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Effect of multimode ultrasound assisted extraction on the yield of crude polysaccharides from *Lycium Barbarum* (Goji)

Rahma MUATASIM[1], Haile MA[1]*, Xue YANG[1]

Abstract

The goals of this exploration were to find out the optimum conditions of ultrasound assisted extraction (UAE) of *L. Barbarum* crude polysaccharides. Furthermore, to research the impacts of three multi-frequency ultrasound assisted extraction modes on the yield of *L. Barbarum* crude polysaccharides. The results showed that by applying the traditional single-frequency UAE mode, the optimum extraction time was 30 min, extraction temperature of 60 °C, and solid/liquid ratio of 20 g/600 mL, at a power density of 300 W/L, and ultrasound frequency of 28 kHz. Secondly; a comparison was carried out between three UAE modes using the optimum extraction conditions obtained previously. The energy aggregation counter flow dual-frequency UAE mode gave the highest yield of 38.93% of crude polysaccharides. Followed by the Opposite-sit dual-frequency UAE mode and the energy aggregation counter flow single-frequency UAE mode with yields of 33.60%, and 26.38% of crude polysaccharides, respectively. As a result the ultrasound assisted extraction with dual-frequency mode is more effective for the extraction of *L. Barbarum* crude polysaccharides. Furthermore, the yield of crude polysaccharides increased by 73.41% using the dual-frequency ultrasound extraction compared to traditional hot water extraction.

Keywords: extraction; *Lycium Barbarum*; crude polysaccharides; ultrasound.

Practical Application: The extraction of *Lycium Barbarum* crude polysaccharides was examined utilizing ultrasound assisted extraction technique. The optimum extraction conditions were studied and applied on three extraction modes to discover the best extraction procedure.

1 Introduction

Lycium (Boxthorn) is a class of the nightshade family (Solanaceae), containing around 80 types of plants local all through the temperate and subtropical areas of the world (Xin et al., 2013). *Lycium Barbarum*, commonly named Wolfberry, Goji (*Gouqi)* in Chinese, is mostly found in dry, semi-saline environments. An extensive variety of *Lycium Barbarum* products have been produced in types of cosmetic products, dietary supplements, tea (Amagase, 2014), milk, juice, seed oil, and so on (Potterat, 2010). For more than 4000 years *Lycium Barbarum* has been consumed as nourishment and in traditional prescription (Amagase & Farnsworth, 2011). Furthermore, the agriculture of *Lycium Barbarum* has been recorded for over 600 years in the Northwestern region of China, particularly Ningxia province which is additionally the authentic district of Chinese medication Lycii Fructus (Li, 2007; Potterat, 2010). Within the chemical composition of *Lycium Barbarum*, water-soluble glycoconjugates, (*Lycium Barbarum* polysaccharides or LBP) are the most researched components, which are evaluated to involve 5-8% of the dried Goji (Amagase & Farnsworth, 2011; Potterat, 2010).

Developing number of investigations of *L. Barbarum*, have led different clinical and fundamental examinations to look at the traditional impacts of the fruits given as a juice that is standardized for *L. Barbarum* polysaccharides (LBP) (Amagase & Farnsworth, 2011). Supporting the conventional uses and properties, recent investigations show that concentrates from *L. Barbarum* fruit, and its dynamic compounds, polysaccharides (LBP) have a scope of natural biological activities, counting impacts on neuro-protection, aging, diminish cholesterol level (Li, 2007), expanded digestion, glucose control in diabetics (Li, 2007; Potterat, 2010) glaucoma, anti-oxidant properties, improved immune responses (Bo et al., 2016), anti-tumor activity and cyto-protection (Amagase, 2014; Cui et al., 2012). *Lycium Barbarum* can be utilized as pharmaceutical for treatment and also as an ingredient in Chinese cooking (Xie et al., 2016).

