UNIVERSIDADE FEDERAL DO PARANÁ

DAMIAN ESTUARDO LOPEZ FETZER

OIL EXTRACTION FROM BARU (*Dipteryx alata* Vogel) SEEDS USING COMPRESSED SOLVENTS TECHNOLOGY

CURITIBA

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Orientador: Prof. Dr. Marcos Lúcio Corazza

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Os membros da Banca Examinadora designada pelo Colegiado do Programa de Pós-Graduação em ENGENHARIA DE ALIMENTOS da Universidade Federal do Paraná foram convocados para realizar a arguição da Dissertação de Mestrado de **DAMIAN ESTUARDO LÓPEZ FETZER** intitulada: **Oil extraction from baru (*Dipteryx alata* vogel) seeds using compressed solvents technology**, após terem inquirido o aluno e realizado a avaliação do trabalho, são de parecer pela sua APROVAÇÃO no rito de defesa.

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Curitiba, 08 de Março de 2018.

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RESUMO

Este estudo trata da extração de óleo de sementes de baru (Dipteryx alata vogel) usando propano comprimido, CO_2 supercrítico ($scCO_2$) com e sem etanol como solventes. Os resultados são comparados à extração convencional (Soxhlet) com etanol e hexano. Os resultados obtidos indicaram que o propano comprimido apresentou um maior rendimento de extração (36,87%) a 10 MPa, 60 ºC, com 10 min de tempo de confinamento e usando um tamanho médio de partícula de 0,5 mm. Também foram obtidos rendimentos de extração mais elevados utilizando $scCO_2$, porém a adição de etanol como co-solvente foi necessária para aumentar a eficiência da extração. As análises de tocoferóis e atividade antioxidante em todas as amostras apresentaram melhores resultados em comparação com a literatura. O perfil de ácidos graxos foi semelhante para todas as amostras, onde ácido oleico (variando de 50-54%), ácido linoléico (23-25%), ácido palmítico (cerca de 5%), ácido esteárico (cerca de 5%) e ácido araquidônico (cerca de 4%) são os principais ácidos graxos encontrados nas amostras avaliadas no presente estudo. Por fim, os resíduos da extração apresentaram alto teor de proteínas (32%). Como resultado geral, observa-se que o óleo obtido é bastante promissor para aplicações nas indústrias de alimentos e farmacêutica.

Palavras-chave: Dióxido de carbono supercrítico, propano comprimido, semente de baru, óleo livre de solvente, estabilidade oxidativa.

ABSTRACT

This study reports the extraction of oil from baru (*Dipteryx alata* vogel) seeds using compressed propane, supercritical CO_2 ($scCO_2$) with ethanol as solvents and the conventional (Soxhlet) extraction using ethanol and hexane. Results indicated that the compressed propane presented the highest extraction yield (36.87%) at 10 MPa, 60 ºC, with 10 min of confinement time and using an average particle size of 0.5 mm. Higher extraction yields were also reached using $scCO_2$ but the addition of ethanol as co-solvent was needed. Tocopherols and antioxidant activity in all samples presented better results in contrast with the literature. The fatty acid profile was similar for all samples, where oleic (within 50-54%), linoleic (23-25%), palmitic (around 5%), stearic (around 5%) and arachidonic acid (around 4%) are the major fatty acids found. Finally, residues of the extraction presented high content of proteins (32%). The oil obtained is very promising for food and pharmaceuticals applications.

Keywords: Supercritical carbon dioxide, compressed propane, baru seed, solvent-free oil, stability oxidative.

RESUMEN

Este estudio reporta la extracción de aceite de semillas de baru (*Dipteryx alata* Vogel) usando propano comprimido, dióxido de carbono supercrítico ($scCO_2$) con etanol como solventes y la extracción convencional (Soxhlet) empleando etanol y hexano. Los resultados indicaron que el propano comprimido presentó el mayor rendimiento de extracción (36.87%) a 10 MPa, 60 ºC, con 10 min de tiempo de confinamiento y usando un tamaño de partícula promedio de 0.5 mm. Además se alcanzaron mayores rendimientos de extracción utilizando $scCO_2$, pero se necesitó la adición de etanol como cosolvente. Los tocoferoles y la actividad antioxidante en todas las muestras presentaron mejores resultados en contraste con la literatura. El perfil de ácidos grasos fue similar para todas las muestras, donde el ácido oleico (dentre de 50-54%), linoleico (23-25%), palmítico (alrededor del 5%), esteárico (alrededor del 5%) y el ácido araquidónico (alrededor del 4%) fueron los principales ácidos grasos encontrados. Finalmente, los residuos de la extracción presentaron alto contenido de proteínas (32%). El aceite obtenido es muy prometedor para aplicaciones alimentarias y farmacêuticas.

Palabras claves: dióxido de carbono supercrítico, propano comprimido, semilla de baru, aceite libre de solventes, estabilidad oxidativa.

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LIST OF ABBREVIATIONS

EMBRAPA - *Empresa Brasileira de Pesquisa Agropecuária*

SFE - Supercritical Fluid Extraction

$scCO_2$ - Supercritical carbon dioxide

FFA - Free Fatty Acid

RI - Refractive Index

MPa - Megapascal

AOAC - Association of Official Agriculture Chemical

FDA - Food Drug Administration

USDA - United States Department of Agriculture

LACTA - *Laboratório de cinética e termodinâmica aplicada*

LABTECAL - *Laboratório de Tecnologia de Alimentos*

CEPPA - *Centro de Pesquisas de Processamento de Alimentos*

CO_2 - Carbon Dioxide

pH - potential Hydrogen

P - Pressure

T - Temperature

MAMSL - Meters Above Mean See Level

PS - Particle size

GC - Gas Chromatography

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1 INTRODUCTION

The Cerrado Biome is one of the highest diversity regions in the world. Aside from the Amazon rainforest, it is the second largest biome in South America covering about 24% of Brazil territory (LEWINSOHN; LEWINSOHN, 2008; OLIVEIRA-FILHO; RATTER, 2002). On the other hand, the Chiquitanian dry forest is one of the best preserved tropical dry forest of the American continent. Its name is native from Bolivia, specific in the region of Chiquitos (COLLEVATTI et al., 2013; DEVISSCHER et al., 2016; VIDES-ALMONACID; REICHLE; PADILLA, 2007). One economically important product from Cerrado and the Chiquitanian dry forest is *Dipteryx alata* Vogel, commonly known as the “baru” tree in Brazil (CORRIÀ-AINSLIE; JULIO CAMARERO; TOLEDO, 2015; SIQUEIRA et al., 2012). It is a species that presents high-quality wood and the contents of the fruit, pulp, and seed, have potential human uses including food and medical application. Fruits are collected on the forest ground after the vigorous shaken of the trees. The fruit has a strong shell and inside it a seed that, according to TAKEMOTO; OKADA, 2001, presents high levels of lipids (40.27%), protein (29.59%), total fibers (19.04%) that 4.95% are soluble and 14.10% are insoluble, total sugar (7.28%) and starch (0.99%). Besides, it contains higher energy value with 476-560 kcal/100 g, as is mentioned in some studies (FERNANDES et al., 2010; TOGASHI, 1993). In addition, the oil is rich in unsaturated oleic (50%) and linoleic (28%) fatty acid and contains α-tocopherol (5 mg/100 g) (TAKEMOTO; OKADA, 2001).

As presented in the literature, the baru seed oil was extracted using different methods (MARTINS et al., 2013), mostly of them employing conventional processes using organic solvents. Several laboratories and industrial extraction units have been proposed to reduce the use of organic solvents as well as the use of alternatives extraction methods and green chemistry which are environmentally friendly and sustainable (BERNAL; MARTÍN; TORIBIO, 2013; KNEZ et al., 2014). Therefore, there is considerable interest in replacing processes such as extraction with organic solvents, for instance the hexane, traditionally used in the oil extraction industries. High pressures technologies, including sub and supercritical fluid extraction offer the opportunity to obtaining new products with interesting characteristics that can present high efficiency as much as traditional methods (KNEZ et al., 2014), and in this sense supercritical CO_2 ($scCO_2$), with or without co-

solvents, and compressed propane have risen as a promising techniques for oil extraction. These techniques can be considered clean technologies because at the end of the extraction the solvent is completely removed and separated from the oil by depressurizing the system, thereby obtaining solvent-free oil with high purity (BRUNNER, 1994). Furthermore, the solvent can be easy recovered and reused in a closed loop in the process. Subcritical propane allows high extraction rates of oilseeds extraction (AHANGARI; SARGOLZAEI, 2012) due to the high solubility of the triacylglycerols (nonpolar compounds) in this solvent (CORSO et al., 2010; NDIAYE et al., 2006). CO_2 has the advantage that is cheaper, nontoxic and abundant, but due its quadrupolar moment it presents low solubility with non-polar molecules as triacylglycerols, even though the addition of a co-solvent can overcome this drawback increasing its efficiency and applicability. In the study presented by DOS SANTOS et al., 2016 employing $scCO_2$ to recover cumbaru oil, better extraction conditions found by those authors were 40 ^{o}C and 50 ^{o}C at 35 MPa, in which 22.6% and 22.8% of extraction yield were obtained, respectively. However, those authors mentioned that $scCO_2$ partially recovered the cumbaru seeds oil, around 60%. Thus, considering as demonstrated in some studies that adding a co-solvent to the $scCO_2$ the extraction yield can be significantly improved (CRUZ et al., 2017; SODEIFIAN; SAJADIAN; SAADATI ARDESTANI, 2016), the addition of a co-solvent to the $scCO_2$ could be an alternative to improve the extraction yield of baru seeds operating at lower pressures than 35 MPa. In this sense, the aim of this study is to evaluate different extraction methods and to improve the extraction yield of baru seed oil using compressed propane and scCO2 with ethanol as co-solvent ($scCO_2$+EtOH). In addition, the oil samples obtained from baru seeds were characterized by physicochemical properties, fatty acid (FA) profile, antioxidant activity (AA), tocopherol content, total phenolic content (TPC). Differential scanning calorimetry (DSC) and thermogravimetric (TG) were also analyzed. Finally, some characteristics of the baru seed residues after the extractions were evaluated.

