Potential of Szechuan pepper as a saltiness enhancer
Tram Hong Le Bao, Siree Chaiseri, and Yaowapa Lorjaroenphon

Department of Food Science and Technology, Faculty of Agro-Industry, Kasetsart University, Bangkok, Thailand

ABSTRACT
The flavor profile, flavor enhancement, and saltiness modulation of Szechuan pepper (Zanthoxylum simulans) were analyzed to evaluate its effectiveness in lowering salt usage. The saltiness enhancing efficiency of Szechuan pepper and its salt-reduction properties were determined to be 28.74% and 22.32%, respectively. The saltiness enhancers were detected in the polar fraction and taste dilution analysis combined with half-tongue test determined the most potent saltiness enhancing components in chromatographic subfractions. Both spectroscopic analysis and sensory evaluation disclosed NaCl as the unexpected primary contributor of saltiness enhancement in this spice. It is the first known study revealing Szechuan pepper’s high salt content.

ARTICLE HISTORY
Received 28 July 2017
Accepted 5 January 2018

KEYWORDS
Half-tongue test; Szechuan pepper (Zanthoxylum simulans); Saltiness enhancer; Taste dilution analysis; Sodium chloride

Introduction
The global overconsumption of salt is widely recognized for contributing to hypertension and cardiovascular disease.[1,2] Strazzullo et al. (2009)[3] also concluded that high salt intake increases stroke risk. Therefore, an effective saltiness enhancer which reduces consumers’ desire for additional salt would undoubtedly prove beneficial to health worldwide. There are three strategies for reducing salt intake: consumer awareness of salt-related health risks, product-structural modification, and chemical stimulation for increased food flavor.[4] The first two strategic elements would likely lower certain product’s sales as consumers either switch to low-sodium diets or no longer enjoy the flavor of products with reduced salt. The ultimate solution is to increase consumers’ perception of food saltiness and overall flavor.

Physical applications have been introduced to enhance saltiness perception. Saltiness intensity of sodium chloride solution increased with the presence of a weak cathodal current to the tongue[5] or by reducing solution temperature.[6] At a more practical level for consumables, several chemical compounds are capable of enhancing the perception of saltiness and is therefore, the preferred method in improving food taste while minimizing sensory quality. The mechanisms involved that enhance saltiness is still under investigation as the biology of saltiness perception remains incomplete. Some pathways, however, have been discovered. Methyl xanthenes heightened saltiness among humans by blocking the receptors of the relaxant adenosine.[7] Bretylium tosylate increased the number of open epithelial Na\(^+\) channels which enhanced saltiness.\(^{[8,9]}\) Condiments and other food ingredients have also been studied regarding the saltiness enhancing characteristic. Both soy sauce and meat broth containing amino acids and esters, such as ornithyl-β-alanine,\(^{[10]}\) glycine methyl,\(^{[11]}\) and alapyridaine\(^{[12]}\) provided not only saltiness but also enhanced its effect. Szechuan pepper (Zanthoxylum simulans), when mixed with chili, lowered the amount of salt desired for flavor as well.\(^{[13,14]}\) Rubemamine, rubescenamide and zanthosinamide in Zanthoxylum rubescens were patented to potentiate tastes in a mixture of umami, kokumi, and salty.\(^{[15]}\) The combination of rubemamine and monosodium glutamate (MSG) also

CONTACT Yaowapa Lorjaroenphon fagiypl@ku.ac.th Department of Food Science and Technology, Faculty of Agro-Industry, Kasetsart University, 50 Ngamwongwan Road, Chatuchak, Bangkok, 10900, Thailand

Color versions of one or more of the figures in the article can be found online at www.tandfonline.com/ljfp

© 2018 Tram Hong Le Bao, Siree Chaiser, and Yaowapa Lorjaroenphon. Published by Taylor & Francis. This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.
increased the saltiness intensity of beef extract.[16] However, there is limited information regarding Szechuan pepper on the overall matter of perceived saltiness. Prior to this research, Szechuan pepper salt-enhancing components had neither been isolated nor structurally identified. Additionally, the worldwide popularity of exotic spices for food flavoring prompts further investigation regarding its effectiveness and mechanisms in taste perception. The aim of this study was to identify the saltiness enhancers in Szechuan pepper via chromatography combined with taste dilution (TD) analysis. The research outcomes could lead to reduced salt content in foods, lower sodium consumption in consumers, and therefore lower the global risk of sodium-induced diseases.

Materials and methods

Four Thai brands of Szechuan pepper (A, B, C, and D) were purchased from local markets (Bangkok, Thailand). Two Chinese brands (E and F) were collected from Szechuan, China. Samples A, B, and C were leading retail brands and sample D was a top wholesale brand. Sample A was used for the entire study. Only the pericarp was used in this research after removing the black seeds. The foods and references used in sensory evaluation were obtained from a local supermarket. The solvents for chromatographic separation were high-performance liquid chromatography (HPLC) grade. The sensory evaluation in this research was approved by the Kasetsart University Research Ethics Committee (code number COE60/002).

Analysis of flavor properties

Evaluation of flavor profile

Szechuan peppers obtained from different manufacturers and forms (powder, semi-ground, and whole) were used for the training session following the same procedure as Meilgaard et al. (2007).[17] The semi-ground and whole samples were powdered via blender. Individual samples were added to drinking water (2 g/100 mL) and the mixtures were filtered to collect homogeneous clear solutions. The samples in both powder and solution forms were served to a panel in 2-oz capped white disposable plastic cups labelled with random 3-digit codes. The panel was first asked to evaluate the aroma of powder samples and then to detect the taste and flavor of the solution while using nose clips. The palate cleaning process was carried out using white bread and drinking water. Sensory descriptive terms were developed and defined. Reference samples were also selected according to the panel’s suggestion and agreement. The intensities were rated on a typical 15-cm line scale (0 = none and 15 = extreme) according to the literature.[17] Once the attributes (Table 1) were established and reproducibly applied for all flavor characteristics, the testing session began. Sample A was prepared and provided to the panelists in the same manner. The 10 panelists (3 males and 7 females, between 22 and 34 years old) scored all attributes in individual booths. Evaluations were performed in duplicate on different days. Attribute means were illustrated using a radar chart.

