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Research Article

The Extraction and Properties of *Carica papaya* Seed Oil

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Abstract: The main objective of the present study was to evaluate the suitability of Ultrasound-Microwave synergistic Extraction (UMAE) for the recovery of papaya seed oil as compared to Ultrasound-Assisted Extraction (UAE). The efficiency of these two methods was assessed by comparing the physicochemical properties and oxidative stability of papaya seed oil. The analytic tests were color, unsaponifiable matters, iodine value, acid value and peroxide value. The fatty acid components were analyzed by GC. Results indicated that the Ultrasound-Microwave synergistic Extraction (UMAE) considerably impact on the physicochemical properties of the extracted papaya seed oil, UMAE provided papaya seed oil with obviously lighter color, lower unsaponifiable matters (2.53%) and higher oxidative stability (PV, 0.98 m mol/kg) than that of UAE. Analysis of fatty acid composition revealed that 13 kinds of components are identified and the total amount of fatty acids accounted for 93.13% of papaya seed oil, the predominant fatty acids in papaya seed oil were oleic (18:1, 72.60%), palmitic (16:0, 18.00%), linoleic acid (18:2, 5.80%) and stearic (18:0, 3.60%).

Keywords: *Carica papaya* seed oil, extraction, fatty acid composition, properties

INTRODUCTION

It would be produced a mass of waste came from the by-products of processing while engaged in agricultural production and utilized resources. Chinese agriculture has occupied an important place in the world that generated a great variety of agricultural residues. The agricultural waste were not handled properly which would resulted in serious pollution of environment and wasting of resources. With the improvement of agricultural production, the adjustment of the structure of agricultural industry and the depth development of agricultural resources, the new waste would be increased more and the possibility of taking advantage of Agricultural by-products (including waste) as traditional way would be reduced which led to the pressure of development and utilization of agricultural waste would be built-up.

In recent years, bio-recovery of valuable byproducts from agro-biomass wastes and underutilized products has become noticeable. Most fruit processing units have massive disposal of biomass waste issues (i.e., seeds, skin, pulp, etc.). Several studies have suggested the bio-recovery of different byproducts like enzymes, essential oils, ethanol and pharmaceuticals from fruit wastes (mango, banana, pineapple and papaya).

Papaya, known as *pawpaw*, pumpkin, which belongs to the family *Caricaceae* from *Carica* genus. According to research, *Caricaceae papaya* are originated in from tropical and subtropical America and Africa. *Papaya* as a perennial evergreen arbor has no branch tree, 3 to 10 height. Fruits and leaves grow from the trunk. Currently, many varieties of *papaya* (i.e., Sekaki, Eksotika and Eksotika II) are cultivated during last decades. *Caricaceae papaya* is mostly available in Hainan local markets that have medium size fruit with 1 to 2 kg weight. It appeals to attractive orange color and the fresh firms with green. It is one of the world's excellent fruit. *Papaya* is regarded as a good source of riboflavin and carotene, an excellent source of ascorbic acid and a considerable source of calcium, iron, thiamin, pantothenic, niacin and vitamin K (Chan, 2008). Being a short duration and highly remunerative fruit crop, it has enormous impact on economic, but it has restraint on export and is mostly available in the local markets cause of storage.

Papaya seed constitute a significant portion of fruit weight about 15 to 20%, which represent a considerable amount of papaya fruit waste in processing units. Papaya seeds have the potential to produce 30 to 34% oil with nutritional and functional properties highly similar to olive oil. However, the edibility of papaya seed oil has not been confirmed by previous studies. Besides, there is an excellent source of amino acid (about 24.91%), especially in the sarcotesta and fiber in seed (Saran *et al*., 2013). Papaya seed oil is liquid with reddish brown color. It is suggested that the oil was potential to be edible and industrial uses as which was made up of palmitic acid, oleic acid and linoleic acid.

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Moreover, *papaya* seed oil is steady against oxidation with vital antioxidant activity (Bouanga-Kalou *et al*., 2011).