1.1 OBJECTIVES

1.1.1 General objective

The main subject of this work is to obtain the higher yield of baru seed oil (*Dipteryx alata* Vogel) by low-pressure extraction and supercritical fluid extraction as the primary technique as well as subsequently analyze and identification of the chemical compounds presented in each different extraction sample.

1.1.2 Specific Objectives

The specific objectives of this work can be delineated as follow:

a) Determine the extraction yields of the baru seed matrix by Soxhlet extraction using ethanol and hexane as solvents;
b) Identify the extraction yields of baru seed oil using supercritical CO_2 and compressed propane and compare the results with Soxhlet extractions;
c) Evaluate the cosolvent addition (organic solvents as ethanol and/or hexane) aiming the enhancement of extractions with supercritical CO_2 and compressed propane;
d) Define the kinetics extraction of baru seed oil using supercritical CO_2 fluid and compressed propane;
e) Analyze and compare physicochemical characteristics of the extracts;
f) Establish and compare the lipid profiles of the extracts.

2 LITERATURE REVIEW

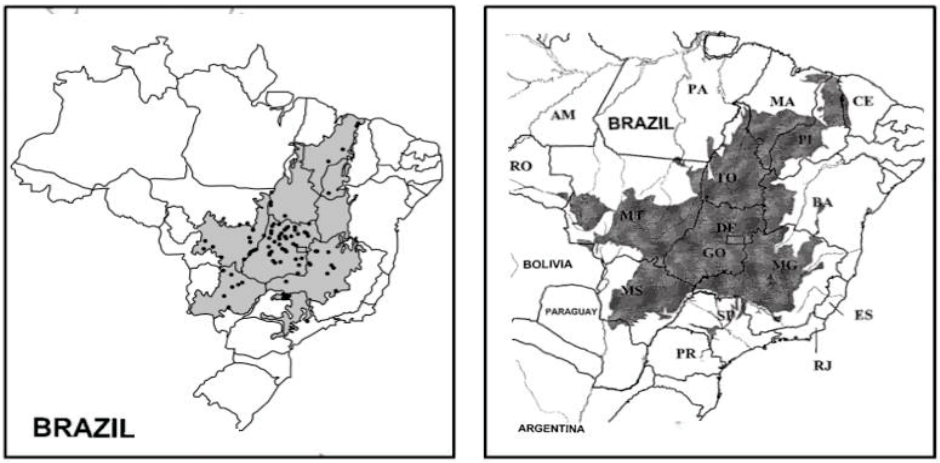
This chapter presents a short literature review of the dissertation focusing the place where baru is endemic and common area; also, the matrix studied, the oil and the extraction methods used are defined and discussed.

2.1 CERRADO BIOME

The term Cerrado (Portuguese for “half-closed,’’ “closed,’’ or “dense’’) was probably applied to this vegetation because of the difficulty of going through it on horseback. The Cerrado Biome is one of the regions to highest diversity in the world, being the second largest biome in South America, behind the Amazon rainforest, covering about 24% of Brazil (2 million Km^2). The Cerrado holds 6000 species of trees, besides other types of life forms. Approximately 40% of these trees are natives of the Brazilian's Cerrado. This Biome includes some territories from Bolivia and Paraguay (LEWINSOHN; LEWINSOHN, 2008; OLIVEIRA-FILHO; RATTER, 2002).

In Brazil is located from the southern borders of the Amazonian forest to boundary areas in the southern states of São Paulo and Paraná. The Cerrado is part of these states, as shows Figure 1: Bahia (BA), Ceará (CE), Distrito Federal of Brazil (DF), Espírito Santo (ES), Goiás (GO), Maranhão (MA), Minas Gerais (MG), Mato Grosso (MT), Mato Grosso do Sul (MS), Pará (PA), Paraná (PR), Piauí (PI), Rondônia (RO), São Paulo (SP), Tocantins (TO). Lying between 2° to 25° of latitude and from 300 to 1800 height above mean sea level (AMSL) (EMBRAPA CERRADOS, 2008). On the biome, more than 121,000 species had been identified with some use as food, fodder, ornamental, medicine, oil, wood, and among others.

FIGURE 1 - GEOGRAPHIC DISTRIBUTION OF THE CERRADO BIOME IN SOUTH AMERICA: BRAZIL, BOLIVIA AND PARAGUAY.



SOURCE: Paulo S. Robert J. (2002).

Nowadays, the main species that have nutrients and commercial value are: the araticum (*Annona crassiflora* Mart.), the mangaba (*Hancornia speciosa* Gomes), the pequi (*Caryocar brasiliense* Camb) and the cagaita (*Eugenia dysenterica* Mart. ex. D.C.). They have been marketed regionally with reasonable success. In addition, several other species with economic potential of the narrow sense Cerrado physiognomy are widely distributed, for example: the sucupira preta *(Bowdichia virgilioides),* the faveira *(Dimorphandra mollis)*, the pacari *(Lafoensia pacari)*, the mama-cadela *(Brosimum gaudichaudii),* the pimenta-de-macaco *(Xylopia aromatica),* the gonçalo-alves (*Astronium fraxinifolium),* the murici (*Byrsonima verbascifolia),* and the baru *(Dipteryx alata* Vog.). Science has shown the economic importance of these species. Some studies have demonstrated that the investment cost could overcome the profit (EMBRAPA CERRADOS, 2008).

Cerrado gives more than 30% of the gross domestic product (GDP) of Brazil and employing approximately 40% of the economically active population. One economically important product from Cerrado species is *Dipteryx alata* Vogel (Fabaceae), commonly known as the “baru” tree, shown in the figure 1. It is widely distributed as large tree species endemic to the biome, normally restricted to seasonal savannas habitats and growing in eutrophic and drained soils. The species is hermaphroditic, and pollination is mainly performed by large and medium-sized bees. Seeds have a very woody endocarp, with edible nuts that are eaten and dispersed by mammals, such as bats and monkeys, and are a source of raw material for small- and middle-sized food industries, playing an important role in the local

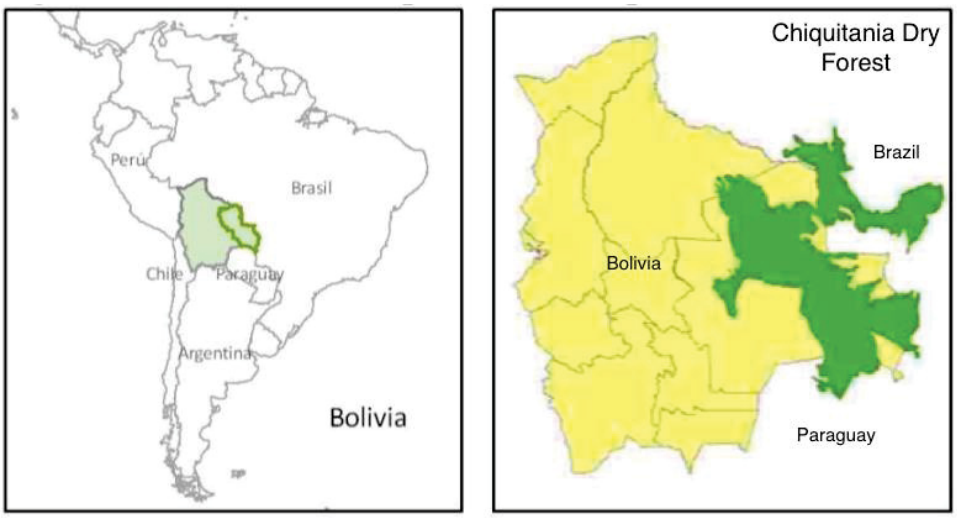
economy of Brazil and Bolivia (DE CAMPOS TELLES et al., 2014; MAGALHÃES, 2014).

2.2 CHIQUITANIAN DRY FOREST

The Chiquitanian dry forest is the best preserved tropical dry forest of the American continent. Its name is native from Bolivia, specific in the region of Chiquitos. The forest area has 24,748,850 ha, which cover Bolivia, Brazil, and Paraguay. In Bolivia, the largest area (16,449.475 ha) is in the region known as Chiquitania, which cover most of the department of Santa Cruz and the south of Beni (Bolivia). In Brazil at 6,547,427.64 ha are part of Mato Grosso, Rondonia e Amazonas states, and In Paraguay. The area is 1,751,176.26 ha of the High-Paraguay department (VENNETIER; PELTIER; COIMBRA, 2012; VIDES-ALMONACID; REICHLE; PADILLA, 2007).

Moreover, the longitude of the Chiquitanian dry forest is amid 14 and 19.5 degrees. The forest is a lower region with 400 metres above mean sea level (MAMSL). Temperatures average 25 °C, it has warm humid tropical climate and with high humidity, above 60% (VENNETIER; PELTIER; COIMBRA, 2012).

FIGURE 2 - LOCATION OF THE CHIQUITANIAN DRY FOREST



SOURCE: Fernández (2010).

The dry forest has the limit to the north with the Amazon rainforest (Brazil and Bolivia); the western with Santa Cruz of the Sierra city (Bolivia); the East limits to be Brazil, and Paraguay as show the Figure 2. The 11% of the land is acceptable to agriculture use; the major area is used by monocultures as wheat and cotton. The

main activity is occupation by livestock and cattle system (60% of the flat land). More than 246 tree species live in the forest, although only 36 covers about 95% of the forest area. Likewise, one of these species is *Dipteryx alata* Vogel, that represents economic importance in the region, principally for the almond. This tree may contribute to the economic development of the region creating a balance between the forest and the beef cattle (DEVISSCHER et al., 2016; VIDES-ALMONACID; REICHLE; PADILLA, 2007).