Evaluation of food flavor enhancement

Food samples including fried potato stick, chicken broth, chicken creamy soup, and pasta sauce were used to observe flavor enhancement by Szechuan pepper. All samples were prepared according to manufacturer instructions. Ground Szechuan pepper A was added to reach 1% (by weight) in all samples. Ten grams of sample was prepared in 6-oz capped white disposable plastic cup labeled with a random 3-digit code. The pasta sauce was served with 10 g of pasta. Samples with and without Szechuan pepper were served in balanced random order to 30 untrained panelists (10 males and 20 females, between 22 and 40 years old). The panel selection and rating test were conducted according to the literature.[17] The panel rated the overall food flavor intensity using a 9-point category scale (0 = none, 2 = light, 4 = moderate, 6 = strong, and 8 = extreme). A 2-min forced break was given between samples and a cleaning palate process was performed to reduce carryover effect. Paired
- A test using SPSS (IBM, version 16) was carried out to compare the intensities of food samples with and without the addition of Szechuan pepper.

**Investigation of saltiness enhancing property**

**Evaluation of saltiness enhancing characteristic**

The saltiness enhancing ability of Szechuan pepper was measured for fried potato sticks via a 2-alternative forced choice (2-AFC) test using 30 untrained panelists (10 males and 20 females, between 22 and 40 years old) following method by Kroeze.\(^{[18]}\) Frozen potato sticks were deep-fried in vegetable oil and seasoned with 1% salt (NaCl) (Thai Refined Salt, Bangkok, Thailand) and/or 1% ground Szechuan pepper. Panelists wore nose clips during tasting in darkened individual booths. A cleaning palate process was performed before testing each sample. The saltiness intensity of fried potato sticks with a 1% salt was compared to those with the combination of 1% salt and 1% Szechuan pepper. Another 2-AFC test compared unseasoned potato sticks with potato sticks seasoned with 1% pepper. The significant differences of saltiness intensity in both tests were assessed using a chi-squared analysis.

**Determination of saltiness enhancement level**

A 2-step rating test\(^{[19]}\) was performed to assess the level of saltiness enhancement in fried potato sticks. First, 11 trained panelists (3 males and 8 females, between 22 and 34 years old) worked on four randomly coded samples of seasoned potato sticks of different salt concentrations (0.5%, 1.0%, 1.5%, and 2.0%). The panelists rated the saltiness intensity of the randomly served samples on a 9-point category scale (0 = none, 2 = light, 4 = moderate, 6 = strong, and 8 = extreme). The mean intensities of

---

**Table 1. Descriptive attributes of Szechuan pepper A.**

| Attribute   | Definition                                                                 | Reference                                                                 | Intensity* |
|-------------|---------------------------------------------------------------------------|---------------------------------------------------------------------------|------------|
| Aroma       | Woody: Aroma sensation with dry wood or bark                              | 1 g of Ice cream wooden stick                                             | 12         |
|             | Camphor: Aroma sensation with camphor which obtained from camphor tree or pine | 1 g of 100% Camphor powder                                                | 12         |
|             | Citrus peel: Aroma sensation with peel of citrus fruit                    | 1 g of fresh-cut kaffir lime peel                                         | 12         |
|             | Clove: Aroma sensation with dried clove bud                               | 0.5 g of Clove powder (Cooking for Fun; Central Food Retail Co., Ltd., Thailand) | 11         |
| Taste       | Salty: Taste sensation associated with sodium chloride                     | 5 mL of NaCl aqueous solution (Prungthip; Thai Refined Salt Co., Ltd., Thailand) | 10         |
|             | Bitter: Taste sensation associated with caffeine                           | 5 mL of Caffeine aqueous solution (Sigma-Arich, MO, USA)                  | 10         |
|             | Astringent: Shrinking or puckering of the tongue surface and associated with tea | 5 mL of Tea (2 g of tea soaked in 100 mL hot water for 1 h) (Lipton; PT Unilever Indonesia Tbk, Indonesia) | 8          |
|             | Numb: Impermanent loss of sensation or lack of feeling                     | 5 mL of Clove powder in water (0.2 g/100 mL) (Nguan Soon; Artchit International Pepper and Spice Co., Ltd., Thailand) | 9          |
|             | Cooling: Cool sensation in the mouth produced by substances associated with menthol or mint | A piece of chewing gum (1.7 g) (Double Mint; William Wrigley Jr., Thailand) | 9          |
|             | Burnt-aftertaste: Burning aftertaste sensation in the mouth associated with black pepper | 5 mL of Grounded black pepper in water (0.02 g/100 mL) (Nguan Soon; Artchit International Pepper and Spice Co., Ltd., Thailand) | 3          |

*Rated on a 15-cm line scale: 0 = none and 15 = extreme (n = 10).
different salt concentrations were plotted as a calibration curve. Fried potato sticks seasoned with the combination of 1% salt and 1% Szechuan pepper were presented to the panel in the second step. The panel rated saltiness intensity using the same category scale. The estimated salt concentration was calculated using a linear equation of the calibration curve. Saltiness enhancement efficiency and salt reduction were assessed by the Eqs. (1) and (2), respectively.

$$\text{Saltiness enhancement efficiency (\%)} = \frac{x_2 - x_1}{x_1} \times 100$$  \hspace{1cm} (1)

$$\text{Salt reduction (\%)} = \left(\frac{x_2}{x_1} - 1\right) \times 100$$  \hspace{1cm} (2)

Where $x_1$ was the percentage of salt used (1%) and $x_2$ was the estimated percentage of salt according to the calibration curve.

