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Effect of Extraction Solvent on Capsaicin Content of Chinda Peppers

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Abstract

Chinda pepper (*Capsicum annuum* L.) is classified as a large chili pepper that is an important economic crop of Thailand. The substance in the chili pepper responsible for its spicy taste is capsaicin. Presently, capsaicin in Chinda peppers has been extracted for use in food and medical products. This research compared the capsaicin content from dried Chinda peppers in four different solvents: H_2O, acetone, ethanol (95% v/v), and a binary solvent of acetone and water at a ratio of 1:1 v/v. For all treatments, the ratio of chili peppers to the solvent was 20 g:240 ml. After analyzing the amount of capsaicin content by a NanoDrop spectrophotometer, it was found that the capsaicin content from dried Chinda pepper by the H_2O:acetone (1:1 v/v), acetone, H_2O and ethanol (95% v/v) were 0.48 ppm, 0.26 ppm, 0.22 ppm, and 0.18 ppm, respectively. These results indicated that the extraction of capsaicin with a binary solvent of H_2O:acetone at a ratio of 1:1 v/v had the highest extraction concentration. This can be explained theoretically that the presence of H_2O in acetone impacted the hydrophobic properties of the solvent and the interaction between capsaicin compound and the solvent.

Keywords: Capsaicin, Extraction, Chinda pepper

1. Introduction

Peppers are one of the most popular ingredients used to add a spicy flavor in several food preparations. They play an important role in the food industry in Thailand. Thailand is among the top three countries-India, China, and Thailand-which has grown the most chili peppers worldwide with 176,873 metric tons being grown and harvested in Thailand (Food and Agriculture Organization of the United Nations [FAO], 2021). Chili peppers are not only mainly utilized for a spicy flavor in food, extractable chemical compounds such as oleoresins were also used in cosmetic and dye industries (Advanced Biotech [ABT], 2021; Imbarex, 2021). Generally, their spicy flavor is due to a main group of organic compounds, capsaicinoids. Capsaicinoids are soluble in moderate polar organic solvents; e.g. methanol, ethanol, acetonitrile and binary solvent among others (Kurian & Starks, 2002). They are also soluble in water at 25°C (28.93 mg/l) and are practically insoluble in cold water (National Library of Medicine [NIH], 2021). The major capsaicinoid compound present in most varieties of the chili peppers is capsaicin (trans-8-methyl-N-vanillyl-6-nonenamide) (Figure 1). In addition, other capsaicinoid compounds were also found in relatively small amounts; such as nordihydrocapsaicin, homocapsaicin, normorcapsacin, etc. (Kobata et al., 1998) Even though capsaicin can cause irritation to skin and respiratory system, it has been extensively studied both experimentally and clinically for its ability to stimulate sensory nerves and to treat bladder inflammation (Kaale, Schepdael, Roets, & Hoogmartens, 2002). Determination of capsaicin content in chili peppers has received an increasing interest for many reasons. Extraction methods of the capsaicin from peppers have been conducted using various extraction techniques including liquid-liquid extraction (LLE) (Tapia, Garcia, Escamilla, Calva, & Rocha, 1993), enzymatic extraction (Santamaria et al., 2000), solid-phase microextraction (SPME) (Spicer & Almirall, 2005) and ultrasonic assisted extraction (Karnka, Rayanakorn, Watanesk, & Vaneesorn, 2002). In addition, the efficiency of capsaicinoids extraction from chili peppers is influenced by the parts of chili peppers used, tissue preparation, contact time, sample particle size, storage time, as well as the presence of interfering substances. Moreover, extraction solvents play an important role in extraction efficiency. In an extraction, one has to priorly consider the chemical nature in different varieties of chili peppers and the type of extraction solvent. In this research, the extraction of capsaicin from Chinda peppers was investigated using solid-liquid extraction methods with different solvents: water, acetone, ethanol, and their combination.

