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# Influence of the bed height on the kinetics of watermelon seed oil extraction with pressurized ethanol

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ABSTRACT

The influence of the bed volume on the kinetics of watermelon seed oil extraction was evaluated in this study. Watermelon seed oil was extracted using different extraction cells ($S_1$ = 34 mL, $S_2$ = 66 mL and $S_3$ = 100 mL) at different temperatures (40, 60 and 80 °C) using the sample mass/solvent volume ratio as the fixed sizing criteria (w/s = 0.30). The extraction kinetics were mathematically described using the Peleg, Fick and second-order models. Samples were extracted in batches using pressurized liquid extraction (PLE) with ethanol as the solvent for different extraction times. Oil extraction yields ranged from 24.69 to 37.21 g oil/100 g of seeds, and the concentration of total phenolic compounds ranged from 2.44 to 3.84 mg of gallic acid equivalents (GAE)/g of seeds. All kinetic models showed a good fit to the experimental data, but the second-order model better predicted the behavior of data, with high coefficient of determination ($R^2_{ajd}$) and low root-mean-square deviation (RMSD) values. The different extraction cells did not affect the total extraction yield, but affected the extraction parameters obtained in the models. The effective diffusivities were dependent on temperature and ranged from $9.10 \times 10^{-6}$ to $2.07 \times 10^{-5}$ m$^2$/s. The activation energy ranged from 11.43 to 18.54 J/mol.



## 1. Introduction

Watermelon is a fresh fruit consumed worldwide and is used in the production of juices, jellies, marmalades, sauces and salads [1]. In addition, watermelon has been widely used as a medicinal plant in African and Asian cultures due to the presence of several compounds with phytochemical activities [2]. Watermelon seeds have strong antioxidant activity [3,4], and due to their diuretic and purgative activity, have also been used in the treatment of gastrointestinal diseases [5], urinary infections [6], gonorrhea, leukorrhea [7] and prostatic hyperplasia [8]. Watermelon seed oils are classified as high quality oils due to the presence of ω-3 and ω-6 fatty acids [9] and phenolic compounds such as gallic, protocatechuic, p-hydroxybenzoic, vanillic, caffeic, syringic, p-coumaric and ferulic acids [10]. The processing of the watermelon fruit yields a large amount of seeds, which are usually treated as waste. The use of these seeds to produce oil and other functional ingredients will add value to this waste produce that currently has no specific use, so that it can become a raw material used for the generation of a product with active compounds.

The extraction and purification of active compounds from natural sources are important steps in the production of phytochemicals for use in food supplements or nutraceuticals, functional foods and pharmaceuticals [11]. In industry, oil extraction processes are based on conventional methods that involve the use of organic solvents which are heated at atmospheric pressure conditions, and production can also occur via mechanical processing such as pressing. High-pressure extraction processes, such as pressurized liquid extraction (PLE) and extraction with supercritical $CO_2$, are extraction techniques that are becoming more prominent due to their ability to obtain specific target molecules and reduced loss of solvents during the production process [12]. PLE is a technique that has emerged as an alternative to conventional extraction methods, such as maceration, percolation or reflux, as it offers advantages for parameters including extraction time, solvent consumption, extraction yield and reproducibility [13]. PLE uses organic solvents at elevated pressure and temperature in order to increase the extraction process efficiency, reduce viscosity and improve solvent penetration and diffusivity, thereby reducing extraction times and avoiding possible thermal degradation [13,14]. PLE can be used for the extraction of polar compounds such as polyphenols, however, this is a little studied technique which still requires more research before its implementation on a larger scale [12]. An advantage of PLE is the possibility of using

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**Nomenclature**

| | |
|---|---|
| $A$ | average yield of oil extracted in the washing step (g oil/100 $g_{db}$) |
| $A_n$, $B_n$ | model-fitting parameters ($A_n$, dimensionless; $B_n$, $s^{-1}$) |
| $B_0$ | extraction rate at the beginning of extraction ($t = t_o$) |
| $C_0$ | yield of oil extracted in the washing step (g oil/100 $g_{db}$) |
| $C_s$ | extraction capacity at equilibrium (g oil/100 $g_{db}$) |
| $C_{sp}$ | extraction capacity of the Peleg model at equilibrium (g oil/100 $g_{db}$) |
| $C_t$ | extraction yield at any time (g oil/100 $g_{db}$) |
| $D_0$ | pre-exponential diffusion constant ($m^2/s$) |
| $D_e$ | effective diffusivity coefficient ($m^2/s$) |
| $IC_{50}$ | antiradical activity (mg/L) |
| $h$ | initial extraction rate for the second-order model (g/100 $g_{db}$ minute) |
| $k$ | second-order extraction rate (100 $g_{db}$/g oil minute) |
| $K_1$ | constant of the Peleg model (min 100 $g_{db}$/g oil) |
| $K_2$ | constant capacity of the Peleg model (100 $g_{db}$/g oil) |
| $R_p$ | average radius (m) |
| $R_g$ | gas constant (J/mol K) |
| $T$ | temperature (°C or K) |
| $t$ | extraction time (min) |
| $\Delta E_a$ | activation energy (J/mol) |