**Identification of saltiness enhancing compounds**

**Sequential solvent extraction**

Szechuan pepper A was extracted by a series of solvents adapting from the procedure of Hufnagel and Hofmann (2008).\textsuperscript{[20]} Sixty milliliters of distilled water was added to 20 g of Szechuan pepper. Non-polar extract was obtained by using $n$-pentane (RCI Lab-scan, Bangkok, Thailand) (4 times × 20 mL) with a shaker (G24; New Brunswick Scientific, NJ, USA) at 200 rpm for 30 min at room temperature, followed by a centrifuge (Rotofix 46; Hettich Zentrifugen, Nevada, USA) at 5,000 rpm (4,844 × g) for 5 min. The sample was further extracted via ethyl acetate (EtOtAc) (Fisher Scientific, Loughborough, UK) (4 times × 20 mL) followed by water (4 times × 20 mL) using the above procedure to collect semi-polar and polar fractions, respectively. All fractions were evaporated at 60°C, 72 mbar (Rotavapor R-114; Buchi, Flawil, Switzerland) and freeze-dried (Heto FD 2.5; Heto-Holten, Allerod, Denmark). The headspace solid-phase microextraction (HS-SPME) coupled with gas chromatography-flame ionization detection (GC-FID) (HP-6890; Agilent Technologies, MA, USA) was performed to ensure that the dried samples were free from any solvents. Samples were prepared in 20-mL vials and incubated at 45°C for 10 min. SPME fiber, 50/30 µm DVB/CAR/PDMS (Sigma-Aldrich, MO, USA), was used for 20 min to absorb the volatiles and was desorbed at 250°C of GC injection port. The volatiles were separated on a HP-5 column (30 m × 0.32 mm × 0.25 µm) (Agilent Technologies, MA, USA). The oven temperature was programmed to begin at 35°C and increase 10°C/min to 250°C. The initial and final hold times were 5 and 5 min, respectively. Helium was used as a carrier gas at a constant flow rate of 1 mL/min.

After dilution with 100 mL of ethanol (EtOH) (QReC Chemical, Auckland, New Zealand), the enhancing properties of each freeze-dried fraction was analyzed via half-tongue test by Schiffman et al.\textsuperscript{[9]} and Bader et al.\textsuperscript{[21]} Filter paper (1 cm × 2 cm) (Whatman, GH Healthcare Bio-Science, Uppsala, Sweden) was immersed in the sample before adding 10 µL of salt solution (1% w/w). The paper was placed in a hot air oven (ED 115; Binder, Tuttinglen, Germany) at 38°C and HS-SPME was used to check for complete EtOH removal. The blank sample was prepared in the same manner, with EtOH and a salt solution. Each fraction and blank sample were placed in balanced random order on the right or the left side of the anterior tongue of panel ($n = 2$). Trained panelists were asked to indicate the side with the higher saltiness intensity. The fraction indicated by all panelists was considered for further analysis.

**Gel permeation chromatography**

Five grams of the chosen freeze-dried water-soluble fraction was diluted with 10 mL of 40% (v/v) methanol (Avantor Performance Materials, Gliwice, Poland) aqueous solution (MeOH/H$_2$O). It was then micro-centrifuged (Eppendorf 5415 R; Sigma-Aldrich, MO, USA) at 10,000 rpm (19,376 × g) for 5 min and next membrane-filtered (0.45 µm) (Minisart RC 15; Sartorius, Hannover, Germany). Gel permeation chromatography (GPC) was performed via a glass column 50 mm × 500 mm (Bio-Rad, CA, USA) filled with
Sephadex LH-20 (GH Healthcare Bio-Science, Uppsala, Sweden). The sample was eluted sequentially with mixtures of 40%, 50%, 60%, 80%, and 100% MeOH/H₂O (1 L each) at a flow rate of 3 mL/min. GPC subfractions were collected every 50 mL, evaporated, freeze-dried, and checked for complete solvent removal as described above. They were re-dissolved in water to form 2 mL samples. The salty enhancing subfractions were analyzed via half-tongue tests and the taste quality of each was also recorded.

**Taste dilution analysis**
The saltiness enhancing subfractions were step-wise diluted with water at a 1:1 ratio for TD analysis. The serial ascending concentrations of each subfraction was presented to the panel for half-tongue testing. The TD factor, the last dilution at which the panel could detect the saltiness enhancing property, was recorded for each subfraction. The TD factors which did not differ by more than two steps were averaged from three panelists.

**High performance liquid chromatography**
The subfraction with the highest TD factor was freeze-dried and then dissolved to 0.05 g/mL in a 40% MeOH/H₂O. The sample was micro-centrifuged, membrane-filtered, and injected onto a HPLC with a diode array detector (DAD). The components were separated on a Varian C18 reversed-phase column (250 × 4.6 mm × 0.25 µm) (Agilent Technologies, MA, USA). A gradient mobile phase, starting at 5% and increasing to 100% (v/v) MeOH in an aqueous formic acid solution (0.1%) (RCI Lab-scan, Bangkok, Thailand), was used at a flow rate of 1 mL/min. The holding times at the initial and final gradients were 5 and 60 min, respectively. UV wavelength was scanned from 200 to 400 nm.

**Determination of chloride salts**
The amount of chloride salts in the highest TD subfraction was determined by Mohr method. Freeze-dried GPC subfraction was dissolved in 100 mL of distilled water. This mixture were shaken vigorously and filtered to collect the clear solution. Two milliliters of potassium chromate were added and the solution was titrated with a silver nitrate (AgNO₃) solution until the first permanent appearance of red silver chromate precipitate. The concentration of chloride salt was calculated as NaCl. Szechuan pepper samples from various manufacturers (A, B, C, D, E, and F) were also analyzed.

**Spectroscopic and ion selective electrode techniques**
The presence of Na⁺ and K⁺ in sample A powder was quantified by inductively coupled plasma-atomic emission spectroscopy (ICP-AES) (Perkin Elmer Optima 2000; Plasma Atom Comp, Jarrell-Ash, Boston, MA, USA) based on the Association of Official Analytical Chemists methodology. Fifteen milliliters of water was added to 1.5 g of Szechuan pepper and the mixture was shaken vigorously in a 100-mL Kjeldahl flask. The sample was then acid-digested with 10 mL of nitric and perchloric acids at a 2:1 ratio at 60°C. After cooling, this mixture was reheated to 200°C and digested several times. Elemental determination was reached using ICP-AES at wavelengths 589 nm for Na⁺ and 766.5 nm for K⁺. Chloride ion content was detected via ion selective electrodes (ISE) based on American Public Health Association methodology. Fifty milliliters of water was added to 1 g of Szechuan pepper powder in a 100-mL screw cap bottle. This mixture was shaken for 1 h and adjusted to 100 mL in a volumetric flask before filtering. The solution was stirred well and measured by the PerfecTion™ combined ISE filled with the Ion Electrolyte B Reference (Mettler-Toledo, Greifensee, Switzerland).

