Original Research Article

Microwave Extraction of Polyphenol from Pomegranate Seed

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ABSTRACT

The effects of different synthetic conditions on the yield of polyphenols in pomegranate seeds were studied by controlling the solvent concentration, liquid / liquid ratio and microwave heating power by microwave heating method using pomegranate seed as raw material and ethanol as solvent. The results showed that the yield of pomegranate seed polyphenols was 3.14% when the solvent concentration was 40%, the ratio of material to liquid was 1:20 and the power was 400 W.

KEYWORDS: plant polyphenols; microwave method; extraction

1. Introduction

1.1. Overview

Plant polyphenols are abundant in the plant, second to cellulose, lignin and hemicellulose, it is mainly in the plant fruit's peels, edible fruits parts, seeds, it present in some plants' branches and roots in large number [1].

Plant polyphenols are not limited to hydrolyzed tannins as thought in traditional consideration, tannins contained in plants and compounds associated with tannins, collectively referred to as plant polyphenols. According to tannins, can be divided into hydrolyzed tannins and condensed tannins. According to the structural characteristics, it also can be divided into poly gallate and polypentanol polyphenols. At the same time, plant polyphenols can be subdivided into: simple phenolic acid, flavonoids, hydrolyzed tannins, procyanidins.

Simple polyphenols are: catechol, resorcinol, hydroquinone, caffeic acid, ferulic acid, chlorogenic acid. Their structure is as follows:

Catechol  Resorcinol  Hydroquinone  Caffeic acid  Ferulic acid  Chlorogenic acid.
Flavonoids [16] are: flavonoids, flavanols, dihydroflavones, isoflavones, anthocyanins, chalcone, flavonols. Representatives are the following:

- Luteolin
- Quercetin
- Catechins
- Hesperidin
- Phlorizin
- Genistein
- Pelargonidin

Hydrolysis of tannins are: gallic acid and ellagic acid. The main structure is as follows:

- Gallic acid
- Ellagic acid
- Hydrolyzed Tannin
The proanthocyanidins are as follows:

\[
\text{N is 2-4, it is the OPC (oligo proanthocyanidins); } n \geq 5 \text{ is the PPC (polymeric proanthocyanidins) where OPC is an antioxidant, which can remove a lot of free radicals in the human body [3].}
\]

1.2. Extraction method of plant polyphenols

The existence of plant polyphenols is mainly in three kinds: free state, binding state and esterification state. Free plant polyphenols are mainly proanthocyanidins and flavonoids, phenolic acids are mostly phenolic and esterified phenols. In terms of the content of polyphenol in plants, different plants have different content of it, even the same organisms have significant difference of content under the non-stop growth and different growth conditions. Therefore, for different plants, plant polyphenols extraction methods are different, the main extraction methods are: Soxhlet extraction method, solvent extraction method, high pressure fluid extraction method, supercritical extraction method, ultrasonic extraction method.

1.3. Application of plant polyphenols

With the increasing demand for living standards, the study of plant polyphenols has received more and more attention, which is the most widely used aspect of health care products and cosmetics additives. In addition, its unique antioxidant properties of plant polyphenols in food, seafood processing, colored pastries, lactic acid drinks played an important role.

The above is the common aspects that can be seen in our daily life. The main direction of the future development are: fine chemicals, chemical modification, synthesis and biological control. In the fine chemical industry, mainly to classify the plant polyphenols based on different characteristics and molecular weight, and then applied to different areas; in the field of chemical modification, due to the plant polyphenols contain more phenolic hydroxyl which is strong hydrophilic, this hydrophilicity feature cause malpractice in its physiological activity, it can be modified to a certain level of hydrophobicity through chemical modification. The main chemical modifications are: derivatization reactions such as acylation, etherification, esterification, azoization, etc.; synthetic and biological control, mainly use the biosynthesis and chemical synthesis methods to obtain a special structure of plant polyphenols. For example, the structure of functional group in plant polyphenols is controlled to regulate the nutrient in plant, thereby causing the pests to be resistant to the plant.

Application of plant polyphenols in medicine

Plant polyphenols have anti-virus, antibacterial, anti-tumor [4], anti-cancer, anti-virus, anti-cardiovascular disease, anti-oxidation, relieve osteoporosis [5] and other pharmaceutical mechanisms.