Currently, the most commonly methods used for the recovery of oil from natural products are extrusion expelling extraction, Soxhelt (Bimake *et al*., 2012), solvent extraction, aqueous enzymatic extraction. Recently years, a high-efficiency extraction method such as Supercritical Fluid Extraction (SFE) has been used for the extraction of the liposoluble compounds from various plants. Subcritical (or superheated) Water Extraction (SWE) has been applied as a technique for extraction in numerous of recent studies. The superheated water occurs between 130 to 374°C which used to denote the region of the condensed phase (Hameed, 2009). Previous research have pointed out that superheated water is an available alternative method for extraction on account of it is capable of rapid extraction. The short-term and rapid effects result in avoiding the loss and degration of thermo labile compounds. In addition, the positive aspects of the application of superheated water extraction are its low cost, simplicity and no undesirable environmental effects.

The ultrasound-assisted extraction method has been applied for the bioactive ingredient and oil from plant source as an efficient cost effective way for the past few years. As certified by Bimake *et al*. (2012), the merits of ultrasound-assisted extraction, such as short extraction time, convenient procedure and high extraction yield, determined that it is an available extraction method. Ultrasound power results in extra vibration in sample molecules, enhances contact surface and cavitations between matrix and liquid solvent consequently improves the yield in the short time. Besides, ultrasonic device with the temperature controlling facilitates the recovery of the thermo sensitive ingredients.

Ultrasound-microwave synergistic extraction combines the microwave with advantages. Ultrasound and microwave radiation could expedite the extraction process and that might enhance extraction of bioactive ingredients. There exists an obvious inhomogeneous phenomenon during microwave treatment (Kwiatkowska *et al*., 2011). The dominate advantage of employ microwave approached is achieving time saving. The benefits of ultrasound are mass transfer intensification and cell disruption, enhanced penetration and capillary effects.

There is rarely published report about *papaya* seed oil extraction. The main purpose of this reach was to evaluate the extraction efficiency of papaya seed oil between ultrasound-assisted extraction and ultrasound-microwave synergistic extraction. It was hypothesized that Ultrasound-Microwave synergistic Extraction (UMAE) might be a more efficient method for the recovery of papaya seed oil than conventional extraction methods. The efficiencies of different extraction techniques were assessed by determining the recovery yield, physicochemical properties, oxidative stability and Fatty Acid Composition (FAC) of papaya seed oil obtained under different extraction conditions.

MATERIALS AND METHODS

Chemical and materials: *Papaya* (*Carica papaya* L.) seeds were collected from the maturity in Hainan.

Petroleum ether, Sulfuric acid, methyl alcohol, n-hexane was purchased from Guangzhou chemical reagent factory. All reagents were of analytic grade.

The pure standards of Fatty Acid Methyl Esters (FAME) were obtained from Sigma-Aldrich (St. Louis, MO, USA).

Ultrasound-microwave synergistic extraction apparatus (CW-2000, Shanghai Xintuo Microwave Instrument Co. Ltd., China).

CS101-E Electricity Eeat Drum Wind Drying Oven (Chongqing SD Experiment Instrument Co., Ltd., China).

DC Low Temperature Circulator Bath (Ningbo Scientz Biotechnology Co., Ltd., China).

Freeze Dryer FDU-2100 (EYELA, Japan)
Ratary Evaporator N1200 (EYELA, Japan)

Agilent Gas Chromatograph (GC) 6890N (Palo Alto, CA, USA).

DB-23 capillary column (60 m×0.25 mm×0.15 μm) (J and W Scientific, Folsom, CA, USA).

Sample preparation: Mellow *papaya* fruit was selected according to the maturation stage. Collected seeds were washed and dried by vacuum freezing at -80°C for 3 days. The waterless seeds were smashed to the powder and achieve uniform particle size. The seed powder was kept in 4°C refrigerator until extraction. Petroleum ether was used as extractant in all extraction procedures.

Ultrasound-assisted extraction: In the current research, ultrasound-assisted extraction was used for the recovery of oil from *papaya* seeds. An ultrasonic bath system was applied for the extraction. The experiment was executed under different condition (i.e., time: 20, 40 and 60 min; temperature: 30, 40 and 50°C; the ratio of material to solvent: 1:10, 1:20 and 1:30, respectively) in triplicate. Petroleum ether was used as the solvent and extraction was performed in duplicate.