**Validation of identification**
The saltiness intensity of fried potato sticks with the combination of 1% salt and 1% ground Szechuan pepper A was compared to those with 1.1% salt by the 2-AFC test. The additional 0.1% salt in the latter sample was adjusted to the same content of NaCl in 1% Szechuan pepper A as the result from the chemical analysis. The test was conducted with 30 panelists (10 males and 20 females, between 22 and 40 years old).
Results and discussion

Flavor properties

Flavor profile
Flavor attributes with definitions, references, and standard intensities were generated (Table 1). The Szechuan pepper A flavor profile is shown in Fig. 1. There were four aromas, two basic tastes, and four flavor characteristics. Aromas were described as woody, camphor, citrus peel, and clove. In general, the food aroma corresponded to the volatile components present. Some volatile aroma compounds of the steam-distilled oil and liquid carbon dioxide extract of *Zanthoxylum simulans* fruit were α-pinene, sabinene, campene, β-caryophyllene, β-myrcene, α-terpinene, limonene, β-phellandrene, 1,8-cineole, β-caryophyllene, p-cymene, terpinolene, linalool, and α-humulene. [26] Despite insufficient information on the human detection of active aroma compounds in Szechuan pepper, odor descriptors of the aforementioned volatiles could be found in existing literature. The citrus peel scent of Szechuan pepper may be associated with limonene, described as having an orange-like and citrus odor [27] and was present in more than 95% of citrus peel oils. [28] Some of the above compounds contribute to more than one aroma attribute. For an instance, 1,8-cineole was woody, camphoraceous, galangal-like and fruity. [27,29] In contrast, some aromas may be the result of a combination of compounds. For example, β-pinene was described as woody in some mandarin cultivars [30] and α-humulene was labelled mildly woody [27] as well. Sabinene and campene were simultaneously described as camphoraceous. [27] Consequently, these compounds, being present in Szechuan pepper [26], may contribute to these woody and camphor aromas.

For taste attribute analysis, nose clips prevented physicochemical interactions between taste and aroma. [31] Further, the panel’s taste sensitivity increased when using nose clips in comparison with those without. [32] Bitter and salty were the two basic tastes of Szechuan pepper (Fig. 1). These two attributes along with umami, sour, metallic, and astringent, were also found in the study of alkyl aromatic amides in the stem and root extract of *Zanthoxylum*. [32] The presence of the taste-active compounds rubemamine and podocarpamamide in that study probably contributed to the astringent flavor of Szechuan pepper.

Hydroxy-α-sanshool found in *Zanthoxylum* [33] might cause the reported numbing effect (Fig. 1). This compound was identified as the main sanshool in *Zanthoxylum* fruits while α-sanshool was dominant within their leaves and flowers. [34] Hydroxy-α-sanshool, having one cis and three trans double bonds in the molecule, was also described as pungent. [35] Pungency was defined by the ability to generate irritation and a sensation of chemical burning. [36] This may introduce a burnt-aftertaste in the flavor profile (Fig. 1). In addition, the cooling effect of Szechuan pepper was noted in an article concerning the development of taste enhancement. [14]

![Figure 1. Flavor profile of Szechuan pepper A from descriptive sensory analysis on 15-cm line scale (n = 10).](image-url)
Food flavor enhancement

The flavor intensities of food samples with and without Szechuan pepper A are presented in Table 2. All samples without Szechuan pepper scored at near-moderate intensities. The addition of Szechuan pepper significantly increased overall flavor intensities \((p \leq 0.05)\). Panelists found the greatest flavor increase in the creamy chicken soup and it should be noted that some commented on the chicken broth aroma with the Szechuan pepper addition. Flavor perception is a multimodal interaction between an individual’s taste, aroma, and trigeminal systems. Changes in these components will affect the overall quality of perceived flavor. For example, an increase in the concentration of NaCl or MSG significantly enhanced food flavor in cheese models\(^{[37]}\), chicken stock, and soy sauce.\(^{[38]}\) High salivary flow rate contributed to increased sodium release in foods and a faster saltiness perception.\(^{[39]}\) Moreover, hydroxyl-\(\alpha\)-sanshool, hydroxyl-\(\beta\)-sanshool, and Szechuan pepper extract were shown to provoke substantial saliva flow.\(^{[21]}\) Thus, the increase of flavor intensity in this study might be due, at least in part, to the increased saliva induced by sanshool compounds. In addition, the \(\textit{Zanthoxylum}\) family contained taste active compounds, such as \((E)-N-(3,4\text{-dimethoxyphenethyl})-3\text{-}(3,4\text{-dimethoxyphenyl})\text{-acrylamide (rubemamine)},^{[15,32]}\) \((E)-3\text{-}(3,4\text{-dimethoxyphenyl})-N\text{-}(3\text{-methoxyphenethyl})\text{-acrylamide, and } (E)-3\text{-}(3,4\text{-dimethoxyphenyl})-N\text{-}(3\text{-propoxyphenethyl})\text{-acrylamide,}^{[32]}\) which enhance umami and saltiness in foods. These taste sensations might have the enhancement impact of Szechuan pepper in food flavor.

Not only did flavor improve, but also saltiness increased when Szechuan pepper included as a food ingredient. It was reported to reduce the amount of salt used in food when mixing with chili.\(^{[13]}\) The saltiness enhancing property of Szechuan pepper, therefore, has attracted great attention due to the health concerns of sodium overconsumption in the world population.

Saltiness enhancing property

Saltiness enhancing characteristic

Fried potato sticks from the previous experiment were selected as the food model in this analysis being a common and highly consumed snack food.\(^{[40]}\) Consumers usually add substantial quantities of salt, herbs and spices into the potato sticks. Snacks and sweets accounted for 8% of all sodium consumption.\(^{[41]}\) In addition, Adam et al. (1995)\(^{[42]}\) reported that the fewer ingredients contained in the food item, the greater perceived saltiness when salt is present. The relative plain taste of fried potato sticks would eliminate the other taste interference from the test result.