Antibacterial effect is mainly manifested as it inhibits the growth of certain microorganism under the premise of it maintain the normal function of the body, so as to achieve the effect of detoxification. For example: Hydrolyzed tannins which contained in the rhizome of water lilies can be used to treat some diseases of pharyngitis and ocular infections. The well-preserved persimmon tannin can control the reproduction and growth of diphtheria, staphylococcus and tetanus and thus to achieve antibacterial effect. The new application of plant polyphenols in anti-AIDS has aroused people's attention. Plant polyphenols can inhibit the activity of reverse transcriptase of HIV RNA, which can effectively
prevent the replication of virus in the cell, so the virus cannot be expressed so that the disease is well controlled. At the same time, you can also prevent adhesion of the virus on the cell, to prevent more cells infected by the virus.

Plant polyphenols contain oxygen free radicals which have a strong ability to scavenge oxygen [2], thus it has a strong antioxidant function. Intake of plant polyphenols can remove a lot of free radicals in the body to maintain the activity of the skin and the elasticity of blood capillaries wall. Plant polyphenols can chelate with metal ions with their unique functional groups, which then reduces the catalytic effect of metal ions on other oxidative reactions in the body. Plant polyphenols can also selectively regulate and control the synthesis of certain enzymes, for example that many plant polyphenols can usually inhibit the related enzyme activities during the production of oxygen free radicals. At the same time, it can activate some antioxidant enzyme activity to promote the production of antioxidant enzymes, thus can reduce or eliminate free radicals, reduce oxidative stress [15]. Plant polyphenols are associated with the hydrogen bonds of organic acids to form a large number of stable hydrogen donors, which can improve antioxidant properties. In addition, plant polyphenols interact with a variety of vitamins, not only can maintain the level of vitamins, and can extend the antioxidant time.

Anti-tumor and anti-cancer effect of plant polyphenols is receiving more and more attention. The inhibition of plant polyphenols towards tumors and viruses is staged, cannot be completed within a step. For example: if the polyphenol can inhibit the enzyme activity during the precursor activation of the pathogen, then it can directly kill carcinogens. Although many polyphenols do not have the ability to kill mutated cancer cells, they mostly have the potential to reduce the incidence of cancer or inhibit the proliferation of cancer cells. In vivo experiments of polyphenols show that caffeic acid and ferulic acid can combine with nitrogen to prevent the formation of nitrosamines, thus to inhibit the 7,12-dimethyl phenylpropanate-induced skin cancer in mice.

The application of plant polyphenols in anti-cardiovascular and cerebrovascular diseases is particularly extensive. The specific principle of preventium is that plant polyphenols can inhibit platelet aggregation, improve blood rheology, reduce blood lipid concentration, induce vasodilatation, anti-thrombosis, inhibit collagenase activity, and reduce the risk of atherosclerosis and hypertension. Plant polyphenols can also prevent the occurrence of diabetes, mainly by inhibiting the activity of enzymes to regulate postprandial blood glucose levels. Plant polyphenols can also regulate the immune system, inhibit hypersensitivity reactions, with anti-allergic activity.

Application of plant polyphenols in functional foods

There are a lots of food are rich in plant polyphenol, such as honeysuckle contains chlorogenic acid has antibacterial and benefits gallbladder properties; Broadleaf Holly leaf contains small amount of acteoside which with antioxidant, protects liver and anti-hepatitis; purple daisy contains chlorogenic acid which has the role of inhibition of HIV integrated enzyme activity; mulberry contains glucose, tannin and malic acid which can improve functions of liver and kidney [11], nourishing blood and body fluid; large number of polyphenols in pomegranate's peel, flesh and seeds can be used as raw materials for health care products; coumaric acid in spinach is a good antioxidant. Thus, the plant polyphenols can be used as edible pigments (sorghum pigment, lycopene, lutein), antioxidants [13] (tea polyphenols, grape polyphenols, apple polyphenols), preservatives (tea polyphenols, grape polyphenols, apple polyphenols), nutritional fortifiers (lycopen, pomegranate polyphenols, apple polyphenols).