2.3 BARU (DIPTERYX ALATA VOGEL)

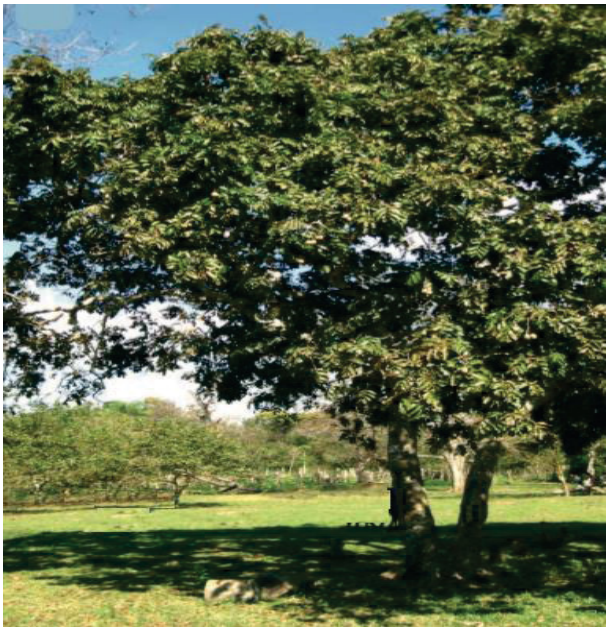
The etymologic of *Dipteryx* is because of a flower shows two wings, and *alata* means winged. These tree species present high-quality wood, and the fruit contains pulp and seed. The plant has also a potential for human uses including food, medical application, and so on. The baru has the next scientific nomenclature: Division; Magnoliphyta (Angiospermae), Class; Magnolipsida (Dicotiledanoe), order; Fabales, Familiar; Fabaceae (Leguminosae: Papilionoideae), species; *Dipteryx alata* Vogel and synonymy botany: Coumarouna alata (Vogel) taubert (EMBRAPA CERRADOS, 2008). Besides, it is called by many names in Brazil, such as baruju, in Mato Grosso; Baruzeiro, in Federal District; bauí, in Goiáis; and in Mato Grosso; cumaru, in Bahia and the São Pablo estate; cururana; cumbaru, in Goiás, in Mato Grosso do Sul, In Mato Grosso and São Paulo state; emburena-brava; fava-de-cumaru, in the Bahia; feijao-coc; Pau-; sucupira-branca, in Piauí and its most famous name is baru. In Addition, it´s also known at Bolivia as amend chiquitana, and Nókümonish (Aimara language). Moreover, there´re records that exist in Peru and Paraguay (LORENZI, 2003; NABOUT et al., 2010; OLIVEIRA-FILHO; RATTER, 2002; RODERJAN, 1983; VENNETIER; PELTIER; COIMBRA, 2012).

2.3.1 Baru tree

Baru tree presents from 5 to 12m height, even so, reaching up to 25m on the good land. Generally, the wood has a diameter between 0.15-0.40m, it is hard with a density high (900-1200 $kg.m^3$), and due to the low moisture (15%), is resistant to termites and fungi. After 5 years old produces fruits, but has life expectancy of around 9 years. Some trees could live up to 60 years and until measure up to 0.7cm

in diameter. The trunk is rarely straight and its color is gray with variable formats desquamation plates as show the Figure 3 (BREEDING; SANTOS; OLIVEIRA, 2014; JAVIER; MOLINA, 2016; SANO; RIBEIRO; BRITO, 2004).

FIGURE 3 – BARU TREE



SOURCE: Coimbra (2016).

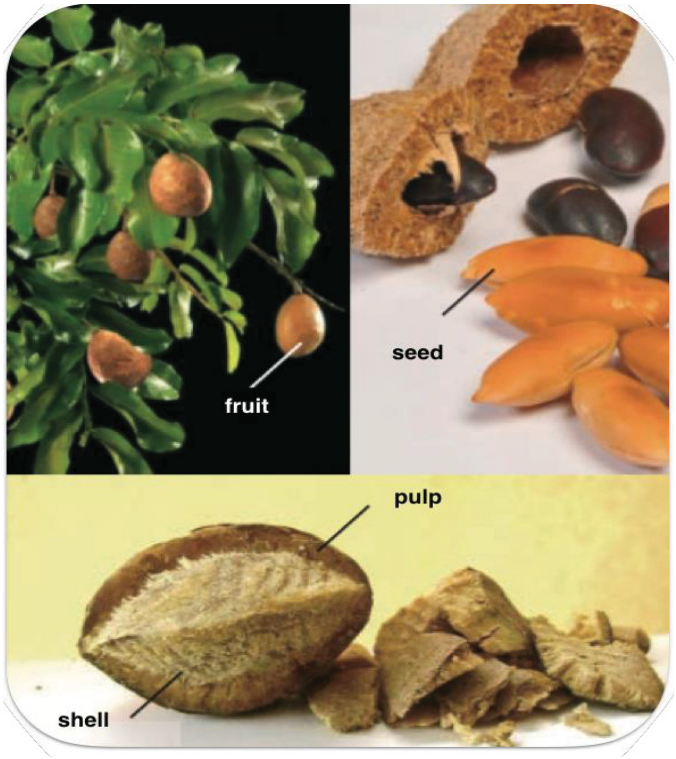
The baru tree was identified between 110 to 1200 MAMSL. The tree harvests around 1000 to 3000 fruits per year (LORENZI, 2003).

In the other hand, the flowers are small, hermaphrodite, and white with a pink or purple stain. The species blooms from May to November (JAVIER; MOLINA, 2016)

2.3.2 Baru fruit

The fruit consists of a seed protected by a hard-woody endocarp shell with a pulpy. The *Dipteryx alata* Vogel is a legume drupóide type and smooth texture, usually ovoid; the color is opaque brown as in Figure 4. When the fruit is open, the pericarp is well defined; the epicarp is a thin, smooth and brittle consistency; woody endocarp, greenish yellow or brown with a spongy layer inside (SILVIA, DANIELA CANUTO, 2015).

FIGURE 4 – BARU FRUIT, SEED, PULP AND SHELL



SOURCE: Orellana and Coimbra (2010).

At the present time, the fruit was recollected on the floor after the agitation of tree (LORENZI, 2003) with a diameter between 3 to 5 cm, and depend on the latitude of the place the fruit crops vary from May to October and from August to September in Brazil (FERREIRA et al., 1998; SILVIA, DANIELA CANUTO, 2015).

2.3.3 Baru pulp

Pulp has around 15mm until the shell. The baru pulp consists of ash (2.99%), protein (5.59%), total lipids (3.46%), total fibers (29.5%) that 28.2% are insoluble and 1.30% are soluble, and carbohydrates (63%) predominating starch with a 38%. Also content 300 kcal/100g of calories (ROCHA; SANTIAGO, 2009; TOGASHI, 1993).

In the composition of the pulp, amino acids showed very high levels of proline (17.91 g/16gN)) and low concentrations of methionine (0.41), tyrosine (0.87) and tryptophan (0.53) (TOGASHI, 1993)

Unfortunately, antinutritional substances were found in baru pulp as phytic acid, trypsin inhibitor, and especially the tannin content was high in the pulp.

2.3.4 Baru seed

By obtaining the seed, regularly is used a knife or others mechanic methods by removing the baru seed of the shell, the most of them made by hand (TOGASHI, 1993). The seed has a natural moister from 6.14% to 8.25% and possess a middle of 1 gram for seed (BOTEZELLI; CLAUDIO; MALAVASI, 2000; SANO; JOSE; ROBERTO, 1999).

In other hand, Takemoto exhibits that the seed presents high levels of lipids (40.27%), proteins (29.59%), total fibers (19.04%) that 4.95% are soluble and 14.10% are insoluble, total sugar (7.28%) and starch (0.99). Besides mineral's seed as calcium (140 mg/100g), potassium (827 mg/100g), phosphorus (358 mg/100g), magnesium (178 mg/100g), copper (1.45 mg/100g), iron (4,24 mg/100g), manganese (4.9 mg/100g), and zinc (4.1 mg/100g). As well, it contains higher energy value with 476-560 kcal/100g.; data that has not changed significantly in recent research (FERNANDES et al., 2010; TOGASHI, 1993).

The seed has elevated ringed of unsaturation as to olive oil. The composition of fatty acids, the saponification index, and iodine are qualitatively very similar to peanut oil as presented the Table 2. Show α-tocopherol content (5 mg/100 g), oleic fatty acid (50%) and linoleic (28%) (TAKEMOTO; OKADA, 2001).

Takemoto showed changes of some compounds when heat treatment of seeds to 200 °C for 15 minutes. Result in losses some amino acids as the l-lysine (26%), tryptophan (27%), tyrosine (10%), histidine (7%), isoleucine (7%), serine (4%) and arginine (3.3%).

2.4 NATURAL EXTRACT AND VEGETABLES OILS

Knowledge of oils began before the Christian era, around 2000 B.C. Ancient records show that in Egypt and China it was used as medicine. Documented oil extraction show that olives were pressed by hand in Egypt using wooden and stone mortars. By 184 B.C., the Romans developed better technology using mills with mechanical pressure systems (FRANK D. GUNSTONE; HERSLÖF; MANAGING, 2004; SHAHIDI, 2005).

Extracts obtained by extraction of the plant (flowers, fruits, seeds, leaves, stems, and roots) or animal tissues (MÓNICA; SALGUEIRO, 2015).

The natural extracts are secondary metabolites that could be: essential oils, vegetal oils, and oleoresins. Plants have two kinds of oils: essential oils and fixed oils. Fixed oils consist of esters of glycerol and fatty acids, whatever essential oils are a mixture of organic compounds, volatile (around 100 u) to semi-volatile compounds (around 300 u), especially hydrocarbons and oxygenated, often with a strong odor, colored, soluble in organic solvents and insoluble in water. Today, analytical techniques present the total separation and identification of all compounds of the volatile mixture unattainable on account of the large number of compounds, structural similarities, isomeric forms, and concentration range of the compounds present in essential oils, with the objective of discover unique properties of each extract, natural antioxidants, microbial agents and among others that can contributed to humanity (ISENGARD, 2010).

In other hand, vegetable oils are in liquid form and present long chains, predominating unsaturated fatty acids in its composition, but that quantity frequently depends on the kind of the extraction. Nowadays, the method for identification of fats and oils are through fatty acid composition analysis determined by Gas Chromatography as show in Table 1 (HIRSCHMANN et al., 1976; OWEN R. FENNEMA, 2008).