Unseasoned fried potato sticks were compared to those seasoned with 1% ground Szechuan pepper A to evaluate whether the salty property existed in pepper. The unseasoned sample’s saltiness intensity was not significantly different from the seasoned ones \((p > 0.05)\) (Table 3). This result was not in agreement with its flavor profile in which the Szechuan pepper at higher doses tasted salty. Although the 2-AFC test was direct, simple, and focused on single attribute,\(^{[43]}\) Szechuan pepper exhibited no saltiness by itself in common dose.

The 2-AFC test was also used to determine the saltiness enhancing property between fried potato sticks with 1% salt and those with the combination of 1% salt and 1% pepper. The latter sample was significantly saltier than the salted fried potato sticks \((p \leq 0.05)\) (Table 3). Therefore, the pepper increased saltiness intensity without a significant salty taste suggesting that Szechuan pepper contained saltiness enhancers when combined with NaCl in fried potato sticks. Taste enhancers are compounds

---

### Table 2. Flavor intensities of various food samples with and without the addition of Szechuan pepper A.

| Food sample      | Without | With    | \(p\)-Value |
|------------------|---------|---------|-------------|
| Fried potato sticks | 3.77 ± 1.30 | 5.00 ± 1.55 | 0.001       |
| Chicken broth     | 4.53 ± 1.52 | 5.93 ± 1.39 | < 0.001     |
| Creamy chicken soup | 3.20 ± 1.79 | 5.53 ± 1.48 | < 0.001     |
| Pasta sauce      | 4.23 ± 1.41 | 6.17 ± 1.26 | < 0.001     |

\(^{a}\)Used a 9-point category scale: 0 = none, 2 = light, 4 = moderate, 6 = strong and 8 = extreme \((n = 30)\).
having the capacity to increase taste intensity of a substance when added to a substance\cite{18} despite its not having that particular taste quality.\cite{44} This finding could lead to salt reduction in foods, thereby decreasing the global risk of sodium-induced diseases.

**Level of saltiness enhancement**

The level of Szechuan pepper’s saltiness enhancing ability was measured through a rating test where the mean saltiness intensity was plotted against different salt concentrations in fried potato sticks, creating a calibration curve (Fig. 2). Regression analysis of the panelists’ responses showed a linear correlation between saltiness intensity and salt concentration ($r = 0.98$, $R^2 = 0.96$). The fried potato sticks with the combination of 1% salt and 1% Szechuan pepper were scored at 4.09, significantly higher than those containing the same amount of salt ($p \leq 0.05$). This was equivalent to 1.29% salt used or saltiness enhancing efficiency of 28.74%. This number was similar to that of arginyl dipeptides having a 26.8% impact in saltiness modulating activity.\cite{19}

Moreover, the addition of Szechuan pepper reduced the need of salt by 22.32%. In terms of public health, the average sodium intake of an American adult was 3,440 mg/day while the recommendation was 2,300 mg/day.\cite{41} Utilization of this pepper could be equal to a 768 mg reduction in daily sodium intake. Laatikainen et al. (2006)\cite{45} postulated that a 30–35% reduction in sodium would lead to a 75% drop in adult coronary heart disease mortality.

**Saltiness enhancing compounds**

$n$-Pentane-, ethyl acetate-, and water-soluble fractions were obtained from Szechuan pepper A by sequential solvent extraction. The saltiness enhancers determined by half-tongue test were present in a water-soluble fraction. The enhancer was successfully localized via the 2-AFC procedure. This study

![Figure 2. Relationship between saltiness intensities and salt concentrations added to fried potato sticks ($n = 11$).](image)

\begin{table}[h]
\centering
\caption{Panel response on the salty and saltiness enhancing properties of Szechuan pepper A in fried potato sticks.}
\begin{tabular}{lll}
\hline
\textbf{Fried potato sticks with} & \textbf{Selection of saltier sample* (%)} & \textbf{$p$-Value} \\
\hline
\textit{Salty property} & & \\
Unseasoned & 36.67 & 0.144 \\
1% Szechuan pepper & 63.33 & \\
\textit{Saltiness enhancing property} & & \\
1% salt & 26.67 & 0.011 \\
1% salt and 1% Szechuan pepper & 73.33 & \\
\hline
\end{tabular}
\end{table}

\*Accessed by 2-alternative forced-choice test ($n = 30$).
showed that the half-tongue test was a suitable method for identifying low-concentration taste enhancers due to simplicity, rapidity, and the small amount of sample needed. Furthermore, this test offers a more direct, less memory-intensive method among test subjects. Two samples were tasted simultaneously side by side without a functionally asymmetrical effect of the tongue. McMahon et al.\[46\] reported that threshold sensitivity was relatively equivalent on the right and left anterior sides.

One hundred subfractions were collected via the GPC from the polar fraction. The half-tongue test revealed 20 saltiness enhancing subfractions (Table 4). Although these subfractions showed the saltiness enhancing property, interestingly they possessed different taste qualities including salty, umami, sweet, bitter, sour, and tasteless. According to TD analysis, the potency of saltiness enhancing compounds were classified into three groups. The first group was subfraction 14 with the highest TD factor of 107. The moderate potency group included subfraction 13, 95, and 96. The other lower TD subfractions belonged to the low potency group that may contribute a weak impact. The TD analysis is used to rank the importance of tastants according to their thresholds in a mixture.\[22,23\] It is also applied in several studies concerning sweet,\[47\] kokumi,\[48\] and saltiness enhancers.\[19\]

Subfraction 14, the highest potency enhancing part, was further analyzed by HPLC-DAD. Chromatography data showed several major compounds observed at $\lambda_{\text{max}}$ 254 and 300 nm. This result was due to GPC technique being the primary separation method for taste analysis. Since this subfraction had extremely salty characteristics (Table 4) as well, the presence of chloride salt as NaCl was determined by the Mohr method. Surprisingly, it was the major component of subfraction 14, being up to 66.91% of the mixture.