Plant polyphenols can be used the precursor of the pigment production, plant polyphenols are easier to be oxidized, in the effect of plant polyphenol oxidase, it can be produced as quinones which are brown or reddish brown and it is an excellent choice for food coloring. The plant polyphenols auxiliary color function has advantages and disadvantages. For example, in the black tea, wine and other dark drinks, the color change of polyphenols oxidation will cause the drinks more beautiful. In addition, polyphenols are good auxiliary materials for some natural flower pigments. But in the presence of some protein or metal ions, polyphenols can easily become brownish, this will affecting the color of food or even taste.

Plant polyphenols can also be used as food additives [6], most of the food are required to add preservatives, flavoring agents, colorants, so plant polyphenols is a green and environmentally friendly food additive. Plant polyphenols can antioxidants by scavenging free radicals, in which tea polyphenols and gallic acid have been widely used in the industry. Most of the plant polyphenols have bitter taste, the polyphenols with smaller molecular weight polyphenols are more bitter taste. Therefore, plant polyphenols will affect the quality of food from both visual and taste aspects.

Application of plant polyphenols in daily cosmetics

With the improvement of living standards, beauty care is part of the lives of most people, pretty face is the ideal pursuit of everyone who likes beauty, especially for the modern female, it is the aspect that cannot be ignored. The contribution of plant polyphenols in the cosmetic aspects [12] cannot be underestimated. Plant polyphenols become consumer favorite due to its unique physiological activity and natural activity.
Plant polyphenols has the special physical and chemical properties that cause it to have anti-oxidation, anti-wrinkle [14], radiation, whitening and moisturizing beauty effect. Plant polyphenols have good astringent and adhesion effect, can make thick pores shrink, skin firmness, wrinkles reduced, to improve the skin from inner part to the outer part, so that the skin is beautiful and shiny. In addition, plant polyphenols can absorb ultraviolet region, thus can be used as anti-aging and sunscreen cosmetics active ingredients. As the phenolic substances can inhibit the activity of catalase, it can be combined with vitamins to prepare whitening agents. Plant polyphenols can also be used in combination with carbohydrates to prepare hair dyes, moisturizers, deodorants and the others.

1.4. Factors affecting the extraction of plant polyphenols

Temperature

Plant polyphenols are more sensitive to temperature, and the effect of temperature on its extracted yield is also obvious. Increased of temperature in extraction can reduce the viscosity of the solvent causing the rate of diffusion of polyphenols in the solvent increase, but too high of temperature may lead to partial decomposition of polyphenols, so the higher the temperature is not the higher the yield, thus in the experiment should try to avoid high temperature to prevent polyphenol decomposition.

Solvents

Plant polyphenols contain hydroxyl, according to the principle of similar compatibility, solvent selection for plant polyphenol mostly are water, low alcohol, ethyl acetate, acetone and so on. According to the previous literature, acetone-water system [8-10] is the best for extraction of plant polyphenols, but acetone is toxic, and the extraction cost is high, and it is flammable and explosive in the reaction process, therefore, water and alcohol are mostly used in extraction.

pH value

When the pH value is low, the plant polyphenols are mostly in the molecular state. When the pH value is high, the plant polyphenols are mostly in the form of negative ions. Therefore, when extracting polyphenols, the pH of the extract is adjusted in real time.

2. Materials and Methods

2.1. Experimental reagents and instruments

Reagents: anhydrous ethanol (Tianjin Daming Chemical Reagent Factory), distilled water (laboratory), ferrous sulphite (Shanghai Fine Chemical Plant), sodium dihydrogen phosphate (Huzhou Linghu Chemical Co., Ltd.), dipotassium hydrogen phosphate Hongrui Chemical Materials Co., Ltd.), potassium tartrate (Samsung Chemical Materials Co., Ltd.), gallic acid (Wufeng Chicheng Biotechnology Co., Ltd.) are analytical pure.

Instrument: UV-2450 UV Visible Spectrophotometer (manufactured by Shimadzu Corporation), AL204 Electronic Balance (METTLER TOLEDO Instruments Shanghai Co., Ltd.), grinder, microwave oven, constant temperature water bath pot, oven (Shanghai Pengshun Scientific Instrument Limited company), conventional glass instruments.

