Extraction of moringa essential oil (Moringa Oleifera) with conventional solvents and supercritical fluid

Extração do óleo essencial de moringa (Moringa Oleifera) usando solventes convencionais e fluído supercrítico

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ABSTRACT
Essential oils (EO) are substances extracted from different parts of plants and have specific characteristics that guarantee their application in different processes. EO extraction processes involve different methods. Among the methods mentioned in the literature, those related to the minimization of effluent production rates are commonly used. In this context, studies were conducted on the extraction of essential oil from the seed of Moringa Oleifera, using a mixture of carbon dioxide (supercritical fluid) and ethanol or acetone (conventional fluids) as a solvent, to evaluate the influence of the flow rates of fluid mixture flow. Several experiments have demonstrated that the process performance is strongly dependent on the flow velocity of the fluid mixture and the type of co-solvent used. In this case, the decrease in CO₂ flow velocity increases the residence and contact time between the fluid and solid particles. The ethanol is more effective than acetone, mainly due to its intrinsic physical properties. The relationship described above results in the highest diffusion rate of the solute contained in the solid pores to the fluid mixture. The results obtained from these studies are similar to those in the literature and
can support scale-up models of the process that aim to enhance the development and proposition of essential oils to meet the country's needs, supported by the use of biomass produced in Angola.

Keywords: extraction, essential oil, co$_2$, supercritical fluid, properties.

1 INTRODUCTION

Essential oils are volatile substances extracted from different parts of the plant and incorporate characteristic as odours and fragrances, with a complex chemical composition that varies among species, place of cultivation, collection, stabilization, and storage conditions (Sartor, R. B (2009) and Miranda et al (2016)).

According to Bezerra et al. (2004), (2013) and Uwineza and Waśkiewicz (2020), these compounds are bioactive, with specific characteristics that guarantee their application as medicines, vitamin supplements, flavours and fragrances. For the above authors mentioned, the oil extracted from plants contains a set of components, such as terpenic hydrocarbons, simple and terpenic alcohols, aldehydes, ketones, phenols, esters, ethers, oxides, peroxides, furans, organic acids, lactones, coumarins, and compounds with sulphur and with biological, antimicrobial and antioxidant emollient properties, in addition to nutritional properties of great relevance to the food, cosmetic and pharmaceutical industries.
Essential oils can be extracted from leaves, fruits, seeds, with the composition and quantity associated with the processes of cultivation, collection, storage and extraction. For the extraction of essential oils from seeds, conventional solvents or supercritical fluids or a mixture of both are used (Calvacante, 2017; and Steffens, 2010).

Extraction methods involve the transfer of a solute contained in the solid structure of the biomass to the solvent, with preferential selectivity. The processes involved in the extraction are based on the principles of liquid-liquid, solid-liquid and gas-liquid equilibrium, depending on the type of extraction used (VEGGI, 2006; SCHNEIDER, 2002).

Solid-liquid extraction (SLE) is based on the principles of mass transfer from the solute, present in the solid phase, to a given solvent with appropriate selectivity. For these cases, the phenomena of leaching, convection, diffusion and dialysis are involved. To ensure the implementation of the referenced mechanisms, extraction may involve steam distillation, hydro distillation, flowering, cold pressing, solvent extraction and supercritical fluid extraction (SFE) (SCHNEIDER, 2002; Ferreira, 2016; Silva, 2019; Scheneider, 2002).

As noted by Ash (2018) the selection of the type of extraction to be adopted in the production of essential oil from oil seeds depends on the physical and chemical characteristics of the seed, mainly concerning the particle size, the solvent involved, the process temperature, and, mainly, the oil content present in the seed.

In this context, Freixo (2017) observed that the extraction, when done using conventional methods, presents disadvantages associated with high costs, alteration of the product's aroma, exposure of the extraction product to solvent boiling temperatures, difficulty in optimizing the process and reduced degree of purity of the products coming from the process.