Chloride salt as NaCl was also investigated in Szechuan peppers from different sources. The peppers A, B and C were from various leading manufacturers, while D was a wholesale brand in Thailand. Samples E and F were from Szechuan, China. The results were in agreement with the subfraction analysis. All samples contained high amount of chloride salt ranging from 12.2 to 65.0 mg/g of Szechuan pepper (Table 5). The $\text{Cl}^-$ is used in plant biochemical and physiological functions with $\text{Cl}^-$ concentrations in different parts of plants at 0.9–25 mg/g.\[49\] Since there is a number of NaCl-sensitive trees within the Rutaceae family,\[50\] the high amount of chloride salt in Szechuan pepper may due to the tree variety involved. Salt contents in all the tested peppers from Thailand (except D) were significantly higher than samples from China ($p \leq 0.05$). The difference

| Subfraction number$^a$ | Taste quality | Taste dilution factor$^b$ |
|------------------------|--------------|--------------------------|
| 11                     | Bitter       | 4                        |
| 12                     | Salty, umami | 8                        |
| 13                     | Salty, umami, sweet | 32                    |
| 14                     | Extremely salty, umami | 107                   |
| 15                     | Salty, sour  | 8                        |
| 16                     | Salty, bitter| 2                        |
| 43                     | Bitter       | 4                        |
| 47                     | Tasteless    | 2                        |
| 55                     | Tasteless    | 2                        |
| 56                     | Tasteless    | 1                        |
| 60                     | Tasteless    | 2                        |
| 82                     | Tasteless    | 2                        |
| 83                     | Tasteless    | 2                        |
| 84                     | Tasteless    | 9                        |
| 92                     | Tasteless    | 5                        |
| 93                     | Tasteless    | 1                        |
| 94                     | Tasteless    | 8                        |
| 95                     | Slightly umami | 21                   |
| 96                     | Slightly umami | 21                   |
| 97                     | Tasteless    | 3                        |

$^a$Referred to the order of subfractions collected by GPC from water-soluble fraction.

$^b$Averaged from three trained panelists.
might be the result of varied soil conditions. Most soil in Thailand is saline and the abundance of ions include Na\(^+\), Ca\(^{2+}\), Mg\(^{2+}\), K\(^+\), Cl\(^-\), SO\(_4\)^{2-}, HCO\(_3\)^-\), CO\(_3\)^{2-}, and NO\(_3\)^-\). Notably, the Szechuan province is located in southwest China, far from the saline soil region. It should be noted that sample D, which had the lowest salt content among Thai brands, might be imported. ICP-AES and ISE techniques were further employed to quantify chloride salts in sample A for specific, sensitive, accurate, and precise analysis. The experiment not only targeted NaCl but also KCl being generally present in trees. NaCl was presumed to be the primary saltiness contributor in Szechuan pepper followed by KCl due to the typical salty taste. DeSimone et al. (2007) reported that Na\(^+\) was prioritized by a salty taste transduction channel. Moreover, the recognition threshold of NaCl (0.047–0.050 g/100 mL) was lower than those of KCl (0.0822–0.107 g/100 mL). It resulted in a higher impact of NaCl at the same concentration according to the taste activity concept. The data from Table 6 showed that Cl\(^-\) was the limiting ion for computing a balanced chemical equation. The result implied that Szechuan pepper contained only NaCl as salty tastant and the presence of KCl was negligible if present at all. Thus, there was 97.6 mg of NaCl in 1 g of Szechuan pepper.

**Validation of identification**

The authentication of chemical analysis was carried out by 2-AFC test. The saltiness intensity of fried potato sticks with the combination of 1% salt and 1% Szechuan pepper A was not significantly different from those with the equivalent amount of additional NaCl (\(p > 0.05\)) (Table 7). This result indicated that NaCl was the most important component in the saltiness enhancement of Szechuan pepper.

**Conclusions**

Szechuan pepper has both flavor and saltiness enhancing properties. The identification of saltiness enhancers is achieved by sensory-guided chemical analysis. The most significant contributor to
saltiness enhancement in the pepper is successfully determined. Even though the enhancement of Szechuan pepper is affected by several compounds, it is dominated by high NaCl content. Hence, consumers who seriously desire to control their daily sodium intake should be aware of that fact when using this seasoning ingredient. Nutritionists and food scientists should consider this when researching and developing food products for specific markets as well. In addition, governmental authorities and health organizations should disseminate this information regarding Szechuan pepper NaCl content to promote and protect public health.

Acknowledgments

The first part of this study received the best track paper award in “Food Structure and Sensory Properties Track” from the 2016 International Conference on Food Properties (iCFP2016) held in Bangkok, Thailand on May 31st to June 2nd, 2016.

Funding

This research was supported by the Kasetsart University Research and Development Institute (KURDI) under grant number 70.59 and the Kasetsart University Graduate School.