2.2. Reaction principle

After the raw materials were ground into powder form using grinder, the extract is obtained under the condition of anhydrous ethanol as solvent and microwave heating and constant temperature water bath. Pomegranate seed polyphenol extract is mainly flavonoids, condensed tannin and hydrolyzed tannin. Most of the flavonoids, tannins compounds contain more carbonyl and hydroxyl, showing weak acidity, thus their solubility will be greater in alkaline solvents.

2.3. Experimental steps

Preparation of experimental materials

Pomegranate seeds were stripped and pressed with a pressure machine, seeds were obtained and the flesh on surface were cleaned using distilled water, put into the beaker, should not put too much of it into the beaker and the datum line is to cover the bottom of the beaker. The beaker is placed in the oven, set the temperature of 40 oC, dried for 12 h after the removal, grind it after cooling using grinder. Put the powder into the reagent bottle, seal it and avoid light.
Preparation of solution

1. Preparation of ferrous tartrate solution

5 g of sodium tartrate powder and 1 g of ferrous sulfate powder were weighed with an electronic balance. The mixture was mixed into a volumetric flask and set to a capacity of 1000 mL in a dark cool environment.

2. Preparation of buffer solution

Accurately weighing 60.2 g sodium dihydrogen phosphate and 5.0 g sodium hydrogen phosphate, mixed with distilled water dissolved in the volumetric flask to set the volume of 1000 mL, placed in a dark environment.

3. Preparation of standard solution and ethanol solution

Accurately weigh 25 mg of gallic acid powder, dissolved in the volume of 500 mL volumetric flask, placed in a dark environment. Cylinder was used to measure the volume of anhydrous ethanol solution into different flasks according the percentage of 20%, 40%, 50%, 60%, 80%, and placed in a dark environment, the bottle stopper was make sure to close tightly to prevent volatilize.

Study on the best extraction conditions

1. Determination of optimum solvent concentration

Six beakers were filled with 2 g of pomegranate seeds powder, in the same condition, 20%, 30%, 35%, 40%, 50%, 60% ethanol solvent was added to the six beakers according to the liquid to material ratio 1:25. Sealed and kept in the dark for half an hour and then heated in a microwave oven (300 W). After heating, shake it in a sonicator for 45 minutes, then heat it in the water bath for one hour at 40 °C. The wet extract was undergoing decompression vacuum and the resulting solution was concentrated. The 5 mL concentrate was accurately pipetted in a 25 mL volumetric flask and 4 mL of distilled water and 5 mL of tartaric acid were added. The solution was finally diluted with a phosphate solution to the mark, and the gallate solution was used as a blank sample instead of the concentrated solution and the same samples were added as above and were measured in an ultraviolet-visible spectrophotometer at the absorbance 540 nm, and record.

2. The best ratio of material to liquid

Seven sets of 2 g gypsum seed powder was accurately weighed, similar to the conditions as above, were filled into seven flask with confirmed concentration of ethanol solution according to the ratio of material to liquid such as 1:5, 1:10, 1:15, 1:20, 1:25, 1:30, 1:35 and were seal and kept in the dark environment for 30 mins. Then it was heated in microwave oven (300W) and placed in an ultrasonic wave for 45 minutes for sonication and then heated in a water bath for one hour at 40°C. The wet extract was undergoing decompression vacuum and the resulting solution was concentrated. The 5 mL concentrate was accurately pipetted in a 25 mL volumetric flask and 4 mL of distilled water and 5 mL of tartaric acid were added. The solution was finally diluted with a phosphate solution to the mark, and the gallate solution was used as a blank sample instead of the concentrated solution and the same samples were added as above and were measured in an ultraviolet-visible spectrophotometer at the absorbance 540 nm, and record.

3. Determination of the optimum heating power

Under the same environment condition, seven sets of pomegranate seed powder were weighed and filled into the beakers, according to the best liquid to material ratio and the best concentration of ethanol solution, sealed in the dark for half an hour, then put into the microwave for heating, adjust the power to 350 W, 450 W, 500 W, 550 W, 600 W, 650 W, heated for half an hour, placed in an ultrasonic wave for 45 minutes and heated in water for one hour at 40 °C. The wet extract was undergoing decompression vacuum and the resulting solution was concentrated. The 5 mL concentrate was accurately pipetted in a 25 mL volumetric flask and 4 mL of distilled water and 5 mL of tartaric acid were added. The solution was finally diluted with a phosphate solution to the mark, and the gallate solution was used as a blank sample instead of the concentrated solution and the same samples were added as above and were measured in an ultraviolet-visible spectrophotometer at the absorbance 540 nm, and record.