It was in the context described by Freixo (2017) that boosted the proposition of modern and more effective processes, resulting in the implementation of the use of supercritical fluids, in the laboratory, bench, pilot, semi-industrial and industrial scales. The first studies point to the implementation of this methodology in the 70s, in Germany, in the processes of extracting caffeine from coffee beans (Vargas, 2005).

The extraction with supercritical fluids is a technique of great interest and that has been consolidated in the last years, as it is characterized as a technological alternative of great relevance in the extraction of vegetable oils.
According to Kevin (1989), a supercritical fluid (SCF) of any substance at a temperature and pressure above its critical point, where distinct liquid and gas phases do not exist, but below the pressure required to compress it into a solid. It can effuse through porous solids like a gas, overcoming the mass transfer limitations that slow liquid transport through such materials. SCF are much superior to gases in their ability to dissolve materials like liquids or solids. In addition, close to the critical point, small changes in pressure or temperature result in large changes in density, allowing many properties of a supercritical fluid to be "fine-tuned". These types of fluids are very attractive for the processing of products rich in bioactive compounds, given the ease of separating the solute from the solvent (CALCANTI, 2013; LEMES, 2018).

The combination of physical-chemical properties, temperature, pressure, specific mass, diffusion coefficients and viscosity of liquids and gases in the supercritical phase makes it possible to use such fluids for various industrial essential oil extraction processes (COSTA, 2015).

Studies conducted by Costa (2015) show that the specific mass of supercritical fluid is similar to that of liquids and the viscosity and surface tension are approximately similar to that of gases. In these studies, it was observed that the diffusivity of supercritical fluids varies between that of gases and liquids, depending on the type of compound evaluated. These characteristics provide high effective solubilisation capacity in the penetration and diffusion of the fluid in pores contained in solid particles and which result in the effective extraction of the solute.

According to Michielin (2002), in the extraction processes, the solute is transported from the solid phase to the supercritical fluid and the performance depends on factors associated with the solubilization power, selectivity of the solvent to the solute, diffusion capacity of the solute to the solvent and the distribution of the solute in the solid particle.

According to Aquino (2018) the pressure of the extracting fluid influences the efficiency of the process. As this parameter reflects on the temperature and the specific mass of the system, which results in the similarity of its characteristics with those of a liquid. Therefore, the increase in temperature decreases the fluid's specific mass at reduced pressures.

According to Costa (2015), processes with high extraction yield should be associated with operations carried out in systems with high pressures, thus ensuring high specific masses of supercritical fluid. On the other hand, the author states that extraction
with supercritical fluids is influenced by fluid properties, such as molecular weight, polarity, density, pressure, temperature and flow.

In this context Costa (2015) and PIES (2017) affirm that the use of carbon dioxide (CO2) is recommended as a supercritical fluid because it is a non-polar, non-flammable, polluting, corrosive, toxic solvent with no risk of occurrence of secondary reactions. When compared to organic solvents, carbon dioxide has relatively low costs, is plentiful and is used at moderate temperatures and pressures. Carbon dioxide is a versatile solvent with limitations for extracting polar compounds. The possibility of modifying the parameters of supercritical extraction processes, with the addition of reduced amounts of other solvents, has driven studies aimed at identifying the best relationship between such fluids and ensuring greater extractive performance.

Dos Santos et al. (2021) developed the study on the extraction of essential oil from an onion using a mixture of ethanol and CO2 as the extraction fluid. The essential oil obtained from the extraction was collected and purified and the mass was determined (by weighing) to evaluate the effect of CO2 flow on the yield. The essential oil extracted and purified was used to determine the acid and refraction indexes, viscosity and specific mass. For the authors, for all cases, when the CO2 was used, there was an increase in the essential oil recovery.

According to Michielin (2002) the ratio between the solvent flow and the solids mass is one of the most relevant factors in the extraction, because the increase in the solvent flow, for a fixed solid mass, reduces the solvent load as a function of reduced residence time. In this case, the extraction speed increases to a maximum threshold. Thus, the resistance to mass transfer inside the particle is dominant. To reduce the intensity of this parameter, the particle size of the constituent particles of the system must be reduced.