*Abbreviations*

| | |
|---|---|
| Abs | absorbance to 765 nm |
| DPPH | 2,2-diphenyl-1-picrylhydrazyl radical |
| GAE | gallic acid equivalents |
| GRAS | generally recognized as safe |
| PLE | pressurized liquid extraction |
| RMSD | root-mean-square deviation |
| $S_1$ | 34 mL extraction cell (5.2 × 2.8 cm) |
| $S_2$ | 66 mL extraction cell (9.8 × 2.8 cm) |
| $S_3$ | 100 mL extraction cell (15.8 × 2.8 cm) |

any solvent for the fractionation of phytochemicals. An example of this is the ability to perform successive extraction steps in the same sample using the same or different solvent for each extraction step, which yields a variety of compounds with different properties. Another advantage of using PLE is that it significantly modifies the properties of the extraction solvents, offering the possibility of using polar solvents for the extraction of compounds with hydrophobic characteristics (e.g. lipids, pigments and vitamins). Pronyk and Mazza [15] reported that pressurized liquids have high versatility due to the physicochemical properties of the solvent, as the density, diffusivity and dielectric constant can be controlled by varying the pressure and extraction temperature, therefore, changing these properties can effectively control the solvation power and selectivity of solvents.

Considering the advantages of using PLE as an extraction technique, it is important to carry out studies to establish equipment design criteria, in addition to the industrial and pilot scale, in order to obtain the information required for its implementation. The design criteria takes into account the extraction processes and the effect of the extraction bed geometry on the kinetic parameters that define the process profile used, and this information can be used in relation to the physicochemical properties of the products obtained. Thus, the aim of this study was to investigate the influence of the bed height on the kinetics of watermelon seed oil extraction with pressurized ethanol at different temperatures, through the use of mathematical models that describe the extraction process. In addition, we also studied the effect of bed height and temperature on the kinetic parameters, total content of phenolic compounds and antiradical capacity of the oil obtained.

## 2. Materials and methods

### 2.1. Sample preparation

Watermelons of the Ruby variety were purchased from a local market in the city of Pirassununga (Sao Paulo, Brazil). The seeds were manually extracted by slicing the watermelons with a stainless steel knife, after which the juice was extracted from the pulp. The seeds were separated from the residue using a sedimentation-flotation system in plastic containers with potable water. The seeds were then dried at 60 °C for 24 h, after which they were ground using a Wiley mill (Thomas Scientific, Philadelphia, PA, USA) with a 1 mm sieve. Finally, the powder (1% moisture) was packaged and stored under refrigeration at 5 °C [16].

### 2.2. Pressurized liquid extraction

Extractions were performed using an accelerated solvent extraction system, ASE 150 (Dionex, Sunnyvale, CA, USA). Extractions were performed at three different temperatures (40, 60 and 80 °C), using three cylindrical cells ($S_1$, $S_2$ and $S_3$), keeping the mass/solvent ratio constant (0.30) over 8 extraction cycles with total solvent renovation at different times (3, 6, 12, 18, 24, 30, 36 and 42 min). The volume of solvent used in each extraction cycle, was approximately 31, 52 and 78 mL for cells S1, S2 and S3, respectively. In this study, ethanol was selected as it is a 'generally recognized as safe' (GRAS) solvent [17,18].

Initially, the fixed bed was packed with ground sample, then solvent was placed into the cell and the system pressure was controlled at 1500 psia (102.4 atm). Temperature conditions were adjusted according to experimental conditions. $N_2$ was used to discharge the cell solvent, and the system was finally depressurized in order to avoid the presence of the remaining extract in the cell. After extraction, the cell was flushed thoroughly before the next extraction cycle. A rotary evaporator (model IKA RV 05 IKA; IKA-Werke, Staufen, Germany) was used to evaporate the solvent from the extract at 40 °C, and the yield was determined as the ratio between the extract obtained from the seed mass present in the fixed bed extractor.

### 2.3. Total phenolic content

The phenolic content was determined using the Folin-Ciocalteau reagent [19]. An aliquot of extract sample was mixed with distilled water (2 mL) and 1 mL of Folin-Ciocalteu reagent. After 3 min, 2 mL of sodium carbonate (20%) was added and stirred with a vortex. Solutions were stored at room temperature for 1 h in the dark and the absorbance was determined at 760 nm. Gallic acid (diluted in ethanol) was used as a standard solution for the preparation of the calibration curve at concentrations from 0 to 80 mg/L ($R^2 = 0.997$). Results were expressed as mg of gallic acid equivalents (GAE) per gram of seed. The quantification of total phenolics was performed in triplicate.

### 2.4. Antiradical activity

The antioxidant activity was determined using the 2,2-diphenyl-l-picrylhydrazyl (DPPH·) reagent as the free radical [20]. For each extract obtained, different concentrations were tested