TABLE 1 - COMPOSITION OF FATTY ACID OF SOME VEGETABLE OILS

Origen	**Saturated fatty acid**			**Unsaturated fatty acid**			
					Enoic		**Dienoic**
	C14	C16	C18	C16	C18	>C18	C18
Corn	0-2	8-10	1-4	1-2	30-50	0-2	34-56
Cottonseed	0-3	17-23	1-3	-	23-44	0-1	34-55
Palm	1-6	32-47	1-6	-	40-52	-	2-11
Peanuts	0,5	6-11	3-6	1-2	39-66	-	17-38
Soybean	0,3	7011	2-5	0-1	22-34	-	50-60

SOURCE: Boyd and Morrison (1995).

Today a lot of researches on oilseeds show the importance for the human. For example, one study about obesity evaluated chronic disease in Brazil, where to determine that nuts intake improved the lipid profile and microvascular function in obese adolescents, perhaps due to its high level of unsaturated fatty acids and bioactive substances (MARANHÃO et al., 2011).

Togashi happily proved that the content of linoleic fatty acid (31.8 mg) of baru almond is higher than the peanut oil, coconut, olive oil and palm oil. The lipid contains fatty acids with chains from 16 to 24 carbons with 1, 2 and 3 double chains (unsaturation), which represent 78.46% of the fatty acids. The most abundant are oleic (44.5%) and linoleic (31.7%).

2.5 PHYSICOCHEMICAL PROPERTIES OF EXTRACTS

The natural extract presents unique physical and chemical characteristic. This part provides background information about the different topics presented mainly in oilseeds.

2.5.1 Moisture content

Nowadays, the moisture content is necessary for the most of the food analysis, it allows: comparing values; convert different types of moisture values; express dry base as received. By this reason, the method must be carefully selected to apply, the same approach does not fit all foods, and some present food changes with differences among of water losing volatiles compounds, such as alcohol, essential oils, fat and so on (MASSON, 1997).

2.5.2 Refractive index (RI)

The RI of oil described as the ratio of the speed of light in the vacuum to the speed of light in oil at a particular temperature. This ratio gives a measure of the purity of oils and is also used as a means of identifying them. It measured with a refractometer, by a temperature control 20 –25 °C to oil and 40 or 60 °C for solid fats (KOH; MIN'S, 1993).

Additionally, if the temperature changes alter the results; refractive index decreases as the temperature goes up but at the same time increases with the length of the carbon chains and with the number of double bonds present in the fatty acids (O'BRIEN, 2004).

Currently, study show than RI of baru oilseeds obtained through hydraulic pressing and continuous screw pressing exhibit 1.468 and 1.469 respectably, as show Table 2 (CARLOS et al., 2012; MARQUES et al., 2015). Furthermore, the baru

amend using mechanically extracted exhibit than iodine value and acidity increased during 120 days of storage without nitrogen. But before of 60 days all the physicochemical analysis, even the sensory profile was acceptable (PINELI et al., 2015).

TABLE 2 - PHYSICAL-CHEMICAL PROPERTIES AND QUALITY CHARACTERIZATION OF BARU SEED OIL OBTAINED BY HYDRAULIC AND CONTINUOUS SCREW PRESSING

Results	**Hydraulic pressing**	**Continuous screw pressing**
Iodine value (g/100g)	89.88 ± 4.42	89.44 ± 2.59
Saponification value (mg KOH/g)	159.92 ± 3.00	156.41 ± 1.32
Refractive index	**1.468 ± 0.00**	**1.469 ± 0.00**
Relative density	0.917 ± 0.03	0.30 ± 0.01
Acid value (mg KOH/g)	**0.41 ± 0.01**	**0.30 ± 0.01**
Relative density (meq/kg)	1.61 ± 0.05	1.36 ± 0.05

SOURCE: Marquez (2015).

In the Table 2 also observed others oil characterization and quality of baru seed oil as acid and peroxide values that have been established as quality characteristics by Codex Alimentarius (2011).

2.5.3 Free Fatty Acid (FFA)

Acidity in oil is a measure of the content of free fatty acids, or in other words, is the result of the degree of breakdown of the triacylgrycerols, due to a chemical reaction called hydrolysis or lipolysis, in which free acids formed. It estimated on the molar mass of a fatty acid or a mixture of fatty acids. Typically it measured by direct titration in solution and a visual indicator (OWEN R. FENNEMA, 2008).

The presence of FFAs in oil is an indication of insufficient processing, lipase activity, or other hydrolytic actions as mention before. Moisture must be present in the oil for hydrolysis to develop. This reaction is accelerated by heat and pressure. In addition, FFA titrations identify all acidic materials in the oil, which includes the acid added to chelate metals, acids leached from the bleaching earth, antioxidant acidity, emulsifiers added, and other acidic materials (CHUAH et al., 2016).

During deep-fat frying, FFA analyses are quality indicators that determine the amount of hydrolysis. FFA development results from the reaction of water and fats at

frying temperature. The rate of hydrolysis progress is due to the amount of moisture in the foods being fried and the frying temperature (O'BRIEN, 2004).

TABLE 3 – COMPOSITION OF FATTY ACIDS IN THE OIL FROM *Dipteryx alata* Vogel USING MECHANICAL EXTRACTED

Fatty acids (%)		**Values**
Myristic	C 14:0	0.06 ± 0.01
Palmitic	C 16:0	6.37 ± 0.01
Palmitoleic	C 16:1	0.07 ± 0.01
Margaric	C 17:0	0.08 ± 0.01
Heptadenoic	C 17:1	0.06 ± 0.02
Stearic	C 18:0	4.95 ± 0.01
Oleic	**C 18:1**	**47.86 ± 0.05**
Linoleic	**C 18:2**	**28.91 ± 0.00**
Linolenic	C 18:3	0.18 ± 0.00
Arachidic	C 20:0	1.29 ± 0.00
Eicosenoic	C 20:1	2.46 ± 0.01
Behenic	C 22:0	3.19 ± 0.02
Erucic	C 22:1	0.26 ± 0.04
Lignoceric	C 24:0	4.26 ± 0.04
∑ Saturated		20.20 ± 0.10
∑ Monounsaturated		50.71 ± 0.13
∑ polyunsaturated		29.09 ± 0.00

SOURCE: Silva (2015).

Furthermore, Table 3 presents the composition of fatty acids of baru seed oil. The high degree of unsaturation was determined in which oleic (C 18:1) and linoleic (C 18:2) acids were the predominant fatty acids (PINELI et al., 2015).

2.6 OXIDATIVE STABILITY

The lipid oxidation is culpable for developing flavor and odors also cause changes that will affect the nutritional quality due to degradation of soluble fatty vitamins, essential fatty acids and safety of food, through the formation of potential toxicity compounds, notwithstanding, in some cases this oxidation is produced intentionally by cooking certain dishes.

Autoxidation occurs via a self-sustaining free radical mechanism that produces hydroperoxides, by then to form various aldehydes, ketones, alcohols, and

hydrocarbons (secondary products). The presence of secondary lipid oxidation products influences the overall quality of a lipid (KOH; MIN'S, 1993).

Unsaturated fats and oils are subject to oxidative; more than saturated fats is called oxidative rancidity that is an oxidative cleavage at double bonds.

The oxidative stability of fats and oils products is determined by the distribution, geometry, and the number of double bonds. Besides this, fatty acid, with only one double bond (monounsaturated), is the most stable of the three unsaturated fatty acids. Investigators have demonstrated that the natural or *cis*-isomeric fatty acid form has less oxidative stability than the corresponding *trans*-isomer (BOYD; MORRISON, 2009)

To conclude the importance of the oxidative stability of a fat or oil products depends on the temperature will be exposed, and the shelf-life or use-life expectancy (O'BRIEN, 2004).

The effects of lipid oxidation apply equally to fats and oils alone as well as to lipids in food. The lowest reaction rate is between 0.2 to 0.3 water activities for most foods. Foods tend to show accelerated oxidation rates below and above this water activity range. Generally, the lowest rate of oxidation occurs near pH 7. As the pH decreases and metals are solubilized, oxidation in many foods is greatly accelerated. In most cases, alkaline pH does not accelerate oxidation (O'BRIEN, 2004).

TABLE 4 – PROFILE OF OXIDATION

Fatty acid	**Relative Oxidation Rate**
Oleic (C-18:1)	1
Linoleic (C-18:2)	12
Linolenic (C-18:3)	15

SOURCE: R. O´Brien (2004).

The inherent oxidation stability uses the relative oxidation rates to calculate stability ratings for individual fats and oils. The formula for this calculation is to multiply the decimal fraction of each unsaturated fatty acid present by its relative oxidation rate and then adding these results to obtain the calculated oxidative stability rating (Table 4).

2.7 EXTRACTION METHODS

The efficiency of the extraction varies according to the location of the oil in the plant and the extraction method use. There are several methods of extraction. The most common processes are the steam distillation and extraction with organic solvents. However, these have some drawbacks and limitations related to the degradation, hydrolysis of esters, rearrangements, isomerization, racemization, and oxidation. Thus, some extractions altering or transforming the composition of the extracts even toxic products depend on the proposed use of it. Besides, there is a restriction on the use of toxic solvents, since vegetable oils used in food, pharmaceuticals, cosmetics, and biofuels. Accordingly, these factors influenced the emergence, development, and applications of new extraction operations, including supercritical extraction (MANIRAKIZA; COVACI; SCHEPENS, 2001)

2.7.1 SOXHLET EXTRACTION

Soxhlet extraction is a standard technique and is the primary method used as a reference for comparison with other methods. In Soxhlet the sample is placed in contact with a certain amount of solvent several times, facilitating the oil transfer matrix for the solvency (EWALD; BREMLE; KARLSSON, 1998).

The main advantages that the Soxhlet method characteristics are: the sample is always in contact with the solvent in constant renewal; the system temperature remains relatively high since the heat applied to the evaporation process is constant; it is a simple method, which enables extraction of a greater amount of oil compared to other methods, and do not need filtration after the extraction, because the sample remains wrapped in the cartridge during the procedure (LUQUE DE CASTRO; PRIEGO-CAPOTE, 2010; WU et al., 2011).

