Effect of oil extraction assisted by ultrasound on the physicochemical properties and fatty acid profile of pumpkin seed oil (*Cucurbita pepo*)

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**A B S T R A C T**

The effects of amplitude and time of ultrasound-assisted extraction on the physicochemical properties and the fatty acid profile of pumpkin seed oil (*Cucurbita pepo*) were evaluated. Ultrasound time (5–30 min) and the response variables amplitude (25–100%), extraction yield, efficiency, oxidative stability in terms of the free fatty acids (FFA) of the plant design comprising two independent experiments variables, peroxide (PV), p-anisidine (AV), totox value (TV) and the fatty acid profile were evaluated. The results were analyzed by multiple linear regression. The time and amplitude showed significant differences (*P* < 0.05) for all variables. The highest yield of extraction was achieved at 5 min and amplitude of 62.5% (62%). However, the optimal ultrasound-assisted extraction conditions were as follows: ultrasound time of 26.34 min and amplitude of 89.02%. All extracts showed low FFA (2.75–4.93% oleic acid), PV (1.67–4.68 meq/kg), AV (1.94–3.69) and TV (6.25–12.55) values. The main fatty acids in all the extracts were oleic and linoleic acid. Therefore, ultrasound-assisted oil extraction had increased performance and reduced extraction time without affecting the oil quality.

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1. Introduction

The *Cucurbita* species are cultivated in the tropics and in arid regions. For many years, particularly in Europe, the seed extract of *Cucurbita pepo* has been used in alternative medicine as a remedy for urination caused by benign prostatic hyperplasia [1]. Pumpkin seed is a rich source of fatty acids and proteins. There are reports that the pumpkin seed has a high content of essential amino acids, so it has great potential for use in food systems, not only as a nutritional supplement but also as a functional agent [2]. In Mexico, pumpkin seed is used in the production of snacks (roasted seed) or as an ingredient in the preparation of traditional dishes. However, in countries such as Austria, Hungary and parts of southern Russia, pumpkin seed is mainly used for the production of oil, which is used as salad dressing, and in alternative medicine as a deworming agent and for the elimination of respiratory mucus [1,3]. Currently, researchers are concentrating their efforts on the search for alternatives to the use of non-conventional oils and the development of methods to minimize the degradation of these oils in the extraction process. Traditional methods of extraction can be detrimental to the original active components, and polyunsaturated fatty acids can be degraded due to prolonged exposure to high temperatures. The efficiency of the extraction of bioactive substances from plants and seeds has been significantly improved by the use of ultrasound waves [4]. Ultrasound is defined as sound waves of a frequency >18–20 kHz. When ultrasound is applied to a liquid, it imposes a sound pressure plus the hydrostatic pressure. In an infinite medium, the sound pressure (Pa) wave is typically considered to be a sine wave and depends on the frequency (*f*), time (*t*) and the maximum amplitude of the pressure wave (Pa, max). In low intensity ultrasound, the sound wave induces movement and mixing within the liquid [5], whereas at high intensities, the sound wave propagates in the liquid medium in alternating cycles of compression and rarefaction [6]. The ultrasound-assisted extraction method, due to its higher efficiency and lower energy and water consumption, has become an appropriate alternative to the conventional extraction methods and is an approved method for processing plant substances, especially for the extraction of compounds with low molecular weights [7]. The additive effect of ultrasound waves on the rate of extracting plant substances is related to the breaking of cells and the release of their contents...
into the extraction environment [8]. Ultrasound technology can improve the process by reducing the processing time, increasing the performance and lowering power consumption [9]. In addition, this method of extraction is considered a green extraction technique, because it is based on the development and design of extraction processes which reduce energy consumption, allows the use of alternative solvents and renewable natural products, and ensure a safe and high quality extract/product [10]. Also, with this green extraction technology it seeks to maximize the value of the raw material for economic sustainability, on the other hand, reduce or eliminate the use of hazardous substances [11]. Therefore, the aim of this study was to evaluate the effect of the amplitude and time of ultrasound on the physicochemical properties and fatty acid profile of pumpkin seed oil (C. pepe).

2. Materials and Methods

Pumpkin seeds were purchased from the local market of San Juan Bautista Tuxtepec, Oaxaca, Mexico. The seeds were cleaned and then dried at 60 ± 2 °C in an oven (Binder) for 24 h to remove the moisture content. The seeds were then ground in a blender (Oster) until the particle size reached 0.59 mm (mesh No. 30) (ASTM). The sifted seed flour was stored in sealed polyethylene bags at 4 °C until further use.