Paes (2011) analysed the influence of pre-treatment on oil extraction with carbon dioxide and observed that particles with reduced particle size favour the effectiveness of the process.

Drawing on the descriptions above, this study was developed to extract the essential oil from moringa using CO2 as supercritical fluid and ethanol and acetone as conventional solvents and evaluated the operating conditions that ensure greater process performance and quality of the products obtained.

Moringa is a medicinal plant with significant amounts of vitamins and minerals, such as iron, carotenoids, quercetin, vitamin C, among others, which provide greater antioxidant and anti-inflammatory effects. The characteristics of moringa as mentioned
here have prompted the development of studies for the treatment of respiratory diseases, reduction of anxiety, weight loss and control of glucose concentration in the blood. The implementation strategy of this study provides the extraction of essential oil from moringa for use as a raw material for the pharmaceutical and food industry.

**2 METHODOLOGY**

The extraction of oil from Moringa seed involved four steps, namely: a) Peeling and drying; b) Crushing and granulometric analysis; c) extraction of moringa essential oil and; d) Purification and characterization of the essential oil produced.

2.1 DRYING

Firstly, moringa seeds were peeled and weighed to determine the mass of waste and raw materials used in the process. Subsequently, drying was performed using a semi-continuous process. The drying involved weighing Moringa seeds using an analytical balance and placed in two heavyweight aluminium trays. The trays were placed in convective dryer Gunt Hamburg EC13. Then, the fan-powered, with the prior definition of the airflow velocity (1.40 m/s) and the drying temperature (40ºC). The dryer was activated, ensuring airflow at the operating temperature, ensuring the contact of the referenced fluid with the surface of the solid particles contained in the tray. Within the drying process, the mass was maintained the solid particles present in the trays as a function of time, for five hours.

2.2 GRANULOMETRY

After drying, moringa seeds were crushed and the particle size analysis was conducted, which consisted of measuring the mass of the crushed moringa sample. Then, the sample was sieved using the Taylor series of standardized sieves, with mesh openings from 0.5 mm to 2 mm. Mechanical agitation made it possible to increase the effectiveness of the process, ensuring the passage and retention of solid particles in the corresponding meshes.

2.3 SUPERCritical FLUID EXTRACTION

Upon completion of the particle size analysis, the samples were placed in a desiccator to minimize the tendency of water absorption, given the hydrophobic character...
of moringa seed. Next, the oil extraction system was structured with supercritical fluid (CO₂) and a co-solvent, high purity ethanol (96%).

The system built for running the experiment is divided into three sections: a) In the first section, the heat exchanger serpentine interconnecting the supercritical fluid (CO₂) to the extraction tank, is preheated with the recirculation of the thermostatic bath until the extraction temperature of 70ºC. Additionally in this section, the co-solvent (ethanol) is heated to the operating temperature referenced above; b) In the second section, the supercritical fluid valve is opened until the desired operating condition, ensuring the flow of CO₂, which is heated by passing through the heat exchanger and bubbling in the co-solvent, forming a fluid mixture that ascends vertically through the mass of particles contained in the extraction tank; c) The third section involves the condensation system and expansion occurs to separate CO₂ and ethanol/essential oil mixture.

In general, the execution of the experiment consisted of measuring the mass of crushed moringa seeds with defined granulometry, and inserting the referenced mass in the extraction tank, as well as adding 500 ml of ethanol to the extraction fluid mixture flask. Then, the CO₂ valve was opened, ensuring the flow of this fluid, passing through the previously heated heat exchanger and subsequently mixing it with the ethanol present in the mixing tank. The mixture of extraction fluids ascends and passes through the particulate system, ensuring a strong interaction between the fluid and solid phases, resulting in the transfer of the essential oil mass contained in the particulate system to the fluid phase.