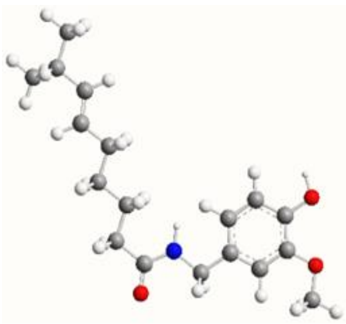

Figure 1. Schematic representation of the structure of Capsaicin. grey: C; white: H; red: O; blue: N.

Moreover, a quantitative analysis of capsaicin content from chili pepper sample can also be done using various techniques; for example, solid phase extraction (SPE) (Attuquayefio & Buckle, 1987), gas chromatography-mass spectrometry (GC-MS) (Thomas, Schreiber, & Weisskopf, 1998), high-performance chromatography (HPLC) (Barbero, Palma, & Barroso, 2006). However, these equipment can be expensive, difficult to use, and uncommon in a routine analysis. For convenience and reliability, a technique associated with UV absorption measured with common wavelength (λ_{max} = 280 nm) has been presently accepted (Juangsamoot, Ruangviriyachai, Techawongstien, & Chanthai, 2012). In this research, a NanoDrop Spectrophotometer was used in the quantitative analysis of capsaicin.

2. Materials and Methods

2.1 Chemicals

All reagents used were at least analytical reagent (AR) grade. Ethanol was supplied by Gammaco (Thailand). Acetone was supplied by Lab Scan (Thailand). The highest purity of standard capsaicin was obtained from Ennagram (France). Aqueous solutions were prepared with deionized water throughout the experiments.

2.2 Instrument

The standard addition method was carried out to determine the amount of capsaicin content. The standard dilute concentrations of 0.02, 0.04, 0.06, 0.08 and 0.10 mg/ml were prepared for the calibration curve. The absorbance of capsaicin was carried out by NanoDrop Spectrophotometer at λ_{max} = 280 nm.

2.3 Plant materials

Chinda peppers (*Capsicum annuum* L.) used in this study were obtained from a fresh market in Thonburi, Bangkok, Thailand. The whole fresh chili pepper was dried in a hot-air oven (at 100ºC for 3 hours 30 minutes until constant weight was achieved). The dried samples were ground and stored in a desiccator until usage.

2.4 Solvent extraction

The ground dried samples were weighed for 20 grams and macerated in 240 ml of 4 different solvents (H_2O, 95% v/v ethanol, and 1:1 v/v H_2O:acetone) at room temperature for 24 hours extraction time. The extracts were filtered through a Whatman No. 42 filter paper. The solvent in the extracts was evaporated to dryness using a water bath (100°C). The residue from the evaporation was weighed for 10 mg and dissolved in suitable methanol solvent to make a final volume of 100 ml, which produced a sample solution of 1.0 mg/ml concentration. The solutions were further quantitatively analyzed for capsaicin content by NanoDrop Spectrophotometer. The experiments were conducted in 3 duplicates for each type of solvents.

2.5 Preparation and analysis of capsaicin by NanoDrop Spectrophotometer

The capsaicin standard solution of 1.0 mg/ml was diluted to 0.02, 0.04, 0.06, 0.08 mg/ml. These solutions were used to graph the standard curve by measuring the absorbance at λ_{max} = 280 nm by NanoDrop Spectrophotometer. This standard curve was used to determine the amount of capsaicin in the unknown samples of each treatment.

3. Results and Discussion

The standard curve for the capsaicin content generated using 0.02, 0.04, 0.06, 0.08 and 0.10 mg/ml capsaicin standard solution versus optical density at 280 nm was recorded. The linear standard curve is shown in Figure 2, R^2 = 1.00.

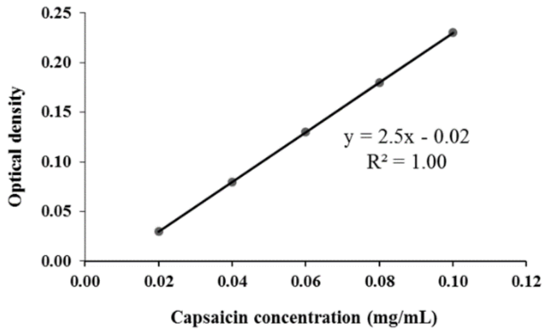

Figure 2. The plot of optical density vs capsaicin concentration shows a positive linear relationship.

