concentration of caffeine. In addition, the extract can be applied as a bitterness masking substance in various food samples including black tea, pomelo juice, bitter melon, as well as KCl. This research provides an interesting point for future investigation that will be very useful for food industry.

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