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A Green Aqueous Extraction of Peanut Oil using Microwave and Demulsifier

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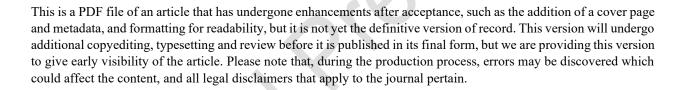
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**Abstract** 

In this study, distilled water was used as a sustainable solvent for extracting oil from peanut using a salt-assisted

microwave technique. In this regard several critical parameters were considered to achieve an optimal oil yield result.

The oil extraction yield was compared to that of obtained by soxhlet (43%) with n-hexan. In aqueous salt-assisted

microwave extraction (ASME) method, NaCl and CaCl2 were considered to promote the oil-water demulsification.

Accordance to the results, an oil extraction yield of 32.08% was obtained at optimum operating conditions including

of the calcium chloride concentration of 0.4 M, water to sample ratio of 20 ml/g, leaching time of 120 min, microwave

radiation time of 8 min and microwave power of 720 W. The GC analysis did not show significant differences between

the fatty acids profile of the oil extracted through the ASME and the soxhlet extraction (SOXE) methods. In both the

extraction methods oleic and linoleic acid were the dominant fatty acids in the peanut oil. Some of the physicochemical

properties of the oil such as acid value, iodine value, peroxide value, density and refract index were considered that

there were no considerable differences between the two methods, just the acid value of the extracted oil by the ASME

method was slightly more than the SOXE method. In addition, the research on the total phenolic content and

antioxidant activity of the extracted oil showed slightly better results for the ASME method compared to the SOXE

method. Therefore, the ASME method is a valid technique to obtain an extra green part of peanut oil to consume in

the food industry.

Keywords: Peanut, Oil, Extraction, Water, Microwave, Demulsifier

#### 1. Introduction

Peanut (*Arachis hypogaea L.*) also known as the groundnut, belongs to the *Fabaceae* (*Leguminacease*) family. This plant is grown principally for its valuable edible oil and protein-rich seeds. The oil content of the peanut seeds is reported to be from 40 to 56% w/w depending upon genotype, variety and plant maturity [1, 2].

In the last two decades, some new extraction methods such as ultrasound-assisted [3, 4] and microwave-assisted [5, 6] extraction were developed to promote the speed and efficiency of the extraction of bio-compounds from plants, but even in these methods, application of chemical solvents has not reached to zero. The chemical solvents, after several purification and reuse, eventually are discharged into the environment as swage that it is a serious threat to living organisms and environmental health. Also, despite the costly post-extraction processes for the complete removal of the chemical solvents, parts of them always remain in the final products, that it is associated with many health risks for humans and animals such as chronic, acute toxicity and carcinogenic effects.

Many attempts have been made to use the water as the greenest solvent for extracting of non-polar compounds from plants. For example, in the water supercritical method, the water was used to extract oil [7]. Salt effect-assisted aqueous extraction process is another method that uses water as a solvent to extract oil. In this method, to break down an oilwater emulsion, a small amount of salt is added to water. This process helps to separate the oil droplets from water, allowing them to be more easily removed or recovered [8-10]. In general, mineral salts, conventional heating and microwave radiation have positive effects on demulsification of the oil-water mixture [10]. One reason for the positive effect of inorganic salts on the oil-water demulsification is the increase in density of the water layer which leads to the forced downward motion of the emulsified water droplets and as a result easier phase separation. Another reason is the decrease in thickness of the double charge layer around the emulsion droplets which reduces the electrostatic barrier to coalescence of the same type of droplets [11]. In conventional heating, increasing the Brownian motion of the emulsion liquid droplets with increasing the temperature leads to disruption of the double charge layers around the droplets, resulting to facilitate the coalescence between same type droplets through the surface tension. Also reducing the viscosity of the mixture at high temperatures leads to more easy coalescence of similar droplets and consequently forced oil-water demulsification [12]. The effect of microwave on the oil-water demulsification is divided to thermal and non-thermal features. The non-thermal effect is due to the electromagnetic field of the microwave that neutralizes the zeta potential on the surface of the emulsion droplets via induced dipole molecule rotation. Microwave thermal effect works by destroying the double charge layers around the emulsion droplets and decreasing the viscosity of the

