Effects of microwave extraction conditions on polyphenol content and antioxidant activity of pomelo extract (*Citrus maxima* (Burm.) Merr.)

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Abstract. The albedo, white peel of pomelo *Citrus maxima* (Burm.) Merr., is by-product of food processing, and has polyphenol and antioxidant activity. However, there are not many studies on polyphenol from pomelo peel in Vietnam by microwave extraction. Microwave-assisted extraction helps more efficient heating, faster energy transfer, increasing or decreasing temperature control, saving solvent and cost to extract active ingredients from plant materials. This research applied microwave extraction to extract polyphenol from pomelo *Citrus maxima* (Burm.) Merr. The factors that affected the extraction process such as solvent concentration, microwave power, extraction time and material to solvent ratio were investigated. The results obtained that the highest polyphenol content (2.46 gGAE/L) and antioxidant activity (1325.85 µmolTE/L) with ethanol concentration of 60%, microwave power of 300W, microwave-assisted time of 2 minutes, and material to solvent ratio of 1:30.

1. Introduction

Pomelo is a kind of citrus fruit, citrus belongs to the rue family, Rutaceae, is one of the important horticultural crops growing extensively in tropical and subtropical southern regions of Asia. In Vietnam, citrus fruits are grown in seven ecological regions in the north, middle and south areas of Vietnam. Vietnamese citrus fruits are diversified and each citrus crop varies with the agroecological region [1]. Pomelo (*Citrus maxima* (Burm.) Merr.) and *Citrus grandis* belong to the genus citrus (family Rutaceae); pomelo is a kind of fruit having the largest size in citrus fruit, is called shaddock or pomelo [2]. Besides, Pomelo (*Citrus grandis* (L.) Osbeck) is one of the most common citrus fruits and is the largest fruit among the citrus fruit varieties. Pomelos are grown mostly in the South of Vietnam including Vinh Long, Ben Tre, Dong Thap, Dong Nai and Binh Duong provinces although some cultivars are found in the Central and the North of Vietnam such as Hue City, Ha Tinh province, Phu Tho province and Ha Noi City. The annual pomelo production in Vietnam is about 50,000 tons with 5,000 ha produce fruit (FAO,2004) [3]. Citrus makes up a large proportion of world agriculture, led by oranges, lemons, tangerines and grapefruit. The two main uses of citrus fruits are fresh and used to prepare juices. As of 2007, 33% of the total processed fruit production, creating 15 million tons of by-products, accounts for nearly 50% of the original fruit volume. This large amount of by-products poses a solution to the problem of waste disposal and financial balance [4]. Pichaityongvongdee et al. (2014) researched total polyphenol content and antioxidant properties (2,2-diphenyl-1-picrylhydrazyl, DPPH) and ferric reducing antioxidant power (FRAP) in pomelo cultivar growing in Thailand [5]. Compounds of the
polyphenol group are known for their antioxidant activity. In addition, these compounds have been proved to be anti-inflammatory, antibacterial, anti-allergic and anti-aging for humans. In grapefruit and bitter orange peel, the main flavonoids are naringin found in high content, up to 1400 mg / 100 g, concentrated distribution in both outer flavedo and white peel (albedo) [6]. Pomelo peel with naringin content accounts for 30–75 mg / 100 mL, which can be seen as a source of strong bitter ingredients. Due to this property, pomelo peel is less used in food field [7], [8]; and so, pomelo peel is an ideal material to extract polyphenol.

Microwave extraction is one of extraction methods having many advantages such as savings in extracting time, solvent consumption and energy [9]. According to author Palash Panja, microwave-assisted extraction is one of the "green extraction" methods that are highly effective in the recovery of polyphenols. In addition, microwaves or custom equipment that is easy to use and low cost. At the same time, the microwave is a modern kitchen equipment for cooking or reheating food, so the use of the microwave in the extraction process makes this process more attractive [10].

Therefore, the aim of research was the study on main factors (microwave irradiation method, irradiation time, microwave power, solvent concentration, the rate of solvent/dried white peels of pomelo) affecting polyphenol content and antioxidant capacity of extraction from white peels of pomelo.

2. Materials and method

2.1 Materials

Pomelo was derived from Ben Tre, Vietnam. White peel of pomelo was received and dried at 60°C, ground into powder and preserved below 5°C to use in extraction. Common chemicals was used such as ethanol, methanol, HCL, KCl, CH₃COONa.3H₂O, Folin-Ciocalteu, Na₂CO₃, gallic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH) and trolox were of analytical grade.

2.2 Procedure

In every experiment, 1 g of sample was extracted at every experiment condition. The extract was then diluted to 100mL and filtered through cloth and subsequently, through Whatman No.1 filter paper before being used for measurement total phenolic contents as well as the antioxidant capacity.

Factors that were investigated during microwave extraction include: solvent concentration, microwave-assisted time, microwave power and material: solvent ratio. In which the microwave-assisted time is investigated in 2 cases: continuous and intermittent extraction. During the intermittent extraction, the microwave energy intermittently affects the mixture of raw materials and the solvent, thereby reducing the temperature of the mixture. Intermitten extraction is carried out for 30 seconds, rested for 1 minute and so on until the end of the investigated interval.

2.3 Analytical methods

2.3.1 Total polyphenol. Folin-Ciocalteau method described by Torres [11] was used to determine total polyphenol content. 1 mL of Folin reagent was added in 1 mL of diluted extract, keep the mixture in the dark for 5 minutes; after that, 1 mL of Na₂CO₃ 20% was added, and samples were incubated in the dark for 30 minutes before being measured for absorbance at 765 nm. Calculated total polyphenol was described as mg acid gallic equivalent per volume of the sample (mg/L).