TABLE 5 – PROFILE OF UNSATURATED AND SATURATED FATTY ACIDS FOUND ALMONDS *Dipteryx alata* Vogel.

Extraction Method	**Saturated**	**Unsaturated**
	% w/w	**% w/w**
Soxhlet	33.1 ± 0.09	66.2 ± 0.01
Blingh & Dryer	23.2 ± 0.01	76.5 ± 0.04

SOURCE: Dos santos (2013).

In the Table 5 can be appreciated a comparison between Soxhlet and Bligh & Dyer method, where the Bligh & Dyer method was more selective for unsaturated fatty acids in comparative relation with the Soxhlet method was more selective for saturated fatty acids. The experiments analyses have the next unsaturated fatty acid: oleic, linoleic, linolenic, gadoleic, erucic and also the follow the saturated fatty acid: arachnid, hyneicosanoic, behenic, lignocerie, palmitic, estesrie (NABOUT et al., 2010).

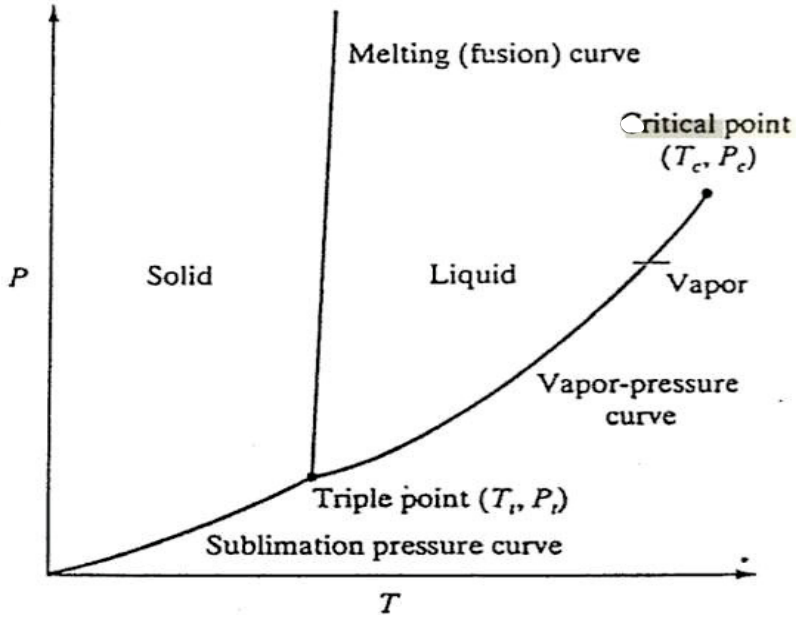
2.7.2 SUPERCRITICAL FLUID EXTRACTION (SFE)

In the last decades, the food industry specifically in the separation processes has tended to improve the food quality and safety. This under the optimization of the unit operation, and stricter legislation related to the residual level of solvent.

In the present work, supercritical fluid extraction is used as an alternative technique for the extraction of natural products, due to the low extraction time, the smaller amount of organic solvents, the production of clean, selective, non-residual and environmentally beneficial extracts (POURMORTAZAVI; HAJIMIRSADEGHI, 2007) (HUANG et al., 2011).

The phase diagram shows in Figure 5 the areas where a pure substance exists as solid, liquid, gas and gas as a supercritical fluid. The curve represents the temperature and pressure where two phases coexist in equilibrium (at the triple point). As the temperature and pressure are increased along the vaporization curve, the liquid becomes less dense due to thermal expansion, and the gas becomes denser due to the increased pressure. Eventually, the densities of the two phases converge and become identical, the distinction between gas and liquid disappears, and the vaporization curve comes to the critical point (KNEZ et al., 2014).

FIGURE 5 - PHASE DIAGRAM P & T, CRITICAL POINT



SOURCE: Sandler (1994).

The SFE process consists of two essential steps: extraction and separation. The most common solvent in SFE is carbon dioxide, due to its characteristics under critical point conditions, as show in Table 6. The CO_2 is used in a lot of works with conditions higher than the critical point (74 bar, 31 °C), and as result an interesting high critical density (0.460 g/ cm^3). These is relatively low critical variables as well as proportionately non-toxic, non-flammable, usable in high purity at relatively low cost, and is easily removed from the extract (DE MELO; SILVESTRE; SILVA, 2014; SAPKALE et al., 2010).

Supercritical CO_2 ($scCO_2$) is selective by lower molecular weight compounds or weakly polar groups such as lipids, cholesterol, aldehydes, ethers, esters and ketones, while high molecular weight (> 400) or polar groups such as hydroxyl, carboxyl, and sugars, polysaccharides, amino acids, proteins, and so on are insoluble in dense carbon dioxide. It is possible to amplify and modify the selectivity and solubility of these compounds in carbon dioxide by the addition of cosolvents. Cosolvents can increase the density as well as by specific chemical interaction with the solute. The cosolvents extend the solubility of CO_2 in different compounds such as egg yolk lipids, fish oils, gluten lipids, carrot, tomato and annatto pigments, tamarind antioxidants, and so on. Commonly is applied to extract color and refining of seed oils due to its economic potential (RAVENTOS; DUARTE; ALARCON, 2002).

A research of baru seed using the supercritical fluid extraction with CO_2 as solvent demonstrated that is possible to obtain oil from the seed. The best yield was 35 MPa and 40 °C, in which 22.8 g/100 g was obtained. The major fatty acids of baru almonds found by those authors was oleic acid, reaching more than 48% of the fatty acid composition (DOS SANTOS et al., 2016).

TABLE 6 – SOLVENTS USED IN SFE

Substance	T(°C)	P (MPa)
CO_2	**31**	**7.29**
Water	374	21.72
Methane	82	4.54
ethane	32	4.82
Propane	**97**	**4.19**
Pentane	197	3.33
Ethylene	9	4.97
Toluene	319	4.06
Methanol	240	7.99
Ethanol	241	6.06
Acetone	235	4.64
Ethyl ether	194	3.59

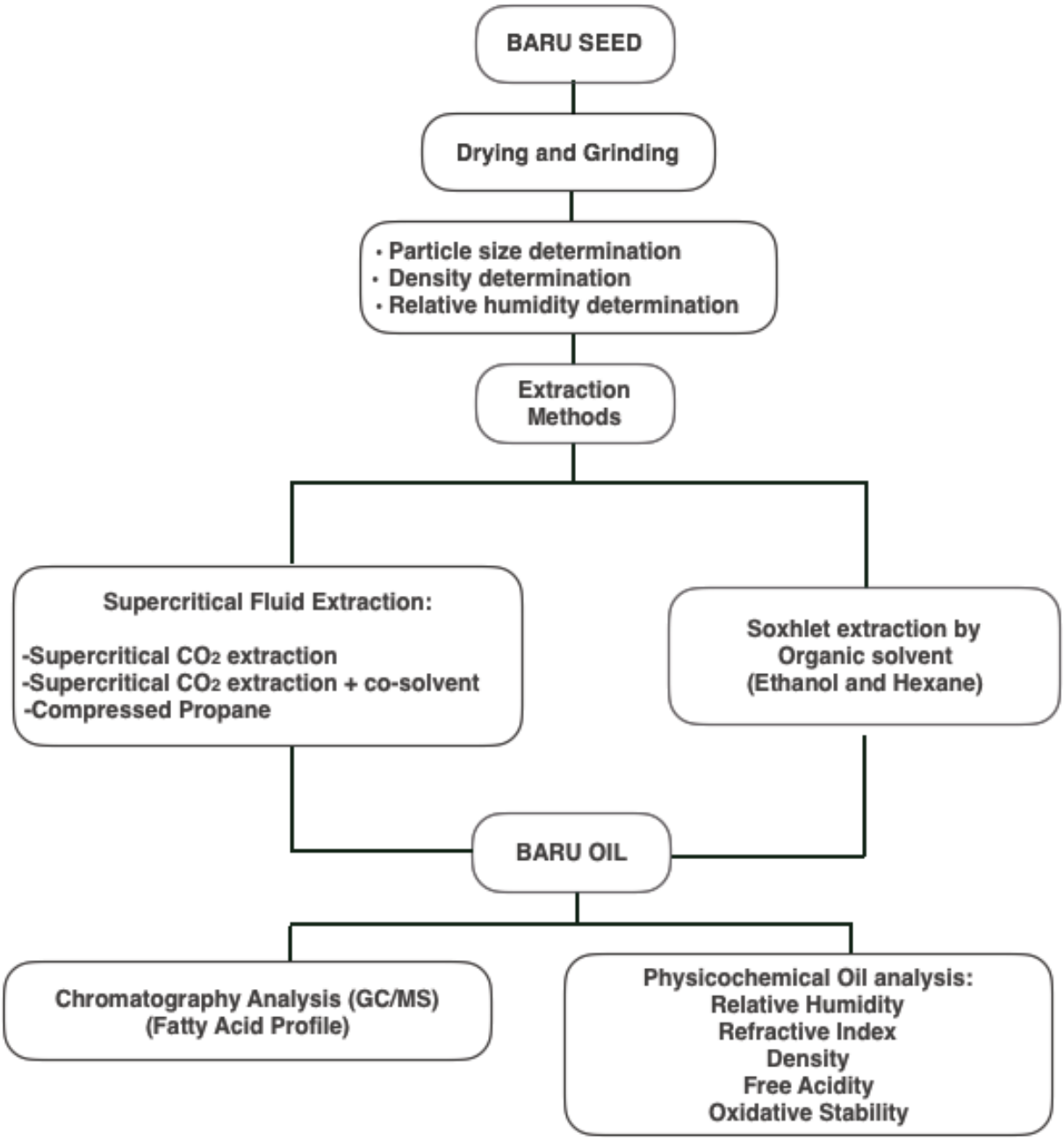
SOURCE: Hierro (1994).