water [13]. Microwave heating originates from two mechanisms including dipole molecule rotation and ionic conduction, that both generate frictional heat. Demulsification through microwave heating is much faster and more efficient than the conventional heating due to its unique heating mechanism. While conventional heating occurs via conduction/convection, microwave heating occurs owing to the direct interaction between polar molecules (or ionic species) and radiation. According to the studies, the presence a very small amount of inorganic salt in a solution enhances the microwave frictional heating due to the increased ion conductivity [11]. Xia et al. [11] reported that the addition of a small amount of inorganic salt (NaCl, MgCl<sub>2</sub>, CaCl<sub>2</sub>, KCl) to a water-oil emulsion, reaches the demulsification efficiency to 100% under 120-150 s microwave radiation (2450 MHz). Martinez-Palou et al. [13] reported 83.6% demulsification for an oil-water emulsion under 10 min microwave radiation (2450 MHz) at the presence of a few amounts of NaCl.

Although there are several papers related to the extraction of oil from peanuts by conventional and modern methods [2, 14, 15], but to the best of our knowledge, no study has been conducted for oil extraction from peanuts using aqueous saline solution as solvent. In this study, an aqueous saline solution was used as a green solvent to extract peanuts oil by microwave radiation technique and the results were compared to the SOXE method with hexane. In the ASME, the effect of NaCl and CaCl<sub>2</sub> and some other main parameters including leaching time, water to sample ratio, microwave power and radiation time were considered and optimized. GC analysis was used to identify the fatty acid profile of the extracted oil. Also, for more qualitative evaluation, some of the physicochemical properties, total phenolic content and antioxidant activity of the extracted oil were measured accordance to the standards.

#### 2. Experimental

#### 2.1. Materials

Peanuts were purchased from a local market. Their brown shells and any other foreign impurities were separated by hand. Clean peanuts were milled and sized in mesh size of 60. Hexane was from Fluka and other reagents and chemicals were obtained from Merck Company.

#### 2.2. SOXE method

The SOXE is a common and standard extraction method that usually is used as a reference to evaluate the efficiency of the other extraction methods. In this study, a classic soxhlet apparatus was applied for the extraction of oil from 10 g peanut powder with 150 ml n-hexane for 8h. Complete removing the solvent from the extract was carried out by a

rota-vapor (BUCHI, R-114) at reduced pressure and temperature of 50°C. The oil extraction yield was calculated as follow:

Oil extraction yield% = 
$$(W_o/W_s) \times 100$$
 (1)

Where  $W_o$  is the mass of the extracted oil (g) and  $W_s$  is the weight of the peanut sample (g). The oil extraction yield by SOXE was obtained 43%.

#### 2.3. ASME method

Microwave-assisted extraction was performed using a home microwave oven (MOD 8084WR-LG). First, peanut powder was leached in distilled water (5 ml/g) in a beaker for a certain time, and then a certain amount of salt water solution with a certain concentration was added to it and placed into the microwave oven and radiated in periods of 30 s with intervals of 15 s. Next, the mixture was centrifuged (VISION, Scientific Co., LTD, VS-400) at 4000 rpm for an average time of 10 min and the crude oil was collected from the surface of the water by a 1ml pipette, transferred into the Petri-dish and oven-dried at 50°C. Some main parameters including leaching time (20-140 min), water to sample ratios (5-25 ml/g), salts aqueous concentration (0-0.6 M), microwave output power (180-900 W) and radiation time (2-10 min) was investigated.

#### 2.4. Fatty acids composition

Gas chromatography (GC) was used to analysis fatty acid composition of the peanut oil. A GC 6890N gas chromatograph equipped with a flame ionization detector (FID) and a HP88 capillary column of 100 m length, 250 μm diameter, 0.25 μm thickness was used. The detector temperature was programmed at 250°C. H<sub>2</sub>, air and He at flow rates of 30, 300 and 25ml/min respectively were used as auxiliary gas for the FID detector. Helium was used as carrier gas at a constant flow rate of 0.5 ml/min. Injector temperature was set at 220°C and 1 μl of the sample was injected with split inlet mode at a split ratio of 100:1. The following oven temperature ramp program was used: Initial temperature of 180°C, maintained for 5 min, followed by an increase of 1°C/min up to 190°C, maintained for 20 min and then 1°C/min up to 200°C, maintained for 37 min.