The procedures outlined in the preceding paragraph were used in the analysis of capacity of essential oil of Moringa extraction is involved performing four experiments, whose operating conditions are described in Table 1.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Exp. 1</th>
<th>Exp. 2</th>
<th>Exp. 3</th>
<th>Exp. 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moringa seed initial mass (g)</td>
<td>235,513</td>
<td>222.72</td>
<td>132.41</td>
<td>100.0</td>
</tr>
<tr>
<td>CO₂ speed (m/s)</td>
<td>2.00</td>
<td>1.62</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td>Particle size (mm)</td>
<td>0.80</td>
<td>0.80</td>
<td>0.80</td>
<td>0.80</td>
</tr>
<tr>
<td>Ethanol (g)</td>
<td>1000.0</td>
<td>1000.0</td>
<td>1000.0</td>
<td>-</td>
</tr>
<tr>
<td>Acetone (g)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1000.0</td>
</tr>
</tbody>
</table>

Upon completion of each experiment, the products were purified by simple distillation for the recovery of co-solvent and obtaining the essential oil with higher
purity. For the control of the purification process, the operating temperature was set at 74ºC, which is an unfavourable condition for possible thermal cracking of the oil.

The process yield was calculated by relating the masses of essential oil produced by the mass of solid particles initially inserted in the extraction tank, as described in this article, following Equation 1.

\[
\eta(\%) = \frac{m_o}{m_s} \times 100
\]  

Where \(m_o\) is the mass of oil extracted, in grams and \(m_s\) is the mass of the sample of solid moringa particles initially inserted into the extraction tank.

2.4 DENSITY

The density of the essential oil produced was determined by measuring the volume and respective mass. In this case, the mass of the beaker was initially determined and, consequently, the mass of the essential oil was inserted into the beaker up to 10 ml and weighed at room temperature. Using Equation 2, the specific mass of essential oil produced was determined.

\[
\rho = \frac{m' - m}{V}
\]  

Where \(m'\) is the mass (g) of the cylinder with oil (g), \(m\) is the mass (g) of the empty cylinder and \(V\) is the volume (ml) of oil.

2.5 ACIDITY INDEX

The determination of the acidity index consisted of adding 50 ml of acetone and 4 drops of phenolphthalein in an Erlenmeyer flask. Then, the titration was performed with a 0.1 M potassium hydroxide (KOH) solution. The volume (ml) spent was noted. Subsequently, 1 g of a moringa essential oil sample was weighed and added to the titrated acetone until homogenization. 4 drops of phenolphthalein were added to the oil/acetone mixture. This mixture was titrated with a 0.1 M solution of potassium hydroxide (KOH) for 30 seconds, until light pink, with stability. The volume of potassium hydroxide (KOH) consumed was noted. The data obtained from this assay were used to determine the acidity index, according to Equation 3.
Acidity index \(\left(\frac{\text{mgKOH}}{g}\right) = \frac{(V_a - V_b) \cdot C \cdot MM \cdot f_c}{m}\) (3)

Where \(V_a\) is the volume (ml) of KOH consumed in the oil blend titration, \(V_b\) is the volume (ml) of KOH consumed during the acetone titration (white), \(C\) is the concentration of potassium hydroxide (KOH), \(MM\) is the molar mass (g/mol) of potassium hydroxide (KOH) and \(M\) is the mass (g) of the oil sample.

2.6 REFRACTIVE INDEX

An Abbe-type refractometer was used to determine the refractive index. To read the refractive index of the essential oil, the equipment was first calibrated and two drops of liquid were inserted into the refraction prism. The scale was then read in the refractometer eyepiece. After calibration with acetone, the refractometer was cleaned with cotton. With this same procedure, the refractive index of moringa seed oil was measured, with the insertion of two drops of moringa essential oil sample in the prism of the refractometer, followed by the reading in the visual field.

3 RESULTS AND DISCUSSION

3.1 DRYING

Using the procedures described in this article, drying was performed with the progressive measurement of time, temperature and mass of moringa seeds, as can be seen in Figure 1.