Due to the nonpolar characteristic of triacylglycerols solvents with similar characteristic are normally used to extract oil from oilseeds raw material. Propane has been proposed as an interesting solvent to oil extraction with the advantage that it is a gas at normal conditions of pressure and temperature (atmospheric conditions), and the condition of pressure to reach propane as compressed fluid are relatively mild. On the other hand, subcritical propane has limited ability to dissolve polar molecules. This characteristic can be modified by adding miscible and polar compounds to the system, which are called modifying solvents. The critical condition of the propane is shown in Table 6, nevertheless, rarely the propane works over its critical condition due the risk of the high temperature degrades the thermolabile compounds. (DÍAZ-REINOSO et al., 2006; MIYAMOTO; UEMATSU, 2007; SILVA et al., 2016).

3 MATERIAL AND METHODS

The methodology of the project since the raw materials collection to the data analysis is showed in flowchart below:

FIGURE 6 – METHODS FLOWCHART



3.1 RAW MATERIAL

Baru (*Dipteryx alata vogel*) used in this work was collect in Barra do Garças City, state of Mato Grosso, Brazil, in June 2016, when the baru shells were mechanically removed. First, baru amend was collected and then transported to

Universidade Federal do Paraná at LACTA Laboratory, located in Curitiba capital of Paraná, Brazil. The total sample was 2,500 g. of baru seed.

The seeds were dried in an air circulating stove at 30 ºC by 72 h until constant moisture content (4.66 ± 0.2 wt %) and therefore stored in a freezer at -6 ºC in a vacuum package of polypropylene plastic bags at the empty equipment at LABTECAL laboratory.

3.1.1 Moisture determination

The moisture content and volatile compounds (MC) of the baru seed samples were determined in triplicated according to AOAC methods (AOAC, 2005). 5 g of the samples were weighed in porcelain capsules and then dried in the oven at 105 ºC for 12 hours. After that, the capsules were weighed hourly until obtaining constant mass. The moisture content (%) was estimated using the following equation:

$$MC = \frac{W\ inicial - W\ dry}{W\ dry} x\ 100 \quad (1)$$

Where:

$W\ initial$ Is the mass sample before dry and $Wdry$ is the mass sample after dry.

FIGURE 7- SEED BARU SAMPLE

3.1.2 Particle diameter

The raw material was ground manually, using a stone, until get fine particles that were classified by the Tyler series sieves with sequential openings of 8,

10, 12, 14, 16, 20, 28, 32 and 35 mesh in a vertical vibratory sieve shaker. The average particle size (PS) distribution was divided in 3 sizes (1.7, 1.0, 0.5 mm) that was estimated using the method presented by Gomide (GOMIDE, 1983). Equation 2, considering the mass fraction of the milled material in the Tyler series (GOMIDE, 1983).

$$dp = \sqrt{\frac{\sum_1^n \frac{\Delta l_i}{d_i}}{\sum_1^n \frac{\Delta l_i}{d_i^3}}} \qquad \Delta l_i = \frac{m_i}{M} \tag{2}$$

Where:

d_i^3 = average diameter of mesh;

mi = mass fraction retained in the mesh;

di = average diameter of the sample;

M = total mass of the sample;

n = total number of fractions.

3.1.3 Density determination for particle

The real density of each particle size sample was measured using a helium pycnometer (Quantachrome Ultrapyc 1200e), at the Analytical Central-Institute of Chemistry/Unicamp, Campinas, Brazil. The density was calculated using the total volume of the extraction cell and the total mass of ginger needed to package it. With the data of real and apparent density, the porosity of the bed was calculated through Equation 3 and 4.

The apparent density (d_a) was calculated using the volume of the batch extractor and the sample mass.

$$d_a = \frac{dp}{\text{be}} \tag{3}$$

$$\emptyset = \frac{1 - da}{d_t} \quad (4)$$

Where:

$\emptyset$ = porosity

d_a = apparent density

d_t = true density

d_p = avarege particle diameter

be = volumen batch extractor

3.2 EXTRACTION METHODS

3.2.1 Soxhlet extraction

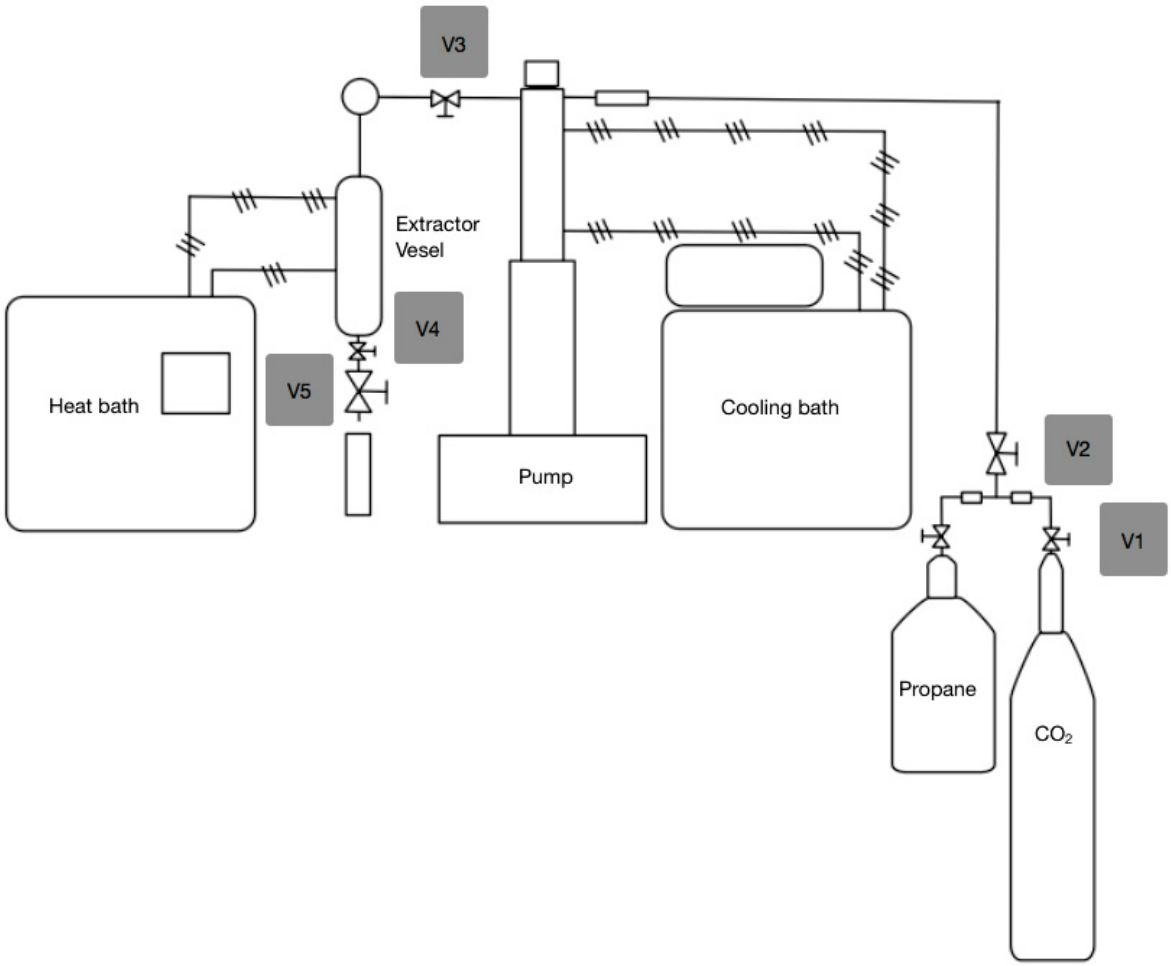
The oil content in the baru seeds was determined in triplicated by Soxhlet extraction using hexane (Neon, 99.5% purity) and ethanol (Neon, 99.8% purity) as solvents (BAÜMLER; CARRÍN; CARELLI, 2015; BHUTADA et al., 2016). And these results were used for comparison with the extractions with compressed solvents. Around 5 g of raw material (baru seeds with different average particle diameters) was used and the solvent was refluxed at the boiling point in a Soxhlet apparatus for 6 h with 150 mL of solvent (hexane or ethanol). After the extraction period, the solvent was removed using a rotary vacuum evaporator (Model RV 10 digital, made by IKA), and thus the extracts were placed in an air circulating oven (Nova Ética, model 400-2, made by IKA) to remove the residual solvent. Additionally, the oil extracted was kept in amber flasks at -6 ºC. The extraction yield (wt%) was calculated as follows:

$$Yield(\%) = \frac{W\ extrated\ oil}{W\ baru\ seed\ sample} x100 \quad (4)$$

3.2.2 Extraction using compressed fluids

The extractions using $scCO_2$ and propane as solvent were performed in a home-made laboratory scale SFE unit (Figure 8), which has been described in other study of our research group Briefly, the experimental setup consists of an extraction vessel with 62.4 cm^3 volume (diameter of 1.9 cm and height of 22 cm). The pressure of extractions was fixed and controlled by the high-pressure syringe pump (ISCO, model 500D) and the temperature by an ultra-thermostatic bath connected to the extractor jacket. The temperature in the syringe pump was maintained at 10 ºC, for all experiments using another ultra-thermostatic bath (MESOMO et al., 2012; PEREIRA et al., 2017). Propane (White Martins SA, 99.5% purity) and CO_2 (White Martins S.A., 99.5% purity) were used without further treatment. In addition, for the extractions with supercritical CO_2 ethanol was added as a co-solvent (named as $scCO_2$ + EtOH) attempting to enhance the extraction yields and increase the extractions rates.

FIGURE 8 - SCHEMATIC DIAGRAM OF THE EXTRACTION UNIT. V1 AND V2: CYLINDER VALVE; V3: BALL VALVES; V4: GAS PRESSURE REGULATOR VALVE; V5: NEEDLE VALVE



For the extraction with $scCO_2$+EtOH, firstly, the raw material was placed into the extractor and then the ethanol amount added at the desired ethanol to raw material mass ratio. The amounts of raw material and ethanol were around 25 g of ground baru seeds to 25 g of ethanol for the ratio of (1:1), around 18 g of seeds to 36 g of ethanol (mass ratio of 2:1), and 12 g of seeds wetted with 36 g of ethanol (mass ratio of 3:1). For the extraction with the propane, the vessel was loaded with approximately 32 g of sample depending on the particle diameter used. When the conditions (pressure and temperature) of the system were ready, the compressed solvent (propane or CO_2) was loaded into the extraction vessel, the amount of solvent injected was read, and the system was kept for the static extraction step (confinement time of 10, 30 or 60 min). After the static period, the dynamic extraction was started using a constant solvent flow around 2.0 $mL.min^{-1}$, which was used for all extractions of this study. The oil extracted from the baru seeds were collected (at atmospheric pressure and room temperature) in test tubes of 10 mL and immediately covered with stopper. The samples were collected at each 2 min for the extraction with $scCO_2$+EtOH, and at each 5 min for the propane until no oil come out of the extractor. For the extraction with $scCO_2$+EtOH, after the sampling the test tubes were placed in an air circulating oven to evaporate the ethanol until constant weight. The oil extraction yield was calculated as is described in Equation 4. section 3.2.1.