3. Experimental results and discussion

3.1. Effect of Ethanol Concentration on Polyphenol Yield

Because most of the plant polyphenols contain strong phenolic hydroxyl functional groups, according to the similar compatibility principle, plant polyphenols are produced more when dissolve in ethanol solution, so the plant polyphenols can react with metal ions, causing the metal ions to show color, thus the yield of plant polyphenol can be obtained. The conversion formula between absorbance and yield is as follows:
Improvement of Classification System of Pterospora and Gametophyte

\[ m = \frac{A \times 3.914 \times 100 \times L_1}{1000 \times M \times L_2} \]

\( m \) is the polyphenol yield, \( A \) absorbance value, \( L_1 \) volume of the sample, \( L_2 \) the volume of the standard sample, and \( M \) mass of the pomegranate seed powder.

Table 3-1 Table of polyphenol yields at different solvent concentrations

<table>
<thead>
<tr>
<th>Ethanol concentration (%)</th>
<th>20</th>
<th>30</th>
<th>35</th>
<th>40</th>
<th>50</th>
<th>60</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absorbance</td>
<td>0.251</td>
<td>0.281</td>
<td>0.287</td>
<td>0.301</td>
<td>0.279</td>
<td>0.272</td>
</tr>
<tr>
<td>Polyphenol production (g)</td>
<td>0.0491</td>
<td>0.0549</td>
<td>0.0562</td>
<td>0.0589</td>
<td>0.0546</td>
<td>0.0532</td>
</tr>
<tr>
<td>Yield (%)</td>
<td>2.46</td>
<td>2.75</td>
<td>2.81</td>
<td>2.95</td>
<td>2.73</td>
<td>2.66</td>
</tr>
</tbody>
</table>

Figure 3-1 Yield of polyphenols at different ethanol concentrations

Figure 3-1 and Table 3-1 showed that:

From the overall trend of the curve, it showed that the yield of plant polyphenols increases with the increases of ethanol solvent concentration when the concentration of ethanol solvent increased from 20% to 40%, but in the interval of 20% to 30%, the yield of plant polyphenols trend was steep and the trend of increasing in the interval of 30% to 35% was moderate. However, when the ethanol concentration continues to increase to more than 40%, the yield of plant polyphenols did not increase, but decreased.

The yield of the polyphenol obtained when the ethanol concentration was 40% was the highest. In the process of increasing the concentration of ethanol from 20% to 30%, with the increase of the percentage of ethanol, the extraction of plant polyphenol is more and more. This is because the plant polyphenols are not in an independent state. They bound to other saccharides, proteins and the others through hydrogen bonds or hydrophobic bonds, and the organic solvents have the ability to break these bonds, when the greater the concentration of ethanol used, the more phenol can be dissolved out. Therefore, within a certain range, with the increase in ethanol concentration, more plant polyphenols can be extracted out.
However, when the concentration of ethanol increased between 40% and 60%, the yield of plant polyphenols did not show the same increases, but with the increase of organic solvent, the yield of plant polyphenols was decreased. There are two main reasons: firstly, the plant polyphenol is a general term of a class of substances, which are not all kinds of compounds are soluble in organic solvents. Most of them are soluble in organic solvents, but some are soluble in water, and some are dissolved in both water and organic solvents. This is why to carry out the experiment to obtain best concentration of ethanol, when the organic solvent concentration increased, those polyphenols which is water-soluble cannot be extracted. Secondly, various substance that extracted by ethanol were mix together in the beginning, as ethanol can react with other substances other than polyphenols, or it can catalyze the reaction between other substances, thus to reduce utilization of ethanol. Therefore, the optimum extraction concentration of ethanol is 40%.