Ultrasound has been utilized as a part of several food industry procedures, for example freezing, dehydrating (Fernandes et al., 2015), tempering, extraction, and cleansing (Chemat et al., 2011) due to its reduction in temperature, energy consumption, and production steps (Chemat et al., 2011). lately, ultrasound innovation has been observed to be a potential sustenance in food processing techniques (Ashokkumar, 2015). Ultrasound assisted extraction (UAE) is applied to recovering bioactive materials such as peptides (Kadam et al., 2015), polysaccharides (Cheung et al., 2013), polyphenolics, aromatic compounds, caffeine, theobromine (Peralta-Jiménez & Cañizares-Macías, 2013), and functional compounds from herbal and animal sources (Vilkhu et al., 2008). Ultrasound waves after interaction with subjected plant

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[1] School of Food and Biological Engineering, Jiangsu University, Zhenjiang, Jiangsu 212013, China
*Corresponding author: mhl@ujs.edu.cn

material modify its physical and synthetic properties and their cavitation impact enhances the extraction yield and upgrades the mass transport by disrupting the plant cell walls (Cravotto & Binello, 2016). UAE is a perfect strategy that limits the usage of considerable amount of solvents close by decreasing the working time (Chemat et al., 2011). Ultrasound can be easily examined on a laboratory scale, giving data suitable for large industrial scale (Cravotto & Binello, 2016). Diverse ultrasound modes have been utilized for the recovery of high value components from numerous raw materials. For instance multi-frequency sonication mode turned out to be more successful to enhance hydrolysis and transformation rate of corn gluten meal (Jin et al., 2015). Ultrasound multi-frequency mode notably affects the extraction and activity of natural products (Yang et al., 2017). Ideal conditions can differ as indicated by the substance of intrigue and crude materials (Azmir et al., 2013; Cravotto & Binello, 2016).

In this model, the optimization of polysaccharides extraction was carried out to find the optimum conditions using a single frequency ultrasound extractor. Later, these optimum conditions were applied on three ultrasound modes with different frequencies. The first mode was the energy aggregation counter flow single-frequency ultrasound extractor. The second mode was the energy aggregation counter flow dual-frequency ultrasound extractor. And the third mode was the opposite-sit dual-frequency ultrasound extractor (Figure 1a, b and c).

2 Materials and methods

2.1 Materials

The *Lycium Barbarum* (Goji) samples were provided by our school and purchased from Ningxia province. Dried *Lycium Barbarum* fruits were grounded by an electrical multi-function grinding machine (model LD-T400A, Meetingpoint trading company, China), into powder and sieved with a manual sieve size (0.4) mm and stored for farther use.

2.2 Methods

Single frequency UAE optimization of crude polysaccharides

As the first step of this study, the optimal conditions for the ultrasound assisted extraction (Time, Temperature, Solid/Liquid ratio) were investigated using an energy aggregation counter flow single-frequency ultrasound extractor Figure 1a. The fixed parameters were ultrasound power density of 300 W/L, On/Off ultrasound time 5 sec/2 sec, and at a chosen frequency 28 kHz.