References

1. Chobanian, A. V.; Hill, M. National Heart, Lung, and Blood Institute Workshop on Sodium and Blood Pressure: A Critical Review of Current Scientific Evidence. Hypertension 2000, 35(4), 858–863.
2. Arcand, J.; Ivanov, J.; Sasson, A.; Floras, V.; Al-Hesayen, A.; Azvedo, E. R.; Mak, S.; Allard, J. P.; Newton, G. E. A High-Sodium Diet Is Associated with Acute Decompensated Heart Failure in Ambulatory Heart Failure Patients: A Prospective Follow-Up Study. The American Journal of Clinical Nutrition 2011, 93(2), 332–337.
3. Strazzullo, P.; D’Elia, L.; Kandala, N. B.; Cappuccio, F. P.; Intake, S. Stroke, and Cardiovascular Disease: Meta-Analysis of Prospective Studies. BMJ 2009, 339, 1–9.
4. Busch, J. L. H. C.; Yong, F. Y. S.; Goh, S. M. Sodium Reduction: Optimizing Product Composition and Structure Towards Increasing Saltiness Perception. Trends in Food Science and Technology 2013, 29(1), 21–34.
5. Nakamura, H.; Miyashita, H. Enhancing Saltiness with Cathodal Current. In Proceedings of the 2013 Changing Perspectives, Paris, France, Apr 27th–May 2nd, 2013, pp. 14.
6. Cruz, A.; Green, B. G. Thermal Stimulation of Taste. Nature 2000, 403(6772), 889–892.
7. Schiffman, S. S.; Gill, J. M.; Diaz, C. Methyl Xanthines Enhance Taste: Evidence for Modulation of Taste by Adenosine Receptor. Pharmacology Biochemistry and Behavior 1985, 22(2), 195–203.
8. Ilani, A.; Lichtstein, D.; Bacaner, M. B. Bretylium Opens Mucosal Amiloride-Sensitive Sodium Channels. Biochimica et Biophysica Acta 1982, 693(2), 503–506.
9. Schiffman, S. S.; Simon, S. A.; Gill, J. M.; Beeker, T. G. Bretylium Tosylate Enhances Salt Taste. Physiology & Behavior 1986, 36(6), 1129–1137.
10. Tada, M.; Shinoda, I.; Okai, H. l-Ornithyltaurine, a New Salty Peptide. Journal of Agricultural and Food Chemistry 1984, 32(5), 992–996.
11. Kikuchi, E.; Okai, H. An Enhancing Effect on the Saltiness of Sodium Chloride of Added Amino Acids and Their Esters. Agricultural and Biological Chemistry 2005, 63(39), 8694–8704.
12. Hofmann, T.; Soldo, T.; Ottinger, H.; Frank, O.; Robert, F.; Blank, I. Structural and Functional Characterization of a Multimodal Taste Enhancer in Beef Bouillon. In Natural Flavors and Fragrances; Frey, C., Rouseff, R. L., Eds.; American Chemical Society: Washington DC, 2005; 173–188.
13. Meilgaard, M. C.; Carr, B. T.; Civille, G. V. Sensory Evaluation Techniques, 4th; Taylor and Francis Group: Florida, USA, 2007; pp. 448.
18. Kroene, J. H. A. Neohesperidin Dihydrochalcone is Not a Taste Enhancer in Aqueous Sucrose Solutions. Chemical Senses 2000, 25(5), 555–559.
19. Schindler, A.; Dunkel, A.; Stahler, F.; Backes, M.; Ley, J.; Meyerhof, W.; Hofmann, T. Discovery of Salt Taste Enhancing Arginyl Dipeptides in Protein Digests and Fermented Fish Sauces by Means of a Sensomics Approach. Journal of Agricultural and Food Chemistry 2011, 59(23), 12578–12588.
20. Hufnagel, J. C.; Hofmann, T. Orosensoric-Directed Identification of Astringent Mouthfeel and Bitter-Tasting Compounds in Red Wine. Journal of Agricultural and Food Chemistry 2008, 56(4), 1376–1386.
21. Bader, M.; Stark, T. D.; Dawid, C.; Losch, S.; Hofmann, T. All-Trans-Configuration in Zanthoxylum Alkylamides Swaps the Tingling with a Numbing Sensation and Diminishes Salivation. Journal of Agricultural and Food Chemistry 2014, 62(12), 2479–2488.
22. Frank, O.; Ottinger, H.; Hofmann, T. Characterization of an Intense Bitter-Tasting 1h,4h-Quinolizinium-7-Olate by Application of the Taste Dilution Analysis, a Novel Bioassay for the Screening and Identification of Taste-Active Compounds in Foods. Journal of Agricultural and Food Chemistry 2001, 49(1), 231–238.
23. Ottinger, H.; Bareth, A.; Hofmann, T. Characterization of Natural “Cooling” Compounds Formed from Glucose and L-Proline in Dark Malt by Application of Taste Dilution Analysis. Journal of Agricultural and Food Chemistry 2001, 49(3), 1336–1344.
24. Association of Official Analytical Chemists. Official Methods of Analysis, 19th edn.; AOAC International Suite: Maryland, USA, 2012.
25. American Public Health Association. Standard Methods for Water and Wastewater, 20th.; American Public Health Association Inc: Washington, DC, 1994.
26. Chyau, C. C.; Mau, J. L.; Wu, C. M. Characteristics of the Steam-Distilled Oil and Carbon Dioxide Extract of Zanthoxylum Piperitum. Flavour Chemistry of Ethnic Foods 2005, 62, 1386.
27. Leffingwell, J. C. Flavor-Base Database; Georgia, USA: Leffingwell and Associates. Version 2004.
28. Karlberg, A. T.; Magnusson, K.; Nilsson, U. Air Oxidation of 6-Limonene (The Citrus Solvent) Creates Potent Allergens. Contact Dermatitis 1992, 26(5), 332–340.
29. Kubota, K.; Someya, Y.; Kurobayashi, Y.; Kobayashi, A. Flavor Characteristics and Stereochemistry of the Volatile Constituents of Greater Galangal (Alpinia Galanga Willd.). In Flavor Chemistry of Ethnic Foods; Shahidi, F., Ho, C. T., Eds.; Springer: Berlin, Germany, 1999; 97–104.
30. Elmaci, Y.; Altug, T. Flavor Characterization of Three Mandarin Cultivars (Satsuma, Bodrum, Clemantine) by Using GC/MS and Flavor Profile Analysis Techniques. Journal of Food Quality 2005, 28(2), 163–170.
31. Poinot, P.; Arvisenet, G.; Texier, F.; Lethuaut, I.; Mehinagic, E.; Vigneau, E.; Prost, C. Use of Sense Masking to Study Sensory Modalities Singly: Interest for the Understanding of Apple In-Mouth Perception. Food Quality and Preference 2011, 22(6), 573–580.
32. Frerot, E.; Neirynck, N.; Cayeux, I.; Yuan, Y. H. J.; Yuan, Y. M. New Umami Amides: Structure–Taste Relationship Studies of Cinnamic Acid Derived Amides and the Natural Occurrence of an Intense Umami Amide in Zanthoxylum Piperitum. Journal of Agricultural and Food Chemistry 2015, 63(32), 7161–7168.
33. Sugai, E.; Morimitsu, Y.; Iwasaki, Y.; Morita, A.; Watanabe, T.; Kubota, K. Pungent Qualities of Sanshool-Related Compounds Evaluated by a Sensory Test and Activation of Rat TRPV1. Bioscience, Biotechnology, and Biochemistry 2005, 69(10), 1951–1957.
34. Mizutani, K.; Fukunaga, Y.; Tanaka, O.; Takasugi, N.; Saruwatari, Y. I.; Fuwa, T.; Yamauchi, T.; Wang, J.; Jia, M. R.; Li, F. Y.; et al. Amides from Huaijiao, Pericarps of Zanthoxylum Bungeanum Maxim. Journal of Agricultural and Food Chemistry 1998, 36(7), 2362–2365.
35. Kashiwada, Y.; Ito, C.; Katagiri, H.; Mase, I.; Komatsu, K.; Namba, T.; Ikeshiro, Y. Amides of the Fruit of Zanthoxylum Spp. Phytochemistry 1997, 44(6), 1125–1127.
36. Ludy, M. J.; Tucker, R.; Tan, S. Y. Chemosensory Properties of Pungent Spices: Their Role in Altering Nutrient Intake. Chemosensory Perception 2015, 8(3), 131–137.
37. Nii, J.; Overington, A. R.; Silcock, P.; Bremer, P. J.; Delahunty, C. M.; Taste, C.-M. Aroma Interactions: Cheese Flavour Perception and Changes in Flavour Character in Multicomponent Mixtures. Food Quality and Preference 2016, 48, 70–80.
38. Linscott, T. D.; Lim, J. Retronasal Odor Enhancement by Salty and Umami Tastes. Food Quality and Preference 2016, 48, 1–10.
39. Phan, V. A.; Yen, C.; Lawrence, G.; Chabanet, C.; Reparet, J. M.; In, S. C. Vivo Sodium Release Related to Salty Perception during Eating Model Cheeses of Different Textures. International Dairy Journal 2008, 18(9), 956–963.
40. van Dam, R. M.; Rimm, E. B.; Willett, W. C.; Stampfer, M. J.; Hu, F. B. Dietary Patterns and Risk for Type 2 Diabetes Mellitus in U.S. Men. Annals of Internal Medicine 2002, 136(3), 201–209.
41. U.S. Food and Drug Administration. 2015–2020 Dietary Guidelines for Americans. https://health.gov/dietaryguidelines/2015/ (accessed January 29, 2017).
42. Adams, S. O.; Mallor, O.; Cardello, A. V. Consumer Acceptance of Foods Lower in Sodium. Journal of the American Dietetic Association 1995, 95(4), 447–453.
43. Methven, L. Techniques in Sensory Analysis of Flavour. In Flavour Development, Analysis and Perception in Food and Beverages; Parker, J. K., Elmore, S., Methven, L., José, M., Eds.; Woodhead Publishing: Cambridge, UK, 2015; 353–368.