Ultrasound-microwave synergistic extraction: An ultrasound-microwave synergistic extraction apparatus (CW-2000, Shanghai Xintuo Microwave Instrument Co. Ltd., China) with maximal microwave power of 800 W at a frequency of 2450 MHz and an ultrasonic transducer with a fixed power of 50 W at a frequency of 40 MHz was used to extract oil. The set-up diagram of ultrasound-microwave synergistic extraction apparatus is shown in Fig. 1. The ultrasound-microwave synergistic extraction procedure were carried out under

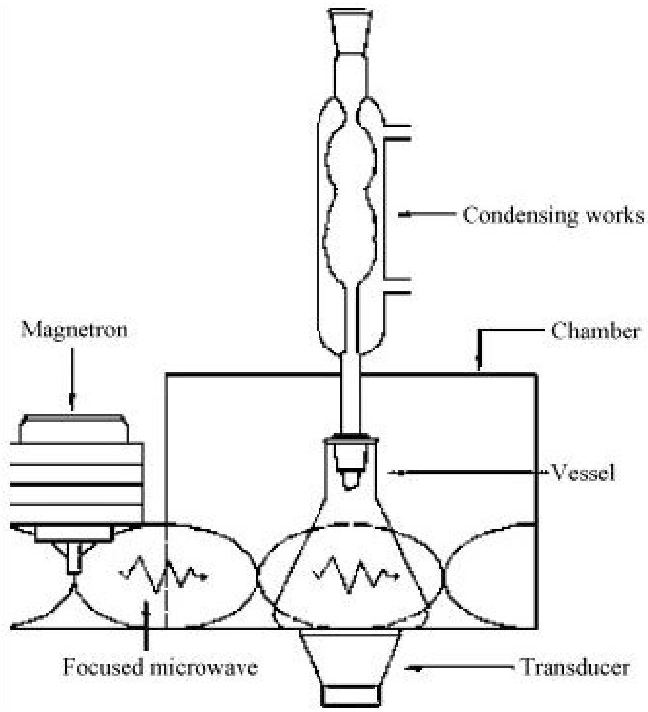

Fig. 1: The set-up diagram of ultrasound-microwave synergistic extraction apparatus

different conditions (i.e., time: 20, 40 and 60 min; temperature: 30, 40 and 50°C; the ratio of material to solvent: 1:10, 1:20 and 1:30, respectively) in triplicate. Petroleum ether was used as the solvent and extraction was performed in duplicate.

Analytic tests: Determination of saturation degree, un-saponifiable matters, color and oxidative stability.

Iodine Value (IV) was determined from fatty acid composition of *papaya* seed oil according to Animal and vegetable fats and oils-Determination of Iodine value (GB/T 5532-2008). Peroxide value was determined from fatty acid composition of papaya seed oil according to Animal and vegetable fats and oils-Determination of peroxide value (GB/T 5538-2005/ISO 3960:2001) and un-saponifiable matters was evaluated according to Animal and vegetable fats and oils-Determination of un-saponifiable matters (GB/T 5532-2008).

Fatty Acid Composition (FAC): Fatty Acid Methyl Esters (FAME) were prepared according to the Animal and vegetable fats and oils-Analysis by gas chromatography of methyl esters of fatty acids (GB/T5538-2005) by using 2 M methanolic KOH and hexane. The analysis of Fatty Acid Composition (FAC) was carried out by using an Agilent Gas Chromatograph (GC) 6890 N (Palo Alto, CA, USA) equipped with a Flame Ionization Detector (FID) and a DB-23 capillary column (60 m×0.25 mm×0.15 µm) (J&W Scientific, Folsom, CA, USA). A liner (i.d., 0.75 mm, Supelco, Bellefonte, PA, USA) was fixed inside the GC injector to minimize peak widening.

The analysis operated under the following conditions: injection volume 0.5 µL, inlet temperature 250°C, split ratio (1:20). Helium was used as a carrier gas with a flow rate of 0.7 mL/min. Oven temperature was set at 50°C and held for 1 min at this temperature. Then it was raised to 175°C with a flow rate of 25°C/min. In the last step, the temperature reached to 230°C with the speed of 4°C/min and held for 5 min at 230°C. Detector temperature was adjusted at 280°C. Hydrogen gas and air were used as the detector gases with the flow rate of 40 and 450 mL/min, respectively. The experiment was carried out in duplicate for each sample.