#### 2.5. Physicochemical properties analysis

The physiochemical properties of the peanut oil were analyzed according to the AOCS official methods with slight modifications [16, 17]. Acid value (AV) was measured via titration of a solution of oil in ethanol with a solution of potassium hydroxide in the presence of phenolphthalein as an indicator. The iodine value (IV) was determined by the Wijs method. Peroxide Value (PV) was determined by adding potassium iodide to a solution of oil in chloroform/acetic

acid (2:3 v/v) and then titration of produced iodine with sodium thiosulphate. The density of the oil was measured by a pycnometer equipped with a thermometer with an accuracy of  $\pm 1^{\circ}$ C. Refract index (RI) was measured by a classic refractometer (Carl Zeiss Abbe type G).

#### 2.6. Determination of total phenolic content

Tocopherols and phytosterols are groups of fat-soluble polyphenol compounds in peanut oil with antioxidant activity. Folin-ciocalteu assay was used to determine the phenolic content in the peanut oil according to the literatures [18, 19] with some modifications. The total phenolic content was quantified using a calibration curve of Catechin (R<sup>2</sup> = 0.99) and results were expressed as milligrams of Catechin equivalent (CAE) per gram of oil (mg CAE/g oil). Peanut oil (1 g) was extracted for phenolic compounds with 10 ml methanol via 10 min shaking (Wisd, VM-10) at room temperature. 0.5 ml of the methanolic extract was diluted 3 times with distilled water, 1.5 ml Folin-Ciocalteu (10%) reagent was added to it and well mixed for 30 s by shaker, next 1 ml NaCO<sub>3</sub> (7%) was added to the mixture and mixed for another 30 s. The solution was kept in the dark at room temperature (23 ± 2°C) for 40 min, next its absorbance was measured at 745 nm using a spectrophotometer (Perkin Elmer Lambda 25).

#### 2.7. Determination of radical scavenging activity

The quality of the extracted oil in term of antioxidant activity was evaluated by free radical scavenging capacity against to 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical. This parameter shows the ability of the sample to reduce free radicals through hydrogen or electron donation. The color change of the DPPH assay solution from violet to yellow indicates the presence of antioxidants in the sample. This test was performed based to the literatures [20, 21] with minor modifications. Briefly, 3 ml of a 0.1 mM freshly prepared methanolic solution of DPPH was added to 1 ml of the oil solution in DMSO (0.5mg/ml) and well shaken vigorously for 10 s. It was kept in the absent of light at a temperature of 30°C for 30 min and then its absorbance was read at 520 nm against a control sample comprising an equal amount of DPPH in 1ml DMSO and the inhibition percentage of the free radical DPPH° (DPPH%) was calculated using the following equation:

$$\text{%DPPH}^{\circ} = [(C - S)/S] \times 100$$
 (2)

Where S is the absorbance of the sample and C is the absorbance of the control sample.

#### 2.8. Statistical analysis

All experiments were repeated 3 times and the results were expressed as a mean value. Standard deviations ranged from  $\pm 0.48$  to  $\pm 1.03$ .

#### 3. Results & Discussion

#### 3.1. Effect of salt type & aqueous saline concentration on the oil extraction yield

The effect of two different salts (NaCl and CaCl<sub>2</sub>) on the oil extraction yield was investigated at operating conditions including of 3 min microwave radiation time, 15 ml/g water to sample ratio, 540 w microwave power and 80 min leaching time. As shown in Fig. 1, both of the salts in dilute concentrations (up to 0.4 M) had a positive effect on the oil extraction yield compared to the control samples with zero amount of salt. The positive effect of a small amount of salt on improving the oil extraction yield by microwave is because of free ions that increase the electrical conductivity of the solution, lead to more frictional heat production under the electromagnetic field of the microwave, and as a result cause more efficient extraction and demulsification. On the other hand, as the results shown, a greater salt concentration had an inverse effect on the oil extraction yield that it may be due to the inverse effects of microwave overheating on the oil extraction and demulsification [22, 23]. Accordance to the results, CaCl<sub>2</sub> had a greater effect on the oil extraction yield compared to the NaCl and was therefore selected for the following experiments. It is because of that each mol of CaCl<sub>2</sub> produces 3 mol ions in the water while about NaCl that is 2 mol, so CaCl<sub>2</sub> creates more ionic strength in water compared to NaCl and thus its effect on dielectric loss and microwave coupling is greater. The positive effect of NaCl and CaCl<sub>2</sub> on improving the oil extraction yield by microwave was in accordance with other reports in the literatures [10, 11].