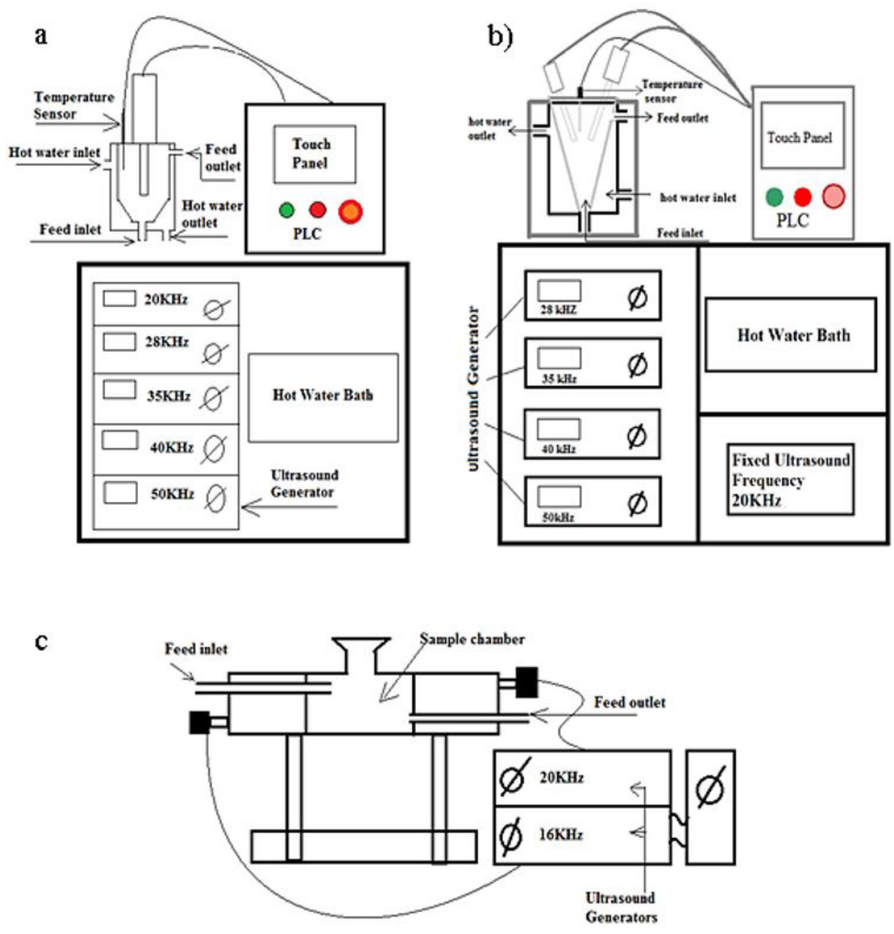

Figure 1. a) The energy aggregation counter flow single-frequency ultrasound extractor, b) The energy aggregation counter flow dual-frequency ultrasound extractor, and c) The opposite-sit dual-frequency ultrasound extractor.

The first condition to be tested was the time, ranged 20, 30, 40, 50 and 60 mins at a temperature of 50 °C and solid/liquid ratio of 15 g/600 mL. The optimum extraction time obtained was applied to find out the perfect extraction temperature between 30, 40, 50, 60 and 70 °C with a solid/liquid ratio of 15 g/600 mL. Two thermostat-controlled water baths were used to maintain the desired temperature controlled and constant. The circulation of the heated water and the sample in and out of the extractor was done by two circulation pumps -one for the sample and another for the temperature control- to maintain the temperature controlled.

And for the last condition, solid/liquid ratio of 5, 10, 15, 20 and 25 g - at a fixed volume of distilled water 600 ml - was investigated using the optimum time and temperature obtained above. These tests were triplicated to reduce error.

Hot water extraction

Hot water extraction process was conducted as a control experiment. The extraction temperature was 90 °C for 60 mins and solid/liquid ratio was 20 g/600 mL using a magnetic stirring hot water bath apparatus (DF-101S, Xiang Tian Experimental Instrument Factory, Changzhou, china) at a speed of 100 rpm.

Extraction of crude polysaccharide

After UAE, samples were centrifuged at 4000 rpm for 15 mins each. The volume of the collected supernatant was then reduced to one-fifth using a rotary evaporator at 60 °C under vacuum. The remaining solution was mixed with four times the volume of Ethanol (ethanol final concentration, 100%) (Wu et al., 2007) and kept overnight at 4 °C (DuBois et al., 1956). Later the sample was centrifuged and freeze dried for 48 hours. The crude polysaccharides were weighed and the percentage yield of the crude *Lycium Barbarum* polysaccharide (L.B.P%), were determined from the Equation 1 as follows (Raza et al., 2017; Wang et al., 2009).

$$L.B.P\ \% = \frac{W}{Wo} \times 100 \quad (1)$$

W (g) is the L.B.P dry weight, $\mathbf{W_0}$ (g) is the raw material dry weight.

The conditions resolving the highest percentage yield of crude polysaccharide were based on the optimum conditions for a single frequency ultrasound extractor.

UAE modes comparison

The second part of the study was to apply the optimum conditions on the three UAE modes to compare the extracted crude polysaccharide. First mode was the energy aggregation counter flow single-frequency ultrasound extractor Figure 1a, consisting frequencies of 20, 28, 35, 40 and 50 kHz. Secondly, energy aggregation counter flow dual-frequency ultrasound extractor Figure 1b with (20/28), (20/35), (20/40) and (20/50) kHz. And the third mode was the opposite-sit dual-frequency ultrasound extractor Figure 1c, having only three frequencies options (20, 16, and (20/16) kHz).