3.3 EXPERIMENTAL DESIGN AND DATA ANALYSIS

Soxhlet extractions were performed at atmospheric pressure of 91.6 ± 0.3 kPa using both hexane and ethanol. The extractions using compressed solvents were performed employing a two-level-two-factor experimental design to explore the best combination of two independent factors affecting the extraction process, including the pressure of the system (X_1: 15.0 – 25.0 MPa using $scCO_2$+EtOH and 2.0 - 10.0 MPa when using propane as solvent) and temperature (X_2: 40.0 - 80.0 °C and 20.0 - 60.0 °C using $scCO_2$+EtOH and propane, respectively). The experimental design consisted of 14 experiments, 7 for each solvent, including 3 center points. Three replicates at center point were used to estimate the sum of squares for the pure error (lack of fit). All the experiments were randomly carried out for minimizing the effect of unexplained variability due to systematic errors. The experimental design and data analysis were performed using the Statistica 7.0 software (Statsoft

Inc., Tulsa, OK, USA). Analysis of variance (ANOVA) was performed for variables used in the experimental design and the physicochemical analysis. Each response was evaluated independently.

TABLE 7- MATRIX PLANNING FACTOR 2^2, USING INDEPENDENT VARIABLES (T AND P) BY OBTAIN BARU SEED OIL VIA $scCO_2$.

Assay	**Variables (real variables)**	
	Pressure (MPa)	Temperature (˚C)
	X1(X1)	**X2(X2)**
1	-1(15)	-1(40)
2	1(25)	-1(40)
3	-1(15)	1(80)
4	1(25)	1(80)
5	0(20)	0(60)
6	0(20)	0(60)
7	0(20)	0(60)

TABLE 8 - MATRIX PLANNING FACTOR 2^2, USING INDEPENDENTS VARIABLE (T & P) BY OBTAIN BARU SEED OIL VIA COMPRESSED PROPANE EXTRACTION

Assay	**Variables (real variables)**	
	Pressure (MPa)	Temperature (˚C)
	X3(X3)	**X4(X4)**
8	-1(2)	-1(20)
9	1(10)	-1(20)
10	-1(2)	1(60)
11	1(10)	1(60)
12	0(6)	0(40)
13	0(6)	0(40)
14	0(6)	0(40)

3.4 OIL SAMPLE ANALYSIS

Different oil samples obtained from baru seeds were submitted to some physicochemical analysis. Free fatty acid content was determined by the titration method according to American Oil Chemist' Society (AOCS) methods Ca 5a-40

(AOCS, 1997). The results were expressed as percentage of free fatty acids as oil acid following the next formula.

$$FFA(\%) = \frac{\text{ml NaOH} * \text{Normality of } KH_8H_4O_4 * oleic\ acid}{\text{mass of the sample (g)}} \quad (5)$$

The saponification index and iodine values of oil were determined using the fatty acid profile according with AOCS methods Cd 3a-94 and Cd 1c-85, respectively. Refractive indexes of the all extracts were measured using an Abbe refractometer (Biobrix) at 20 ºC and estimated error of 0.0001 to follow the official method AOCS Cc 7-25**.** The density was also determined at 20 ^{o}C using an Anton Paar vibrating-tube densitometer, model DMA 500 M (KANDA et al., 2016).

3.4.1 Fatty acids analysis

The fatty acids profile of baru seeds oil was analyzed using a Shimadzu chromatograph (GC 2010 Plus), a capillary column (CP-Wax 58 FFAP CB, 50 m x 0.25 mm x 0.20 mm), Flame Ionization Detector (FID) and split injection mode (1:10). The injector and detector (FID) temperatures were 250 °C and 280 °C, respectively. The oven temperature was programmed to increase from 100 °C to 175 °C at a rate of 25 °C.min^{-1}. After that, it increased to 230 °C at a rate of 4 °C.min^{-1}, and maintained at 230 °C for 15 min. The carrier gas was Helium at 35.6 cm.s^{-1} (linear velocity). The samples were prepared according to official methods Ce 2-66 to convert the oils into fatty acid methyl esters (FAMEs) (AOCS, 1997). FAMEs were identified by comparison with retention times of the standard mixture FAMEs (Supelco, MIX FAME 37, St. Louis, MO 63103, USA). The quantification of fatty acid was conducted by normalization area procedure. Results were expressed as a percentage of each individual fatty acid present in the sample.

3.4.2 Total tocopherols

Total tocopherol composition was determined using high performance liquid chromatography (HPLC) (TASIOULA-MARGARI; & OKOGERI, 2001). Briefly, the tocopherol was extracted from oil samples using 500 mg of oil and 5 mL of methanol

(Panreac, 99.9%) and the mixture was shaken vigorously for 5 min (TASIOULA-MARGARI; & OKOGERI, 2001). The supernatant phase was separated and the extraction procedure was repeated two more times. The supernatant phases were mixed, and the solvent was removed under vacuum (IKA®, HB 10, Germany) at 40 °C and 450 mmHg. The residue was dissolved in 1 mL of isopropanol and analyzed at an Agilent 1200 Series chromatograph with a diode array detector (DAD), using a C18 column (Kinetex-Phenomenex, 4.6 mm x 150 mm x 5 µm) at 292 nm. The injection volume was 20 µL, and mobile phase was methanol and isopropanol (90:10, v/v), using an isocratic mode, with a flow rate of 1 $mL.min^{-1}$. The total of tocopherol was quantified with external standard, using α tocopherol (Sigma-Aldrich), as reference.

3.4.3 Total phenolic content (TPC)

Total phenolic content of the baru seed oil was determined according to the Folin–Ciocalteu method (SINGLETON; ROSSI, 1965). 100 µL of oil extract were weighted and diluted in 6 mL of ethanol into an ultrasonic bath. The reaction mixture was composed by 0.1 mL of oil extracted, 2 mL of distilled water, 0.5 mL of Folin–Ciocalteu reagent (Sigma) (a mixture of phosphomolybdate and phosphotungstate) and 1.5 mL of 20% sodium carbonate. The flasks were shaken, and the absorbance of the mixture was measured at 765 nm, after 120 min of reaction. All runs were performance in triplicated. The gallic acid was used as a standard reference and the results were expressed as milligrams of gallic acid equivalents (GAE) per 100g of extract (mg GAE/100g) ± standard deviation.

3.4.4 Antioxidant activity

The antioxidant activity (AA) was determined from 2,2-azino-bis-(3-ethylbenzotiazoline-6-sulfonic acid) (ABTS) with radical scavenging. It was carried out based on the procedure described by Re (ROBERTA et al., 1999). ABTS radical cations were produced reacting ABTS stock solution (7 mM concentration) with a solution of potassium persulphate (2.45 mM) and allowing the mixture to stand in the dark at room temperature for 16 h before use. The radical $ABTS^{\bullet+}$ is reduced in the presence of a hydrogen donor that is the antioxidant compound. Different dilutions of the oil samples (100 µL of sample and 10 mL of ethanol) were mixed with radical

ABTS and absorbance was measured at 734 nm after 6 min. The 6-hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid (Trolox) (Sigma–Aldrich Co, St. Louis, USA), was used as antioxidant standard. Results were expressed as Trolox equivalent antioxidant capacity (TEAC) (mM concentration of a Trolox solution, which AA is equivalent to the activity of 1.0 mg/mL of sample solution). In order to find TEAC values, a response curve for standard Trolox solutions was prepared. Results are presented by average ± standard deviation of triplicate runs.

3.4.5 Oxidative stability

Oxidative stability of oil samples extracted from baru seeds was analyzed by Differential Scanning Calorimetry (DSC) and Thermogravimetric (TG) analysis in the chemical department. Four samples were analyzed: two of soxhlet extraction (hexane and ethanol), one with the best extraction yield of compressed propane (run 9) and one with the best extraction yield of $scCO_2$+EtOH (run 21). The thermal analyses were conducted in a DSC/TG (Differential Scanning Calorimeter and Thermogravimetric analysis), STA Instruments (NETZSCH, model STA 449F3). The oxidative stability of the baru seeds oil was determined according to Tan et al. (TAN et al., 2002). Oil samples (4.0 ± 0.5 mg) were inserted into an open aluminum pan, placed in the DSC/TG equipment and submitted a heating rate of 12.74 °C/min, from 20 to 400 °C, at atmospheric pressure in contact with a constant flow rate of 50 cm^3/min of oxygen (White Martins S.A., 99.5 % pure). An empty open aluminum pan was used as reference (WETTEN et al., 2014).

3.4.6 Residue analysis

After oil extraction, by compressed solvents and Soxhlet, the residual of the baru seeds were analyzed to determine the content of moisture, protein, lipids and ash. All analyzes were performed in triplicate according to the AOCS methods (AOAC, 2005). For the protein quantification, the total of nitrogen of each sample was analyzed under the Kjeldhal method. In addition, the Soxhlet methods using petroleum ether as solvent for 8 h was used to estimate the total lipids contents. The residues of the extractions were put in an oven at 550 °C for 12 h to measure the ash content.

4 RESULTS AND DISCUSSION

In this study, raw material (baru seeds) with three different average particle diameter (PS), 1.7 mm, 1.0 mm and to 0.5 mm were used for the extractions experiments, as presented in Table 9. The true density was statistically the same for the three different dp, however the apparent density and consequently the bed porosity varied from 536.38 $kg.m^{-3}$ and 0.389 to 430.65 $kg.m^{-3}$ and 0.482, respectively.