Fig. 1. Effect of salt concentration on the oil extraction yield

#### 3.2. Effect of leaching time on the oil extraction yield

The effect of leaching time on the oil extraction yield was investigated at operating conditions including of 3 min microwave radiation time, 15 ml/g water to sample ratio, 0.4 M CaCl<sub>2</sub> and 540 W microwave power. As shown in Fig. 2, the oil extraction yield enhanced with increasing the leaching time up to 120 min. This is due to this fact that with increasing the leaching time, a greater amount of water penetrates into the peanut cells and so greater water vapor creates inside the cells during the microwave radiation and as a result, the cell collapsing take places more efficient. Water molecules are dipole so under microwave radiation begin to fast rotation owing to align themselves with the microwave oscillating electromagnetic field, thus the enormous frictional heat generated results to evaporate the water

molecules inside the cell and subsequently an increase in the internal pressure of the cells take places which leads to the destruction of the cell walls and the release their contents into the solvent bulk.

#### Fig. 2. Effect of leaching time on the oil extraction yield

#### 3.3. Effect of microwave radiation's power on the oil extraction yield

The effect of microwave's power on the oil extraction yield was investigated in the presence of 0.4 M CaCl<sub>2</sub>, microwave radiation time of 3 min, water to sample ratio of 15 ml/g and leaching time of 120 min. As shown in Fig. 3, the oil extraction yield increased with the increasing the microwave radiation power. At high powers microwave radiation, more energy is transferred into the water inside the cells and more frictional heat generated, leading to an increase in the rate and efficiency of the cell wall disruption. Another reason for improving the oil extraction yield with increasing the microwave power is to promote demulsification of the oil-water via thermal and non-thermal effects of the microwave radiation. According to the results, there was no significant difference between the oil extraction yield at 750 and 900 W. Therefore, microwave power radiation at 750 W was chosen for subsequent experiments to prevent overheating which can lead to the reduction of the oil quality due to some undesired reactions such as degradation, oxidation and polymerization. Also overheating can lead to more emulsification because of the violent collision of the molecules inside the mixture [24].

#### Fig. 3. Effect of microwave radiation power on the oil extraction yield

#### 3.4. Effect of water to sample ratio on the oil extraction yield

The effect of water to sample ratio on the oil extraction yield was investigated at the presence of 0.4 M CaCl<sub>2</sub>, microwave radiation time of 3 min, microwave power of 720 w and 120 min leaching time. As shown in Fig. 4, the oil extraction yield increased to some extent with the increasing the water to sample ratio. In ASME method, water acts as a medium to transfer the microwave energy and also as a bearer for extracted oil. The amount of water used should be sufficient to completely immerse and float the sample. If the water is too low, the sample will conglomerate and so radiation will not take place evenly, resulting in inefficient extraction.

#### Fig. 4. Effect of water to sample ratio on the oil extraction yield

#### 3.5. Effect of microwave radiation time on the oil extraction yield

The effect of microwave radiation time on the oil extraction yield was investigated at the presence of 0.4 M CaCl<sub>2</sub>, microwave power of 720 W, water to sample ratio of 20 ml/g and leaching time of 120 min. As shown in Fig. 5, the extraction oil yield increased along with the increasing the microwave radiation time up to 8 min and then it decreased slightly. The microwave heating mechanism is different from the conventional heating methods. Unlike conventional heating, microwave heating is very fast and efficient in such a way that the whole mixture containing dipole molecules or free ions (or ionic species) is warmed during some seconds or minutes of radiation. Prolonged microwave radiation causes overheating which may have destructive effects on the compounds and adverse effects on the demulsification [24, 25].

Fig. 5. Effect of microwave radiation time on the oil extraction yield

#### 3.6. Physicochemical properties of the peanut oil

As shown in Table 1, there were no significant differences between fatty acids profile of the extracted oil by SOXE and ASME methods. Just amount of some certain fatty acids of the extracted oil by the ASME were slightly less than the SOXE method. This could be due to the partial degradation under overheating by microwave.