From the analysis of the data, a 15 g loss of mass can be observed within a drying period of five hours, corresponding to 2.27% of the mass initially inserted in the drying system. Studies conducted by Almeida et al. (2015) confirm the presence of water in moringa seeds with approximately 5.40% water. The difference observed in this work compared to the work developed by Almeida et al. is due to the relative humidity of the air in each region where the studies were implemented.

The graphical analysis contained in Figure 1 below shows a marked loss of mass within the early 150 minutes of drying, due to the difference in moisture concentration in the solid particles and in the drying fluid, parameters that drive mass transfer rates. In this hypothetical analysis, stability is recorded for times greater than 150 minutes, up to the operating time limit. The behaviour described was represented by a second-degree polynomial with a regression coefficient of 97.327%, therefore, with high significance.
3.2 GRANULOMETRIC ANALYSIS

The average diameter of the particles used was 0.782 mm, therefore with an adequate surface area for an effective extraction based on a strong interaction between the fluid and solid phases. For this analysis, it is observed that the distribution of particles by diameter covers 28.3% with a diameter of 1 mm, 34.42% with a diameter of 0.5 mm and 25.0% with diameters smaller than 0.5 mm. Therefore, the largest proportion of particles was retained in the 0.50 mm mash, then in the bottom mash with 22.3% and finally in the 1 mm mash. For the effectiveness of this study, grains retained in the 0.5 mm mash were used with a particle diameter of 0.8 mm.

3.3 EXTRACTION WITH CO₂ AND ETHANOL

With the definition of the particulate system used in this process, the essential oil of moringa was extracted, following the procedures contained in this work. The extraction experiments were performed for an operating time of three hours. The analysis of dates makes it possible to state that the yield in extracted oil was between 6.11% and 17.16%. For this analysis, the yield increases with increasing CO₂ flow velocity, that is, when the CO₂ velocity is 2.0 m/s, the process performance is 6.11%, while for at a speed of 0.1 the yield changes to 17.16%. This behaviour is associated with the residence time of the extraction fluids, therefore, the contact time between the phases, which impact the mass transfer rates, ensuring an increase in the efficiency of the process. Another factor associated with process performance is the operating temperature. When the flow velocity of CO₂ is high, the heat exchanger does not guarantee the heating of this supercritical fluid sufficiently for extraction to take place at the established temperature. For reduced speeds, the heating time increases and therefore the temperature and performance of the process. Studies conducted by Belo et al. (2019) show that the best essential oil extraction temperature for Moringa oleifera is 57ºC and 80MPa pressure. According to Nguyen et al. (2011) the yield of essential oil extraction processes from the seed of Moringa oleifera.
varies between 29.24% and 40.01% and is strongly dependent on the size of the particles used. The works developed by the authors indicate a yield of 29.24% for 30 MPa pressure and 47.5°C temperature, for particles with a diameter of 1.0 mm and an extraction time of 7 hours.

The data obtained by Nguyen et al (2011), if compared to the data contained in this work, show that the extraction time is one of the most predominant factors in this process because when the extraction process is carried out within seven hours the performance is 40% and the linearization of these values lasting three hours, results in a performance of 17.142% yield, similar to the results obtained in this work for reduced flow velocity of CO₂.

The linearization of the lowest performance obtained by Nguyen et al (2011), for an extraction time of three hours, would result in 12.53%. In this case, the CO₂ flow velocity that impacts the residence and contact time between the phases, negatively, predominates in the performance of the process. For this reason, the extraction operations must be optimized to guarantee the necessary parametric domain to increase the essential oil extraction rates.

Aquino (2018) analyzed the tribological performance of the biolubricant based on Moringa oil, extracted with solvent and then added with iron oxide nanoparticles, obtaining an acid index of 0.909 mg NaOH/g and density of 0.834 g/cm³ for pure essential oil. For the essential oil with additives, an acidity index of 0.432 mg NaOH/g and density of 0.867 g/cm³ were determined. In the comparative analysis, the results obtained by Aquino, without additives, show a deviation similar to the deviation observed by Pereira, of 4.13%, therefore, within the deviation margin.