3 Results and discussion

3.1 Optimal conditions of L. Barbarum *crude polysaccharides UAE*

Effect of extraction time on L. Barbarum crude polysaccharides yield

Figure 2 shows the effect of extraction time on percentage yield of crude polysaccharide. The percentage yield increased as the extraction time increased from 20 to 30 min and then decreased as the extraction time increased to 60 min. This shows that the highest percentage yield of crude polysaccharides (23.55%) was obtained at extraction time of 30 min. Similar observations were obtained by (Wang et al., 2016; Ying et al., 2011) who respectively, reported that the yield of *Artemisia selengensis* Turcz polysaccharides and mulberry leaves polysaccharides decreased as the extraction time increased. The Minitab 17 software was used to apply the Tukey's Test to compare to the difference between the means in all treatments. Means that share same letter are (not significantly different).

The greater part of the polysaccharides inside the cells were discharged at the beginning of the extraction, and expanded extraction time would lead to degradation of polysaccharides (Esclapez et al., 2011; Hromádková et al., 1999).

Effect of extraction temperature on L. Barbarum crude polysaccharides yield

Figure 3 shows the percentage yield of L. Barbarum crude polysaccharides at five temperatures (30, 40, 50, 60, and 70 °C) at the optimal extraction time of 30 min. The extraction yield started with 17.26% at temperature of 30 °C, then elevated to 19.46% and 22.95% at 50 °C and 60 °C, respectively. The extracted yield decreased when the temperature exceeded to 70 °C to give 18.20%. (Zhu et al., 2016) studied the extraction of *Polygonum multiflorum* polysaccharide, and stated that high temperature could allow the release of polysaccharides from cells to the solvent. These outcomes were likewise because of the impacts of acoustic cavitation and diffusion through the cell walls,

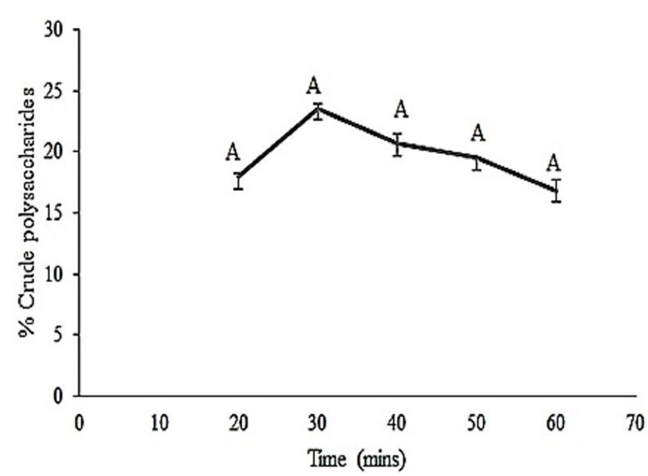

Figure 2. The effect of time on the extracted yield of *L. Barbarum* crude polysaccharides values are means ± SD.

which were improved by the extraction temperature. As the temperature increased, the viscosity coefficient and the surface tension coefficient decreased, which made it less demanding to strengthen the cavitation impact and produce the cavitation bubble (Bai et al., 2017).

However, when the temperature increased further, the vapor pressure increased and this resulted in the decrease of cavitation strength or cavitation effect causing the damping of the ultrasonic wave (Zhao et al., 2007). Resulting, the optimum temperature was 60 °C. Using the Tukey's Test, the results showed (no significant difference).

Effect of solid/liquid ratio on L. Barbarum crude polysaccharides yield

Figure 4 demonstrates the impact of solid/liquid ratio on the yield of *L. Barbarum* crude polysaccharides. The solid/liquid ratio affected the extraction yield significantly. As the solid/liquid ratio increased from 5 g/600 mL to 20 g/600 mL, the percentage yield increased from 11.53% to 26.38%. Under the high temperature condition, the increase of solid/liquid ratio led to increase in liquid viscosity. Because of the expansion in fluid consistency (viscosity), the volume of the cavitation bubbles and the quality of the air bubble fall expanded (Yan et al., 2016), which facilitated the extraction of polysaccharides. Further increase of solid/liquid ratio to 25 g/600 mL led to decrease in the percentage yield. This could be attributed to the fact that when the liquid viscosity was too high, it would cause the decrease of bubble number and the difficulty of collapsing, which decreased the cavitation effect finally. The optimal sample concentration recovered from this test was 20 g/600 mL as shown in (Figure 4).