**Table 1.** Fatty acids profile of the extracted peanut oil

As given in Table 2, there were no significant differences between the considered properties of the extracted oil by ASME and SOXE method. The relatively greater AV of the microwave extracted oil was probably due to the partial hydrolysis of the oil to the glycerol and the free fatty acids. Also as shown in Table 3, differences between the total phenolic content and the antioxidant activity of the extracted oil by the two methods were low and the results for the microwave were slightly better than the soxhlet. Treatment of the plant oily seeds at high temperatures in the process such as roasting or microwave radiation increases the antioxidant activity of the plant oils through inactivation of the oxidative enzymes [26]. Another reason for antioxidant properties of the vegetable oils is the non-enzymatic Maillard reaction in oil, which produces phenolic products with strong antioxidant properties. [14, 19]. The results for physicochemical properties, total phenol content and antioxidant activity of the peanut oil were in an acceptable ranges [14, 18, 26, 27].

Table 2. Physicochemical properties of the extracted peanut oil

Table 3. Total phenolic content and antioxidant activity of the extracted peanut oil

#### 4. Conclusions

The results of this study showed that the ASME is a relatively good method to extract oil from peanut powder. The maximum oil extraction yield by this method was obtained 32.08% at optimum operating conditions including of calcium chloride concentration of 0.4 M as demulsifier, leaching time in water of 120 min, water to sample ratio of 20 ml/g, microwave radiation time of 8 min and microwave power of 720 W. Although the oil extraction yield obtained by this method was not as well as the SOXE, but according to the results ASME is a promising, healthy, clean and economical method for obtaining peanut oil for use in the food industries. This is due to the lack of organic solvents used in the extraction process, making it an eco-friendly option. The consideration of the fatty acid profile and some of the other physicochemical properties of the extracted oil did not show significant differences between the two methods. The investigation of the total phenolic content and antioxidant activity of the extracted oil using the ASME method showed slightly better results compared to the SOXE.

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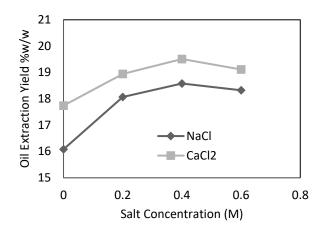


Fig. 1

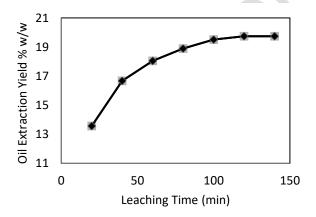


Fig. 2

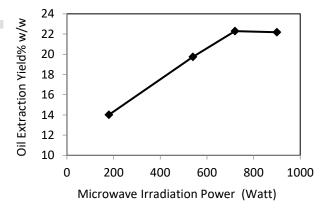


Fig. 3

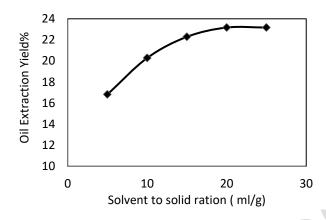


Fig. 4

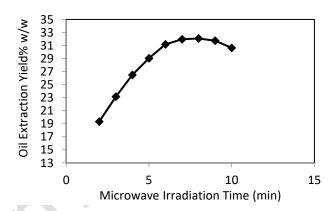


Fig. 5

Table 1

Fatty acid	† Composition (wt %)	‡ Composition (wt %)
Linoleic acid	23.36	23.33
Palmitic acid	10.25	10.19
Oleic acid	52.89	52.85
Stearic acid	2.18	2.18
Arachidic acid	1.32	1.28
Lionolenic acid	0.27	0.25
Behinic acid	0.98	0.98
Myristic acid	0.09	0.06

<sup>†</sup> SOXE, ‡ ASME

Table 2

Parameters	ASME	SOXE
Color (visual)	Light yellow	Light yellow
Density at 25°C (g/cm <sup>3</sup> )	0.90	0.91
Acid Value (mg KOH/g oil)	0.67	0.51
Iodine Value (g I <sub>2</sub> /100 g oil)	93.91	94
Peroxide Value (meq O <sub>2</sub> /Kg Oil)	0.85	0.9
Refractive Index at 23°C	1.461	1.459

Table 3

Parameters	ASME	SOXE
Total phenolic content (mg CAE/100g oil)	38.74	35.86
†DPPH°%	58.2	57.32

<sup>†</sup> Inhibition percentage of the free radical DPPH° (1, 1-diphenyl-2-picrylhydrazyl)