2.3.2 The antioxidant capacity (DPPH). The scavenging free radical (DPPH) method described by Brand-Williams [12] that was used to determine antioxidant activity of the samples. The general principle of the method bases on the ability of antioxidants to scavenge free radical, in turn causing discoloration to the purple color of DPPH solution. An aliquot of 0.2ml of diluted extract first introduced into 3ml of DPPH solution, followed by 30 minutes of incubation in the dark and then measurement for absorbance at 515 nm. The results were expressed as μmol trolox equivalent per volume of the sample (μmol/L).
3. Results and discussion

3.1. Effects of ethanol concentration on the extraction process
Ethanol concentration is one of the factors that significantly affects the extraction process. The effect of ethanol concentration on the extraction of polyphenol from pomelo peel was shown in Figure 1.

Figure 1. Effect of ethanol concentration on polyphenol content and antioxidant activity in the extract from pomelo peel

Figure 1 showed that the ethanol concentration 60% achieved the highest extraction content of polyphenol and DPPH. The polarity of the solvent depends on the dielectric constant and the hydrogen bond value, water has a dielectric constant and the hydrogen bond value is higher than ethanol, so when the two solvents are mixed, the mixture of ethanol - water has different polarity [13]. As a result, ethanol - water mixtures with different concentrations have different polarity and hydrogen bonding values. SC Moldoveanu also mentioned that the solvent mixture was used to obtain the desired properties of the solvent and the extraction efficiency of the mixture was higher than for each individual solvent. If the polarity of the substances in the material corresponds to the polarity of the solvent, that will make the substances that are soluble in the solvent better [14]. Selection of extraction solvents is critical for the complex plant materials as it will determine the amount and type of phenolic compounds being extracted. Spigno, Tramelli, & De Faveri, 2007 used 50% aqueous ethanol that is one of solvents to extract phenolic compounds from Citrus Sinensis peels and the content of polyphenol was $6.29 \pm 0.77$ mg GAE g$^{-1}$[18]

3.2 Effects of microwave extraction time and microwave power on the extraction process
The continuous and intermittent microwave-assisted extraction were done in 5 minutes, the polyphenol content and antioxidant activity of two extraction processes were shown in the Figure 2.
From Figure 2, it can be seen that whether the extraction process was intermittent or continuous, at 300W, the most effective microwave-assisted time were 2 minutes. Continuous microwave-assisted extraction obtained higher polyphenol content and antioxidant capacity than those of intermittent extraction. If the extraction time is too short, the microwave energy may be insufficient; and if the irradiation is too long, energy of microwave will degrade extracted compounds [15]. Every compound that to be extracted will has a specific microwave-assisted time extraction although that period is short [16]. It may be explained that during five-minute extraction process, the essential microwave energy of the continuous microwave-assisted extraction was higher than those of the intermittent extraction. When the microwave-assisted extraction time was 1 minute, the microwave energy was low, but when the extraction time was increased to 3 minutes, 4 minutes and 5 minutes, the content of the extracted substances gradually decreased. When microwave-assisted extraction time was 2 minutes, the content of polyphenol and antioxidant activity were highest, it mean that microwave energy in 2 minutes was sufficient to extract the substances in the material. Therefore, with continuous microwave-assisted extraction, 2 minutes was chosen to perform the next experiment.

With the continuous microwave-assisted extraction, 2 min extraction time, the effect of microwave power on polyphenol content and antioxidant activity was verified and shown in Figure 3.
From the Figure 3, it can be seen that the lowest extracted substance contents were followed by microwave power 800W and the second lowest content by 100W, 300W, 400W and 600W obtained the extracted substance contents with no significance differences at significance levels P <0.05. The results from this experiment are similar to those of authors (Yi Zhang et al.). They studied the microwave-assisted extraction and antioxidant activity of polyphenols from lotus seeds (*Nelumbo nucifera Gaertn.*), and concluded that the 300W output achieves the optimum extraction yield [19] Therefore, microwave power of 300W was chosen to perform the next experiment.

3.3. Effects of solid to solvent ratio

The effect of solid to solvent ratio on polyphenol content and antioxidant activity was shown in Figure 4. Based on the results of Figure 4, 1:30 ratio achieved the highest polyphenol and DPPH content in the extract. According to research by Rostagno [16], if the solvent content used for extraction is too small, it will not flood the whole material, so the material does not absorb enough solvent during extraction, so lower extracted efficiency. The author also explained that, for microwave-assisted extraction, the higher the extraction solvent content, the extraction efficiency is also low because the microwave energy reduces impacting on the solvent, temperature mixture, friction between molecules, resulting in a decrease in the content of the extracted substances. In addition to the large amount of solvents, greater capacity and time are needed to achieve the required temperature for extraction, and the large amount of solvents can also cause the dissolution of unwanted compounds, reduces selectivity for the desired compound. Farid Dahmoune et al., 2014 also studied the optimization of microwave extraction of polyphenol from *Myrtus communis* L. leaves at the material : solvent ratios of 1:10, 1:20 and 1:30 in RSM. The results obtained at the rate of 1:32 that showed the highest polyphenol content [17]. Therefore, the material to solvent ratio of 1:30 was suitable to extraction process.
Figure 4. Effect of solid to solvent ratio on polyphenol content and antioxidant activity in the extract from pomelo peel

4. Conclusion
The microwave-assisted extraction of polyphenol by from pomelo peel to take advantage of by-products in food processing. Research had shown that continuous microwave-assisted extraction was more effective than intermittent extraction. Alcohol content, extraction time, microwave power, material to solvent ratio that significantly influence the extraction process were the parameters to be investigated. Ethanol concentration of 60%, microwave power of 300W, microwave-assisted time of 2 minutes, and material to solvent ratio of 1:30 were significant parameters that obtained in the research.

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