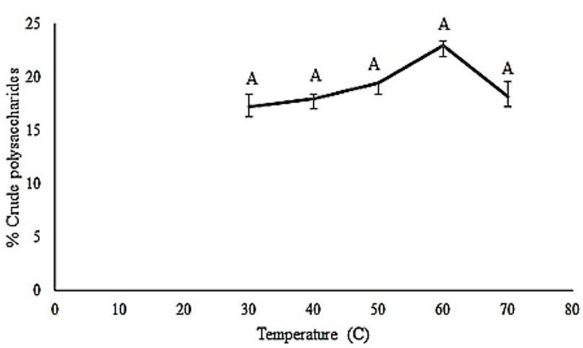

Figure 3. The effect of temperature on the extracted yield of *L. Barbarum* crude polysaccharides, values are means ± SD.

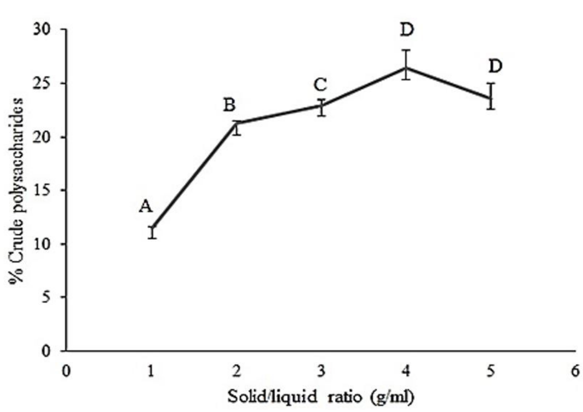

Figure 4. The effect of the solid/liquid ratio on the extracted yield of *L. Barbarum* crude polysaccharides. Values are means ± SD.

The control sample was done by the hot water extraction procedure, using the optimal solid/liquid ratio recovered previously. Since the first, second, third, and forth solid/liquid ratios results don't share letters, thus they are (significantly different). At temperature of 60 °C for 30 mins and solid/liquid ratio 20 g/600 mL, the crude polysaccharides percentage was 22.45% (Figure 5 and 6).

Yield of crude polysaccharides using single-frequency UAE mode

The optimum conditions obtained from the previous part of the study – (extraction time 30 mins, extraction temperature 60 °C and solid/liquid ratio 20 g/600 mL) - were applied to the five frequencies of the single-frequency ultrasound extractor. Figure 5a shows the percentage yield of crude polysaccharides of the control sample and the five frequencies (20, 28, 35, 40, and 50 kHz). Results showed that the crude polysaccharides yield increased from 19.53% at the first frequency 20 kHz, to 26.38% at 28 kHz. With the increase of ultrasonic frequency, the cavitation effect of ultrasound increases (Yusof et al., 2016). The mechanical impacts required in ultrasound can permit more penetration of solvent into the sample matrix, the disturbance of the organic cell walls while the ultrasonically actuated cavitation encourages and increments the release of substance (Dolatowski et al., 2007).

However, when the ultrasound frequency elevated to 35 kHz and 40 kHz the extraction yield dropped to 22.71% and 23.51%, respectively, and finally decreased to 11.88% at the frequency 50 kHz. When the ultrasonic frequency was too high, the time of acoustic expansion became relatively shorter, leading to insufficient time to form the cavitation bubble that generates ultrasonic effect and when the cavitation bubble was formed, the compression phase of acoustic wave was too short, which might be not enough for the cavitation bubble to collapse, thus, a decrease in the cavitation effect occurred (Esclapez et al., 2011; Kentish & Ashokkumar, 2011). The Tukey's Test showed that the results of 28 kHz and 50 kHz are (significantly different). Therefore, from this result, the optimal frequency for single-frequency UAE mode was 28 kHz. (Figure 5a) also showed that the extraction yield at frequency of 28 kHz was 17.5% greater than the control's.