In addition, ethanol is not the only soluble solvent for plant polyphenols, effect of acetone - water system dissolution is also better, as well as ethyl acetate and other low – alcoholic solvent also can be used for dissolving polyphenols. But for the acetone-water system, because acetone is poisonous, it is too volatile, and the by-product after the reaction is more, thus most of the time it is not been used. For other low-alcoholic solvent and ethyl acetate, the market price is mostly higher than ethanol, it is not suitable for large-scale industrial production. The source of industrial anhydrous ethanol for preparation is common, and it is widely used in the preparation of plant polyphenols in industrial, thus reducing the industrial costs.

3.2 Effect of material and Liquid Ratio on Yield of Polyphenols

Table 3-2 Yield and Absorbance Table under Different Liquid Ratio

<table>
<thead>
<tr>
<th>Material-Liquid ratio</th>
<th>1:5</th>
<th>1:10</th>
<th>1:15</th>
<th>1:20</th>
<th>1:25</th>
<th>1:30</th>
<th>1:35</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absorbance</td>
<td>0.261</td>
<td>0.273</td>
<td>0.289</td>
<td>0.321</td>
<td>0.317</td>
<td>0.308</td>
<td>0.299</td>
</tr>
<tr>
<td>Yield (g)</td>
<td>0.0511</td>
<td>0.0534</td>
<td>0.0566</td>
<td>0.0628</td>
<td>0.0620</td>
<td>0.0603</td>
<td>0.585</td>
</tr>
<tr>
<td>Yield rate (%)</td>
<td>2.56</td>
<td>2.67</td>
<td>2.83</td>
<td>3.14</td>
<td>3.10</td>
<td>3.01</td>
<td>2.93</td>
</tr>
</tbody>
</table>

From the above chart, it showed that the yield of plant polyphenols extracted from pomegranate seeds is largest at about 1:20.

It showed from the curve that the yield of plant polyphenol is increased in the range of 1: 5-1: 15, and the yield of plant polyphenol is increased when the ratio of feed to liquid is increased from 1:15 to 1:20. When the ratio of material to liquid increased to about 1:25, the yield of plant polyphenols decreased.
Figure 3-2 The yield of polyphenols at different ratios

The choice of good solvent is a very critical step in the extraction of plant polyphenols, and the choice of solvent volume is also a very critical step, how to determine the ratio of material to liquid into raw materials at certain concentration of solvent, when these problems expanded to the field of industrial production, it will be a very important economic budget and interest rate calculation, so it is cannot be underestimated. The yield of the plant polyphenol extracted from the pomegranate seed is significantly increased when the material / liquid ratio is between 1:10 and 1:20, this is due to the increased of organic solvent ratio and the plant polyphenols can react with the solvent easily. When the material/liquid ratio become smaller and the volume of solvent increases, the volume of extracted plant polyphenol increases. Figure showed that production of polyphenols is not much change in the between of 1:15 to 1:25, this indicating that the amount of solvent added is saturated in dissolving the pomegranate seed plant polyphenol, thus there is no use to add more ethanol. In some other experiment, the excess ethanol can react with the other extraction and caused a series of side effect reaction, this is not only causing the source wasted, but also cause the production to mix with other substances and resulting in impurities of products, so it is best to determine the ratio of extract and solvent. And the best material to liquid ratio is 1:20.

We choose the best material liquid ratio is not only to save resources, in most of the organic chemical reaction, the occurrence of side effects to our extraction and separation has brought a lot of trouble. It is affecting the quality and function of production due to the existance of by products. In order to avoid the above situation, the choice of a suitable material to liquid ratio is a necessary step.

3.3 Effects of Different Heating Power on Plant Polyphenol Yield

Table 3-3 Yield of polyphenols under different heating power conditions

<table>
<thead>
<tr>
<th>Heating power (W)</th>
<th>350</th>
<th>400</th>
<th>450</th>
<th>500</th>
<th>550</th>
<th>600</th>
<th>650</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absorbance</td>
<td>0.232</td>
<td>0.224</td>
<td>0.263</td>
<td>0.241</td>
<td>0.223</td>
<td>0.202</td>
<td>0.189</td>
</tr>
<tr>
<td>Polyphenol Yield(g)</td>
<td>0.0450</td>
<td>0.0438</td>
<td>0.0515</td>
<td>0.0472</td>
<td>0.0436</td>
<td>0.0395</td>
<td>0.0369</td>
</tr>
<tr>
<td>Yield rate (%)</td>
<td>2.25</td>
<td>2.19</td>
<td>2.57</td>
<td>2.36</td>
<td>2.18</td>
<td>1.98</td>
<td>1.85</td>
</tr>
</tbody>
</table>

Figure 3-3 Yield of polyphenols at different heating power
The above figures and tables showed that the yield of plant polyphenols is the largest when the heating power is about 450 W.