Statistical design and data analysis: A Completely Randomized Design (CRD) was considered to prepare different experimental treatments. Two different extraction methods were examined: Ultrasound-Assisted Extraction (UAE) and Ultrasound-Microwave synergistic Extraction (UMAE). In this study, the effects of solvent extraction conditions (i.e., time: 20, 40 and 60 min; temperature: 30, 40 and 50°C; the ratio of material to solvent: 1:10, 1:20 and 1:30, respectively) on physicochemical properties of papaya seed oil were investigated. The efficiency and suitability of this two extraction methods were assessed by determining the recovery yield, Fatty Acid Composition (FAC) of differently extracted papaya seed oils. Microsoft office software (version 2007) was used to create the experimental design and analyze the data through one way analysis of variance.

RESULTS AND DISCUSSION

The effect of extraction time on extraction efficiency of papaya seed oil: Figure 2 shown, at the beginning, the extraction efficiency increases with time in both UAE (22.92%) and UMAE (24.26%). Later on, the efficiency tends to smooth and steady till to extraction maximum (UAE: 26.16% and UMAE: 28.76%). However, known from the Fig. 2, the extraction yield of UMAE (28.76%)) was higher than UAE (26.16%). This might be due to comparison of the ultrasound-assisted extraction and ultrasound-microwave synergistic extraction, the latter combines the microwave with ultrasound forming a new complementary technique and may show the unforeseen advantages on extraction.

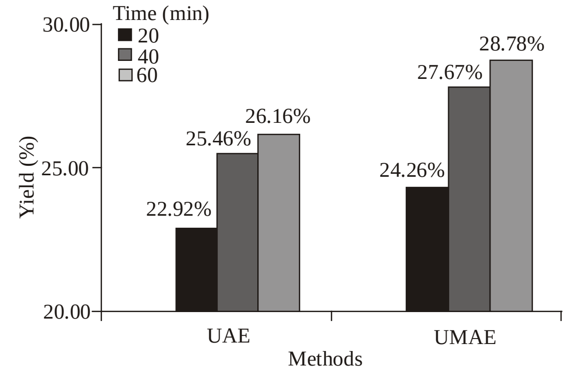

Fig. 2: Comparison of the UAE and UMAE in extraction time, yield of papaya seed oil

The effect of extraction temperature on extraction efficiency of papaya seed oil: From Fig. 3, the extraction efficiency rises with the elevated temperature in both UAE and UMAE. But the efficiency of UWAE (28.78%) was superior to UAE (25.86%). This might own to ultrasound and microwave radiation could expedite the extraction process and that might enhance extraction of ingredients.

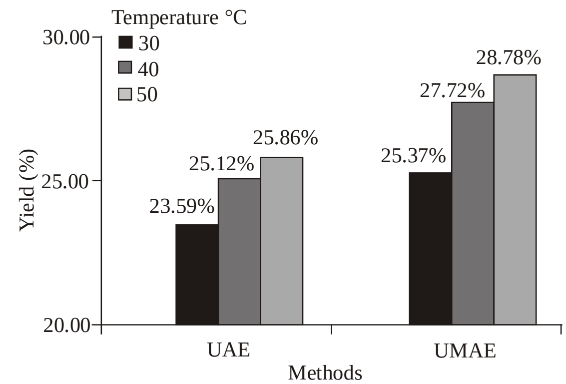

Fig. 3: Comparison of the UAE and UMAE in extraction temperature, yield of papaya seed oil

The effect of the ratio of material to solvent on extraction efficiency of papaya seed oil: Shown as Fig. 4, although the extraction efficiency was not linear relation to the ratio of material to solvent, UWAE (27.74%) was also superior to UAE (25.66%). It might due to obvious inhomogeneous phenomena existed during microwave treatment.

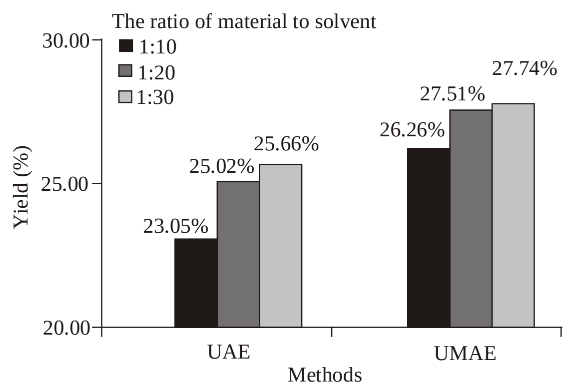

Fig. 4: Comparison of the UAE and UMAE in the ratio of material to solvent, yield of papaya seed oil

Saturation degree and unsaponifiable matters of differently extracted papaya seed oil: Physicochemical properties of papaya seed oil from different extraction methods are shown in Table 1. Results indicated that papaya seed oil from different methods had nearly similar Iodine Value (IV), it mean similar saturation degree. Iodine Value (IV) indicates the degree of saturation in the oil and the alteration could happen in intensive conditions like the thermal oxidation in frying process oxygen molecules saturate the fatty acid chain. In the current research, papaya seed oil was extracted under moderate conditions that could be insignificant effect of methods and conditions on Iodine Value (IV) of papaya seed oil which shown that extraction methods and conditions did not visibly affected the fatty acid composition of papaya seed oil.