Yield of crude polysaccharides using dual-frequency UAE mode

The dual-frequency ultrasound extractor is equipped with four dual-frequencies options, (20/28), (20/35), (20/40) and (20/50) kHz. As shown in Figure 5b, the two frequencies options (20/40) and (20/28) kHz gave the highest results of 38.93% and 38.25%, respectively. The higher extract of *L. Barbarum* crude polysaccharides could be clarified by the expanded cavitation bubble crash which brought on additional diminishment in particle measure and advanced leaching. Chukwumah et al, correspondingly detailed that the effect of multi-frequency UAE, is more proficient than single frequency UAE during the

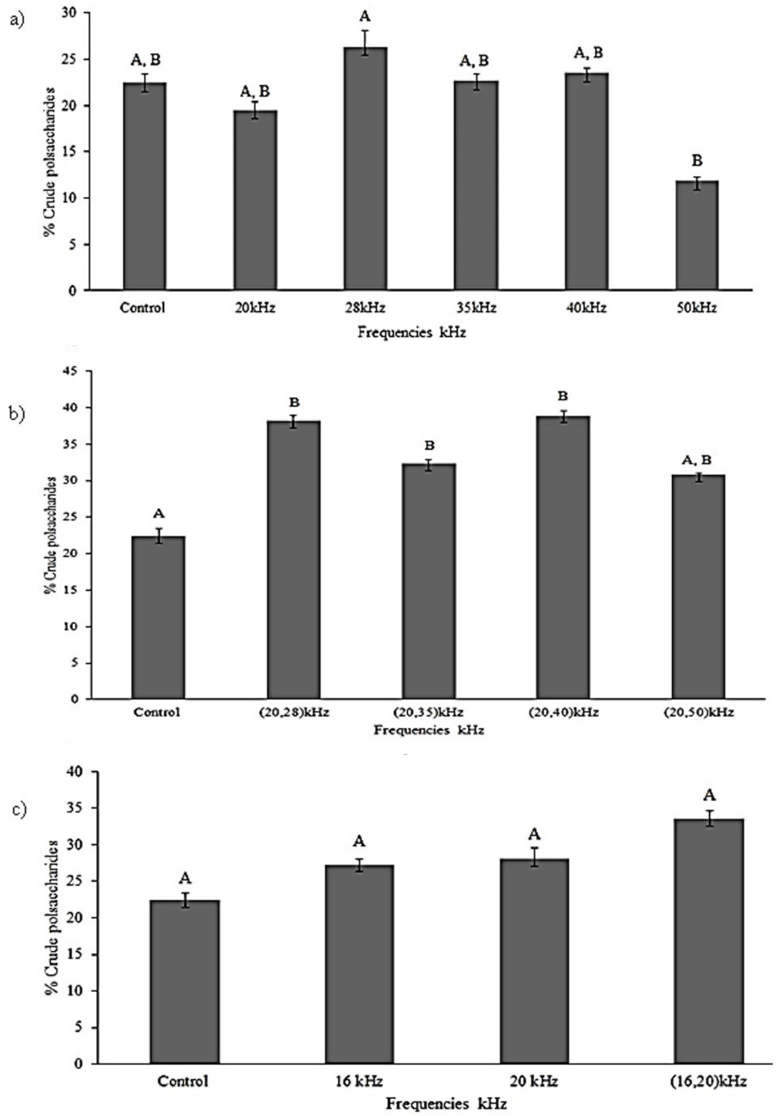

Figure 5. The yields of *L. Barbarum* crude polysaccharides using: a) single-frequency ultrasound extractor, b) dual-frequency ultrasound extractor, and c) opposite-sit dual-frequency ultrasound extractor. Values are means ± SD.

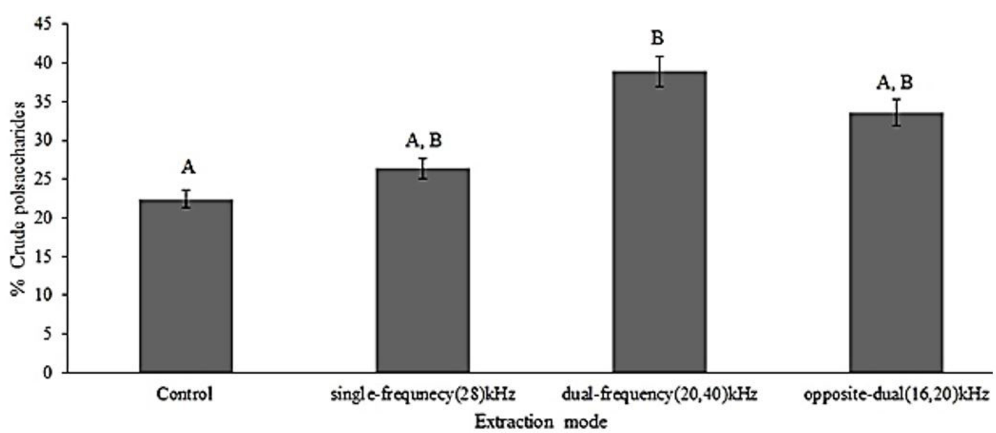

Figure 6. Comparison between control sample and the highest crude polysaccharides extraction yields achieved by each one of the three ultrasound extractors. Values are means ± SD.