When heated at different power, it is clear that the reactant response at different temperature. Temperature is a very important factor for a reaction. The use of microwave to control the different heating power indirectly controls the reaction temperature and heating conditions of the reactants in the solvent. Thus, as the heating power increases, the temperature increases, and the increase in temperature accelerate the motion of the extract molecules and increase the frequency of vibrations, which increases the probability of contact between the plant polyphenols and the solvent. The dissolution of polyphenols will become easier, so there will be a phenomenon as shown in the figure, with the increase in temperature, the production of plant polyphenols was increasing.

But when the heating power is too high, the temperature increases with the power, high temperature accelerated the oxidation and hydrolysis of polyphenols, organic matter will accelerate be decomposed in high temperature, and even being carbonized, which resulted decreased in the yield of plant polyphenols. At the same time, the organic solvent used is ethanol, is volatile faster at room temperature, in high temperature the volatile rate is higher, from the above-mentioned changes, solvent concentration also lead to increase or decrease of plant polyphenols production, so the extraction effect is better when the organic solvent heat power was 450 W. This also avoids the risks when use of higher power for heating.

The principle of microwave heating is very different from the ordinary heating method. The way of transferring energy is different. Microwave heating method is the way of transmitting energy, and the heat is diffused from the inside, which can increase the heating rate and reduce the loss of heat during transmission process, while shortening the reaction time, it improve the efficiency in the reaction in industrial production.

### 3.2. Color principle

In this experiment, tartaric acid method [2] was used to determine the content of plant polyphenol, that is, plant polyphenols could react with metal ions. The principle mechanism of color development can be understood from two aspects: first, the general organic reagent contains chromophores and auxochrome group, ordinary chromophores such as carbonyl, nitroso, nitro, nitrogen, they can be conjugated to the benzene ring or other structures, thereby reducing the molecular excitation energy, the absorption of light waves will move to the long wave direction, the compound will show color or turn into darker; similar, some have the group of solitary electrons can interact with the unsaturated bond of the chromophore, and then the effect of reducing the molecular excitation energy occurs as mentioned above, so that the wavelength of the absorbed light wave moves in the long wave direction and the coloration occurs. Second, the plant polyphenols have more phenolic hydroxyl and aromatic ring structure, so that the color reaction in general conditions will be more prone to occur. In this experimental test method is the phenomenon where Fe3 + or Fe2 + react with auxochrome OH- group and will the above-mentioned result occurs which is the absorption of light wavelength become longer, and color reaction occur, the fundamental reason is the formation of the complex makes the energy level difference becomes smaller, the electron transition absorbs the light wave becomes longer, the energy is reduced, this will show the red's complementary color which is blue violet, it is blue violet complex obtained in this experiment.

Raw material extracts polyphenols can react with metal ions to form complexes, showing purple-brown, the degree of color represents the content of polyphenols, the degree of color is proportional to the content of polyphenols. Since gallic acid is contained in plant polyphenols and gallic acid is almost coincident with the ultraviolet absorption spectra of various polyphenols in UV-visible spectrophotometers, gallic acid is chosen as the standard solution. The obtained extract was added with ferrous tartrate and a buffering solvent, the absorbance was measured at 540 nm, and the total phenol content was calculated by the absorbance value.

### 4. Conclusion

In this experiment, the optimum conditions for the extraction of plant polyphenols from pomegranate seeds were as follows: ethanol concentration 40%, material/liquid ratio 1:20, heating power 450 W, the highest yield rate is 3.14%.

### References

14. Li Zhizhou. Microwave assisted extraction of polyphenols from pomegranate leaves. Food and Machinery, 2009, 25 (4), 72-75.