Leone et al. (2016) determined the acidity index of moringa essential oil in the range between 0.32 and 4.0 mg KOH/g and the refractive index in the range of 1.455 and 1.470, at 40°C. The two parameters evaluated by the authors were located in the same range as the parameters obtained in this study, therefore, with methodological consistency of the procedure used and described in this work.

Bouanga-Kalou et al. (2011) performed the physicochemical characterization of moringa essential oil, when determining the refractive index, at 25°C, of 1.4652, a value similar to that obtained in this work.

The Brazilian National Health Surveillance Agency (Anvisa) establishes the maximum limit of acidity index for crude and unrefined oils at 4 mg KOH/g of oil. This
reference parameter, when compared with the parameters coming from this study, shows the compliance with the limit established by Anvisa.

3.4 EXTRACTION WITH CO\textsubscript{2} AND ACETONE

To expand the scope of the studies, moringa essential oil extraction tests were performed, using the procedures described in this work, but this time using acetone as a co-solvent. In this case, considering the greater performance delivered with a lower flow velocity of the supercritical fluid with the use of ethanol, this condition was explored and the results show a yield of 8.48% along with a lower rate of acidity.

The analysis of the data obtained from the acetone extraction, shows the influence of the type of solvent on the quality of moringa essential oil produced, characterized by lower density, lower acidity index and higher yield, when the data are compared with data from the use of ethanol as a co-solvent. In the comparative analysis with literature data, it is observed that the best performance and quality are associated with the use of ethanol as a co-solvent.

4 CONCLUSION

Based on the results contained in this work, it can be concluded that:

a) The performance of the extraction process is more effective when ethanol is used as a co-solvent, compared to acetone, due to the molecular and physical properties of ethanol;

b) The flow velocity of the fluid mixture, composed of the conventional solvent and supercritical fluid, must be reduced to ensure longer contact time between the fluid and solid phases, which results in higher mass transfer rates and, therefore, greater performance of the process;

c) The performance of the process obtained in this study, when using ethanol as a co-solvent, is similar to the performance described in the literature;

d) The physical properties of the essential oil evaluated in this study are similar to those described in the literature, constituting the quality element for the expansion of the scale for the industrial process.

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REFERENCES


Etiandra dos Santos, Teresa Saleme Tingeira, Vicencia de Fátima Cristovão da Costa, Luana Marcele Chiarello, António André Chivanga Barros; Essential oil extraction from onion using ethanol and CO₂ as an extraction fluid mixture; F1000 Research Journal, 10, 625, 2021.


Paes, Mariana Schincoriol. Influence of Pre-Treatment of Tubercles and Priprioca Rhizome (Cyperus articulatus L.) in obtaining essential oil by extraction with Supercritical CO2. Postgraduate Program in Chemical Engineering, State University of Campinas, Faculty of Chemical Engineering. Campinas, São Paulo, 2011.


Pies, Gustavo. Supercritical technology applied to obtain extracts rich in Phenolic compounds from the bark of Jabuticaba Plinia Trunciflora (O. Berg) Kausel. Dissertation (Masters in Food Engineering) - Federal University of Santa Catarina, Florianópolis, Santa Catarina, 2017.


Silveira, Jeniffer Cristina; Busato, Nathália Viégas; Costa, Andrea Oliveira Souza da; Junior Esly Ferreira da Costa - Survey and analysis of methods for extracting essential oils - Federal University of Espírito Santo, Alegre Campus, Alto Universitário, CEP: 29,500-000. 2012


Veggi, Priscilla Carvalho. Obtaining plant extracts by different extraction methods: experimental study and process simulation. Dissertation (Masters) - State University of Campinas. Faculty of Food Engineering, SP. 2009.