Table 1. The yield of crude polysaccharides by control and three UAE modes.

Sample	control	single-frequency (28) kHz	dual-frequency (20,40) kHz	opposite-dual (16,20) kHz
crude polysaccharides %	22.45	26.38	38.93	33.60

extraction of iso-flavones and trans-resveratrol from peanuts (Chukwumah et al., 2009). The results showed (significant difference) between the control and the results of the dual-frequencies except the last dual-frequency (20/50) kHz which is (not significantly different) from the control and the rest of the dual frequencies. The extraction yield at dual frequency of (20/40) kHz was 73.4% higher than the control yield.

Yield of crude polysaccharides using opposite-sit dual-frequency UAE mode

In Figure 1c, the opposite-sit dual-frequency ultrasound extractor looks completely different from the previous two extractors. The single-frequency options provided by this extractor are (16 kHz, and 20 kHz), and a dual-frequency of (16/20) kHz.

Figure 5c shows the percentage yield of crude polysaccharides for control sample, single and dual frequencies. The single-frequency extracted yield at 16 kHz was 27.28%, and increased gradually to 28.03% at 20 kHz with no (significant difference). As for the dual- frequency application the extraction yield was 33.60% at (16/20) kHz. These results also indicated that the dual-frequency UAE is more effective for *L. Barbarum* crude polysaccharides extraction. The percentage yield at dual frequency of (16/20) kHz was 49.6% higher than the control's yield.

Lernetti et al, stated out to that one possible system for this enhanced impact could come about because of the generation of new bubbles by the low-frequency stimulating field (Lernetti et al., 1997). An alternative could emerge from the diminishing quasi-static pressure during the negative pressure amplitude half-wave of the low-frequency field. This decrease the cavitation threshold of the high frequency field and builds up more bubbles driven by that field. This quasi-static pressure contributes to an increase of the collapse rate during the positive pressure amplitude half-wave of the low-frequency field.

Comparing extraction frequencies among the three UAE modes

The three ultrasound extractors were run under the same parameters, ultrasound power density 300 W/L, On/Off ultrasound time (5 sec/2 sec), using the optimal conditions for *L. Barbarum* polysaccharide extraction: time 30 mins, temperature 60 °C, and solid/liquid ratio 20 g/600 mL. The energy aggregation counter flow dual-frequency ultrasound extractor gave the highest result among the three extractors, 38.93% crude polysaccharides. The Opposite-sit dual-frequency ultrasound extractor was the second best followed by the energy aggregation counter flow single-frequency ultrasound extractor as shown in (Table 1) and (Figure 6).

The yield of crude polysaccharides increased by 73.41% using the dual-frequency ultrasound extraction compared by traditional hot water extraction. The Tukey's Test showed (significant difference) between the control and the dual-frequency ultrasound extraction (20, 40) kHz. This also caused a reduction in temperature and extraction time to 33.3% and 50% respectively.

4 Conclusion

The UAE has been considered an effective method for the extraction of bioactive components from plants. The first aim of this study was to find out the optimal condition for *Lycium Barbarum* crude polysaccharides by UAE. The extraction optimal conditions were as follows: extraction time of 30 mins, extraction temperature of 60 °C, and solid/liquid ratio of 20 g/600 mL. Under these conditions, the *Lycium Barbarum* crude polysaccharides yield was 26.38% for single frequency at 28 kHz.

The second aim of the study was to apply these extraction conditions on three UAE modes to test different frequencies. The energy aggregation counter flow dual-frequency UAE mode gave the highest yield of 38.93% at the dual-frequency of (20, 40) kHz.

This study clearly showed that the dual frequency mode gave a higher polysaccharides extraction compared to the single-frequency mode and the traditional hot water extraction.

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