2.1. Oil extraction

Ultrasonic extraction was performed with an ultrasound probe (Branson Ultrasonics) with a frequency of 20 kHz and a power of 400 W. Twenty grams of pumpkin seed flour (with moisture content of 6.5 ± 0.5%) was mixed with 200 mL of hexane in a beaker. The extractions were performed under different conditions of time (5–30 min) and amplitude (25–100%). The resulting suspension was filtered under vacuum, and the oil was recovered in rotary evaporator (Buchi) and stored until analysis. The extraction yield and efficiency were calculated with the following Eq’s

\[
\text{Oil extraction yield (\%) } = \frac{\text{Mass of extracted oil (g)}}{\text{Mass of oil – bearing material (g)}} \times 100 \\
\text{(1.1)}
\]

\[
\text{Extraction efficiency (\%) } = \frac{\text{Oil extraction yield (g)}}{\text{Total lipid content (g)}} \times 100 \\
\text{(1.2)}
\]

2.2. Fatty acids composition

The fatty acids content was quantified by gas chromatography (GC). Raw pumpkin seed oil methyl esters were prepared following the method of Martinez et al. [12] with 1 N HCl-methanol. The Fatty Acid Methyl Ester (FAMEs) were analyzed with a gas chromatograph (Agilent) equipped with a flame ionization detector and using nitrogen as the carrier gas (4 mL/min). A splitless injector and detector ports (both set at 250 °C) were employed. Samples (1 µL) were injected into a fused-silica capillary column (HP-Innowax, 60 m, 0.25 mm) at a programmed temperature of 130–230 °C at 20 °C/min and were held for a 47 min total run time. Fatty acids were identified based on the retention times of fatty acid methyl esters from the official AOCS methods [13]. Fatty acid profile and oxidative stability (PV, FFA, AV and TV) were calculated based on the following equation [14,15]:

\[
TV = 2PV + AV \\
\text{(1.3)}
\]

2.3. Oxidative stability

The stability of crude oil was calculated according to the techniques of the official AOCS methods [13]; peroxide value (PV) (Method Cd 8-53), free fatty acid (FFA) (Method Aa 4-38), p-anisidine value (AV) (Method Cd 18-90) and totox value (TV) were calculated based on the following equation [14,15]:

2.4. Experimental design and statistical analysis

A central composite design was used with two independent variables, as shown in Tables 1 and 2. Sonication time (X₁) and amplitude (X₂) were the independent variables considered. The response variables were the oil yield, efficiency, fatty acid profile and oxidative stability (PV, FFA, AV and TV). Data were analyzed by surface response methodology using the commercial statistical package Design Expert (Design Expert 8.0.2, Stat-ease Inc., Minneapolis, United States). The results were also analyzed by multiple linear regression. The statistical significance of the regression terms was examined by analysis of variance (ANOVA) and linear correlation analysis for each response. All experiments were performed in triplicate.

2.4.1. Process optimization

Numerical optimization was performed using the superposition of response surfaces methodology. The responses used were the oil yield, efficiency, fatty acid profile and oxidative stability (PV, FFA, AV and TV). Desired values were assigned for all the parameters to obtain the optimum numerical values for the responses. Sonication and amplitude were maintained within the desired range. The response parameters oil yield and efficiency were maintained at maximum. Oxidative stability (PV, FFA, AV and TV) minimum values and fatty acid profile were maintained within a set range.

3. Results and discussion

3.1. Effect of ultrasound-assisted extraction conditions on oil yield

A higher extraction yield was obtained at amplitude of 62.50% and a time of 5 min (Table 3). The regression model fitted to the experimental results had a correlation coefficient $R^2$ value of 0.738 (Table 4). The amplitude had a significant effect ($P < 0.05$) on the linear term, and time proved to be significant ($P < 0.05$) for the quadratic term.

Fig. 1 shows that the oil extraction yield increased with increasing amplitude because of the cavitation phenomenon, in which some bubbles are produced by the propagation of sound waves in the phase cycles of the contraction and expansion of the liquid and solid. These bubbles continue to grow and eventually break, which causes solid and fluid particles to oscillate and gain more speed under the action of ultrasound, resulting in the rapid spread of soluble substances from the solid phase to the solvent phase [16]. Similar behavior was reported in the ultrasound-assisted

<table>
<thead>
<tr>
<th>Variables</th>
<th>Coded Levels</th>
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<tbody>
<tr>
<td>Time (min)</td>
<td>X₁</td>
</tr>
<tr>
<td>Amplitude (%)</td>
<td>X₂</td>
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</tbody>
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$x = 1.414$. |
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