Based on current studies, iodine values of papaya seed oil were different from different kinds of varieties and different extraction methods. It had been covered that the IV of 66.0-69.3 (g I2/100 g oil) for solvent extracted and enzymatic papaya seed oil from Batek-Batu variety (Puansri *et al.*, 2005). Besides, it had reported that the IVs of papaya seed oil from Kaeg-dum and Hamaii, Tainoung and Formosa variety were 72.5-74.9 (g I2/100 g oil), 64.1 (g I2/100 g oil) and 79.95 (g I2/100 g oil), respectively. It means that the stabilized effect of variety on the Saturation degree of the papaya seed oil. In the previous study, IVs for all samples were lower than those different varieties of olive oil (g I_2/100 g oil). This could be resulted from the presence of more polyunsaturated fatty acids in olive oil the papaya seed oil.

Unsaponifiable matters are composed of tocopherol, sterol derivatives and other antioxidant ingredients. Results signified that the considerable effect of extraction methods and conditions on level of unsaponifiable matters in papaya seed oil. In this study

Table 1: Physicochemical properties of differently extracted papaya seed oils

	30°C		40°C		50°C	
	UAE	UWAE	UAE	UWAE	UAE	UWAE
Color						
IV (mg/100 g)	102.12	112.04	98.13	107.21	95.62	105.60
AV (mg/g)	1.98	1.63	2.21	1.89	2.31	2.01
PV (mmol/kg)	1.09	0.98	1.17	1.02	1.23	1.09

Table 2: Oil from different fruit sources

Oil source	Oil content (%)	PUFA[1] (%)	MUFA[2] (%)	SFA[3] (%)	Reference
Papaya seed oil	30-34	2.1-6.3	67.5-77.6	18.6-29.0	Puangsri *et al*. (2005)
Olive oil[4]	22-24	3.5-22.5	55.3-86.5	10.5-20.0	Salvador *et al*. (2001) and Codex Stan 33-1981 (2001)
Grape seed oil	8-15	50.0-83.0	13.7-36.5	5.8-23.5	Passos *et al*. (2009)
Orange seed oil	32-35	43.5-45.0	26.0-27.0	28.0-29.0	Shahidi (2006) and Ajewole (1993)
Apple seed oil	21-24	48.4-65.3	24.7-43.0	6.3-12.4	Tian *et al*. (2010)
Watermelon seed oil	50	60.0	18.4	21.6	Boboli and Kordi (2010)
Pumpkin seed oil	42-45	55.6	20.8	23.5	Shahidi (2006) and Schinas *et al*. (2009)

[1]PUFA: Polyunsaturated fatty acid; [2]MUFA: Monounsaturated fatty acid; [3]SFA: Saturated fatty acid; [4]: Olive oil from the fresh fruit

the ultrasound-assisted extraction papaya seed oil and ultrasound-microwave synergistic extraction papaya seed oil had shown the highest and the lowest levels of unsaponifiable matters among all samples (Table 1). It might be an account on a lighter color of UWAE than others from UAE. UAE papaya seed oil at the temperature of 30°C had higher unsaponifiable matter than it at the temperature of 50°C. This could be attributed to the volatility of the unsaponifiable matters in the anabatic temperature of 50°C which apparently affected the total of unsaponifiable matters of papaya seed oil. Previous researches indicated almost similar content of unsaponifiable matters for the solvent-extracted papaya seed oil (Puangsri *et al*., 2005). However, papaya seed oil from UAE and UWAE indicated lower unsaponifiable matters enzymatic extraction and screw pressed extraction. The consequence underlined the considerable effect of extraction method on unsaponifiable matters of papaya seed oil. The differences might also be due to different varieties considered in all studies.