44. Kilcast, D.; De Ridder, C. Sensory Issues in Reducing Salt in Food Product. In Reducing Salt in Foods: Practical Strategies; Kilcast, D., Angus, F., Eds.; Woodhead Publishing: Cambridge, UK, 2007; 201–220.

45. Laatikainen, T.; Pietinen, P.; Valsta, L.; Sundvall, J.; Reinivuo, H.; Tuomilehto, J. Sodium in the Finnish Diet: 20-Year Trends in Urinary Sodium Excretion among the Adult Population. European Journal of Clinical Nutrition 2006, 60, 965–970.

46. McMahon, D. B. T.; Shikata, H.; Breslin, P. A. S. Are Human Taste Thresholds Similar on the Right and Left Sides of the Tongue?. Chemical Senses 2001, 26(7), 875–883.

47. Hillmann, H.; Mattes, J.; Brockhoff, A.; Dunkel, A.; Meyerhof, W.; Hofmann, T. Sensomics Analysis of Taste Compounds in Balsamic Vinegar and Discovery of 5-Acetoxymethyl-2-Furaldehyde as a Novel Sweet Taste Modulator. Journal of Agricultural and Food Chemistry 2012, 60(40), 9974–9990.

48. Degenhardt, A. G.; Hofmann, T. Bitter-Tasting and Kokumi-Enhancing Molecules in Thermally Processed Avocado (Persea Americana Mill.). Journal of Agricultural and Food Chemistry 2010, 58(24), 12906–12915.

49. Heckman, J. R. Chlorine. In Handbook of Plant Nutrition; Barker, A. V., Pilbeam, D. J., Eds.; CRC Press: Boca Raton, USA, 2006; 279–291.

50. Boman, B. J.; Zekri, M.; Stover, E. Managing Salinity in Citrus. HortTechnology 2005, 15(1), 108–113.

51. Kachondham, Y.; Viravathana, N. Food Security in Thailand. In Food Security in Asian Countries in the Context of Millennium Goals; Vyas, V. S., Ed.; Academic Foundation: New Delhi, India, 2005; 81–110.

52. Pratt, P. L.; Suarez, D. L. Irrigation Water Quality Assessments. In Agricultural Salinity Assessment and Management; Tanji, K. K., Ed.; American Social Civil Engineers: New York, 1990; 200–236.

53. Yu, R. P.; Hseung, Y. Alkaline Soils in China; Institute of Soil Science, Academia Sinica: Nanjing, China, 1983.

54. Du, J.; Luo, Y.; Zhang, W.; Xu, C.; Wei, C. Major Element Geochemistry of Purple Soils/Rocks in the Red Sichuan Basin, China: Implications of Their Diagenesis and Pedogenesis. Environmental Earth Sciences 2013, 69(6), 1831–1844.

55. Mengel, K. Potassium. In Handbook of Plant Nutrition; Barker, A. V., Pilbeam, D. J., Eds.; CRC Press: Boca Raton, USA, 2006; 91–120.

56. Roper, S. D. The Taste of Table Salt. Pflügers Archiv - European Journal of Physiology 2015, 467(3), 457–463.

57. DeSimone, J. A.; Ye, Q.; Heck, G. L. Ion Pathways in the Taste Bud and Their Significance for Transduction. In Ciba Foundation Symposium 179 - the Molecular Basis of Smell and Taste Transduction; Chadwick, D., Marsh, J., Goode, J., Eds.; John Wiley & Sons, Ltd: Chichester, UK, 2007; 218–234.

58. Torrico, D. D.; Sae-Eaw, A.; Srimawattana, S.; Boeneke, C.; Prinyawiwatkul, W. Oil-In-Water Emulsion Exhibits Bitterness-Suppressing Effects in a Sensory Threshold Study. Journal of Food Science 2015, 80(6), s1404–s1411.

59. Schlichtherle-Cerny, H.; Grosch, W. Evaluation of Taste Compounds of Stewed Beef Juice. Zeitschrift für Lebensmitteluntersuchung und - Forschung A 1998, 207(5), 369–376.