To determine capsaicin content in four different extraction solvents, optical activity readings were converted into their respective concentrations using the standard curve in Figure 2, R^2 = 1.00. The average capsaicin content was calculated in ppm as shown in Table 1. The capsaicin content indicates the extraction efficiency. The results revealed that the capsaicin content was related to the type of the solvents. This agrees well with the rationality by which the polarity of solvent affects capsaicin extraction efficiencies (Rostagno, Palma, & Barroso, 2003). Moreover, it inferred that the binary mixture (H_2O:acetone) showed a higher capsaicin concentration when compared to the single solvents; acetone, H_2O, and ethanol. Among the single solvents, acetone has the highest capsaicin content, followed by H_2O and ethanol, respectively.

Nevertheless, different extraction efficiencies have been reported when the same solvent was used on dried versus wet chili pepper samples. Ethanol and Acetonitrile were better solvents for capsaicin extraction from fresh chili pepper samples, while acetone was a better solvent for capsaicin extraction from dried chili pepper samples (Chinn, Sharma-Shivappa, & Cotter, 2011). In other words, acetone alone was expected to have the highest extraction efficiency among the single solvents, which the results of this work agreed upon. On the other hand, the highest extraction efficiency of binary solvent observed in this work infers that the presence of H_2O during the extraction may have an impact on the hydrophobic properties of the solvents and the interaction with capsaicin compound (Barbero, Liazid, Palma, & Barroso, 2008; Rostagno et al., 2003). Whether to use single or binary solvent, it may depend on what is available or convenient to the user. However, regardless of choosing to use a single or binary solvent, the extraction efficiency of the specific solvent chosen still has to be considered. As shown by the results of this work, the yield of capsaicin content extractable from Chinda peppers was significantly affected by the type of the solvent as shown in Table 1, which agreed well with literature (Chinn et al., 2011). Although the parts of peppers used in the extraction (e.g. whole peppers, seeds, shells) might have an effect on the extraction efficiency (Attuquayefio & Buckle, 1987), the whole Chinda peppers were used in this work to mimic a practical production in a large scale processing and industries. To aid with consideration for processing and industrial uses, the comparison of advantages and disadvantages of the single and binary solvents used in this work was summarized in Table 2.

Table 1. The capsaicin content extracted from dried Chinda pepper by different extraction solvents.

Extraction solvents	Capsaicin content (ppm)
H_2O	0.22
Ethanol (95% v/v)	0.18
Acetone	0.26
H_2O:Acetone (1:1 v/v)	0.48

Table 2. The advantages and disadvantages of different extraction solvents.

Extraction solvents	Advantages	Disadvantages
H_2O	- Safe for consumption - Low cost	- Poor extraction efficiency
Ethanol (95% v/v)	- Safe for consumption - Easily obtained	- Slow filtration rate
Acetone	- High extraction efficiency for single solvent	- Possible health effects due to acetone contamination
H_2O:Acetone (1:1 v/v)	- Highest extraction efficiency	- Possible health effects due to acetone contamination

4. Conclusions

The solid-liquid extraction efficiency have been carried out to investigate the capsaicin content extractable from whole Chinda pepper parts using 4 different solvents: H_2O, acetone, ethanol (95% v/v), and H_2O:acetone (1:1 v/v). The results from NanoDrop Spectrophotometer showed that the capsaicin content decreases in the following order: H_2O:acetone (1:1 v/v) > Acetone > H_2O > 95%v/v ethanol. The different extraction concentration generally was related to the different polarity properties between solvents and capsaicin compounds. It was observed that binary mixture H_2O:acetone (1:1 v/v) obtained the highest capsaicin content. The presence of H_2O might have an impact on the hydrophobic properties of solvent and the interaction with capsaicin compounds. Furthermore, acetone alone had the highest concentration of capsaicin followed by H_2O and ethanol for dried Chinda peppers which agreed with reports elsewhere. Finally, to obtain a high capsaicin content from Chinda peppers, the choice of extraction solvent is of the matter.

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Suan Sunandha Science and Technology Journal

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