Color and oxidative stability of differently papaya seed oil extraction: According to the present studies, the color of the papaya seed oil was due to presence of chlorophyll and carotenoids. UWAE papaya seed oil had the lowest reddish brown among all samples might due to its higher stability. In many cases, color is an indicator to weigh the quality for the oil. Besides, papaya seed oil extracted from UAE at temperature 30°C indicated lighter color than which from the same extraction at the elevated temperature which determined the practicable effect of extraction temperature on the color of papaya seed oil. The observation might be explained by the extraction at the elevated temperature might accelerate abundant transfer of pigment to the oil.

Lipid oxidation results from photo oxidation, autoxidation leading to rancidity and objectionable odors. The oxidation tests had shown the oxidative stability of the considerable effects of extraction methods and condition of papaya seed oil in this study. There were obvious difference among the samples in AV and PV which shown extraction methods and conditions apparently impacted on oxidative stability of papaya seed oil. Results indicated that papaya seed oil was stable oil against oxidation as shown by its low peroxide value. The phenomena could be explained by the high levels of monounsaturated fatty acid of papaya seed oil. As shown in Table 1, the papaya seed oil had significant PV and AV might result from the thermal degration of the papaya oil extraction at high temperature. Actually, lipid rancidity occurred when hydroperoxides, accumulated in initial oxidation, change to secondary oxidative ingredients during the extraction at elevated temperature. In addition, UMAE papaya seed oil had shown the low apparently PV and AV different from UAE so that UMAE is more suitable for papaya seed oil extraction.

GC analysis of fatty acid of papaya seed oil: The fatty acid components of papaya seed oil was analyzed by GC. Thirteen compounds was identified by computer retrieval, artificial resolution and consulting standard. The Fatty Acid Composition (FAC) of solvent-extracted papaya seed oil. FAC analysis revealed that, the predominant fatty acids in papaya seed oil were oleic (18:1, 72.60%), palmitic (16:0, 18.00%), linoleic acid (18:2, 5.80%) and stearic (18:0, 3.60%). Other minor fatty acids (such as myristic acid (14:0), palmitoleic acid (16:1), linolenic acid (18:3), arachidic acid (20:0) and gadoleic acid (20:1)) were less than 0.5% in papaya seed oil.

In this study, papaya seed oil showed a slightly different Fatty Acid Composition (FAC) as compared to the fatty acid composition of papaya seed oil reported by previous researchers (Table 2). The variations could be due to the different extraction methods used and the utilization of different papaya varieties in previous studies. The fatty acid composition of papaya seed oil was similar to that of olive oil (Salvador *et al*., 2001; Codex Stan 33-1981, 2001). Papaya seed oil had a lower level of PUFA than grape seed oil (50.0-83.0%) (Passos *et al*., 2009), orange seed oil (43.5-45.0%) (Shahidi, 2006; Ajewole, 1993), apple seed oil (48.4-65.3%) (Tian *et al*., 2010), watermelon seed oil (59.6%) (Baboli *et al*., 2010). And pumpkin seed oil (55.6%) (Shahidi, 2006; Schinas *et al*., 2009). Both olive oil and papaya seed oil are rich sources of oleic acid (18:1), which is beneficial for the human body. Moreover, oleic acid is the indicator of high stability in frying oils (Huertas *et al*., 2010; Abdulkarim *et al*., 2007).

CONCLUSION

The current study investigated the effect of UAE and UMAE extraction and conditions on the recovery yield, physicochemical, oxidative stability and fatty acid composition. The current research also examined the efficiency of UMAE as compared to UAE for the recovery of the oil from papaya seeds. This goal was achieved by comparing the extraction yield and FAC of differently extracted papaya seed oils. The results indicated that both method and condition apparently affected the properties and oxidative stability of papaya seed oil. The results ensured the available of UMAE due to supplied by more stable oil than UAE. Thirteen kinds of components were identified and the total amount of fatty acids accounted for 93.13% of papaya seed oil. The main components were oleic acid (72.60%), palmitic acid (18.00%), linoleic acid (5.80%), stearic acid (3.60%). This confirms that papaya seed is a potential source of high oleic oil. However, the current work suggests a further study on the toxicity and safety issue of the crude papaya seed oil. The present study revealed that the UMAE was an appropriate technique for recovering the oil from papaya and moderate conditions as compared to different extraction methods. The current research also recommends optimizing the ultrasound conditions in order to achieve papaya seed oil with the most desirable quality.

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