TABLE 9 - CHARACTERIZATION OF DIFFERENT RAW MATERIAL SIZES OF BARU SEEDS USED IN THIS WORK.

Average particle size (mm)	True density ($kg.m^{-3}$)	Apparent density ($kg.m^{-3}$)	Bed Porosity
1.7	1190 ± 10	536.38	0.389
1.0	1190 ± 10	489.62	0.428
0.5	1180 ± 10	430.65	0.482

Table 10 presents the results of Soxhlet extraction yield of baru seeds using ethanol and hexane as solvents and different average particle diameters (PS). The ethanol provided better extraction yields when compared to the hexane for all particle sizes (PS) evaluated. The efficiency of both solvents was significantly enhanced using smaller particle diameters providing higher oil yields. However, it is worth mentioning that the raw material with PS = 0.5 mm presented a pasty consistency, which turned its manipulation very difficult and caused clogging in the needle valve during the preliminary experiments of extractions with both compressed fluids (V5, experimental set up presented in Figure 8. section 3.2.2). Thus, aiming to avoid operational problems, we opted to mostly use the raw material with 1.7 mm for further experiments in this study; just few experiments were performed with smaller particle sizes aiming a further comparison as show the Figure 9. The results presented in Table 10 show that baru seed oil is not rapidly available in the particles and the extraction process is limited by the mass transfer phenomena. Ethanol as solvent presented very promising results for the oil recovery from baru seeds considering that it is a green solvent if compared to hexane.

TABLE 10 – RESULTS OF EXTRACTION YIELDS OF BARU SEED USING HEXANE AND ETHANOL AS SOLVENTS (ALL THE EXPERIMENTS WERE PERFORMED BY 360 AND ATMOSPHERIC PRESSURE OF 91.6 KPA)

Run	Solvent	P (MPa)	T (°C)	PS (mm)	Extraction yield (wt%)[a]
1	Hexane	0.09	68.00	1.7	23.98 ± 0.14
2	Ethanol	0.09	78.37	1.7	26.80 ± 0.31
3	Hexane	0.09	68.00	1.0	33.40 ± 0.07
4	Ethanol	0.09	78.37	1.0	36.84 ± 0.05
5	Hexane	0.09	68.00	0.5	39.76 ± 0.22
6	Ethanol	0.09	78.37	0.5	46.10 ± 0.10

[c]Average and standard deviation values of three extractions.

FIGURE 9 - RESIDUE OF SEED 1.7 mm AVERAGE PARTICLE DIAMETER

4.1 EXTRACTION WITH COMPRESSED SOLVENTS

Results of extraction using compressed propane and $scCO_2$+EtOH are presented in Tables 11 and 12, respectively. In order to investigate the influence of different extraction parameters (pressure and temperature) on extraction yield, the raw material was initially used with a fixed average particle diameter of 1.7 mm (runs 7 to 11 in Table 11 and runs 18 to 22 in Table 12). All extraction yields were calculated after a fixed extraction time of 60 min, compressed solvent flow rate of 2.0 $mL.min^{-1}$ and a confinement time of 10 min to allow a comparison of results obtained under different experimental conditions. Additional experiments to the experimental design were performed for both processes using compressed solvents, as it can be observed in Tables 12 and 13, named as additional experiments, where different

conditions and parameters were evaluated aiming to assesses the extraction yield of baru seeds.

4.1.1 Compressed propane

When using propane as a compressed solvent and considering larger PS (1.7 mm), the results showed that the extraction yields were slightly affected by the pressure and temperature changes. Both the temperature and pressure presented a significant and positive effect ($p \leq 0.05$) on the extraction yield (by ANOVA analysis with pure error for the experimental design results). The highest yield of 17.95% was obtained at 60 ºC and 10 MPa (highest P and T condition used), however at the same temperature (60 ^{o}C) and reducing the pressure to 2 MPa the extraction was slightly decreased to 17.90%, showing that the extraction with compressed propane could be operated at low density conditions. On the other hand, the main temperature effect is also demonstrated by the extraction at the lower conditions at 20 ^{o}C, where the yields decreased to 14.64% at 2 MPa and reached a value of 16.90% when the pressure was set to 10 MPa. It is interesting to notice that only the condition at lower parameters (2 MPa/20 ºC) was significantly different in contrast to others ($p \leq 0.05$). These results are similar because the interactions between the solvent (propane) and the oil molecules (triacylglycerols) are little affected by the changes in the density of the solvent (due to the alterations in the pressure and temperature conditions). Changes in the temperature are more effective than in pressure because the temperature affects the solubility rather than pressure, as well as increase the compressed solvent diffusivity and decrease its viscosity.

The static extraction period (confinement time) showed to slightly affects the extraction yield of baru seeds oil with propane. It was analyzed fixing the central point of the experimental design (40 ^{o}C and 6 MPa). Increasing the static extraction time from 10 min to 60 min increased the yield from 17.69 to 19.35% (runs 11 and 13, in Table 11), therefore from the process point of view a static extraction time of 10 min is enough to assure the equilibration conditions previously to the dynamic extraction process. In addition, from Table 11 it is also noticed that varying the average particle size (PS) is possible to enhance the extraction yield. At 10 MPa and 60 °C with a PS of 0.5 mm the extraction yield was significantly increased (36.87%) compared to the extraction at same conditions with PS = 1.7 mm (17.95%). Thus, in process in scale

smaller particles than 1.7 mm must be used, and the process plant must design to support the raw material with smaller diameters. As mentioned before, in this study the average particle diameter was 1.7 mm for all further experiments due to operational problems in our experimental setup.

TABLE 11- EXPERIMENTAL CONDITIONS AND RESULTS FOR BARU SEED OIL EXTRACTION

Run	P (MPa)	T (°C)	PS (mm)	Density**	Solubility # (kg extract.kg^{-1}CO$_2$)	q (g/min)	CT (min)	Extraction yield (wt%)
7	2 (- 1)	60 (+1)	1.7	44.99	0.3236	2.07 ± 0.17	10	17.90
8	2 (- 1)	20 (- 1)	1.7	503.31	0.3142	1.90 ± 0.01	10	14.64
9	10 (+1)	60 (+1)	1.7	463.63	0.3116	2.00 ±0.02	10	17.95
10	10 (+1)	20 (-1)	1.7	495.50	0.2963	1.99 ± 0.01	10	16.90
11[c]	6 (0)	40 (0)	1.7	484.39	0.2464	2.00 ± 0.00	10	17.69 ± 0.23
Additional experiments								
12	6	40	1.7	484.39	0.3560	1.99 ± 0.00	30	18.80
13	6	40	1.7	484.39	0.3642	1.99 ± 0.01	60	19.35
14	10	20	1	495.50	0.4624	1.99 ± 0.00	10	26.77
15	2	60	1	44.99	0.5380	1.99 ± 0.02	10	26.95
16[c]	10	60	1	463.63	0.5382	2.00 ± 0.00	10	28.83 ± 0.39
17	10	60	0.5	463.63	0.7553	1.99 ± 0.04	10	36.87

USING PROPANE AS SOLVENT.*

*Factors and levels, and 2^2 experimental design with independent variables.
**Obtained from the NIST database.
[a] Mass of oil extract by the mass of dried matrix.
[c] Average and standard deviation values of three extractions.
Apparent solubility (kg extract.kg^{-1} propane).
q solvent flow rate
CT Confinement time (min)
PS Average particle diameter.

Figure 3 depicts a comparison regarding the particle size (PS) effect on the overall extraction curves of baru seeds with compressed propane. The extraction rates increased as the particle diameter decreased. The highest extraction rate was achieved at 0.5 mm and almost the extraction oil was recovered in 30 min of extraction (36.87% of extraction yield). These results (Figure 3) show that the mass transfer resistances were minimized when smaller particles were used, and the extractions yields reached values comparable to the extraction in Soxhlet using hexane as solvent. Furthermore, it is possible achieve the same extraction efficiency using compressed propane than hexane but in very shorter extraction time and

producing a solvent-free oil (propane presents a vapor pressure much higher than hexane at room temperature).

FIGURE 10– EXPERIMENTAL OVERALL EXTRACTION CURVES FOR THE BARU EXTRACTION WITH PROPANE AS SOLVENT (AVERAGE PARTICLE SIZE PS = 1.7 mm).

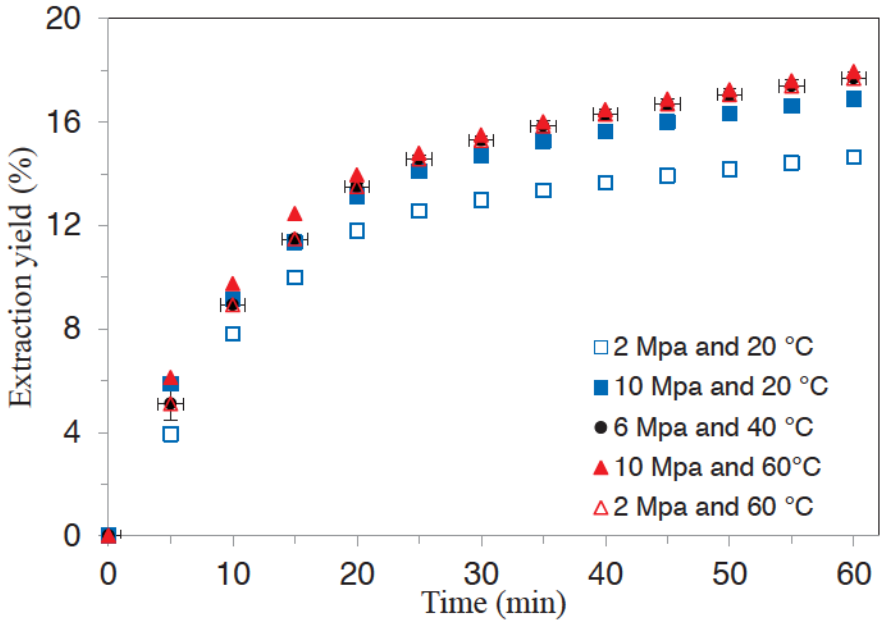
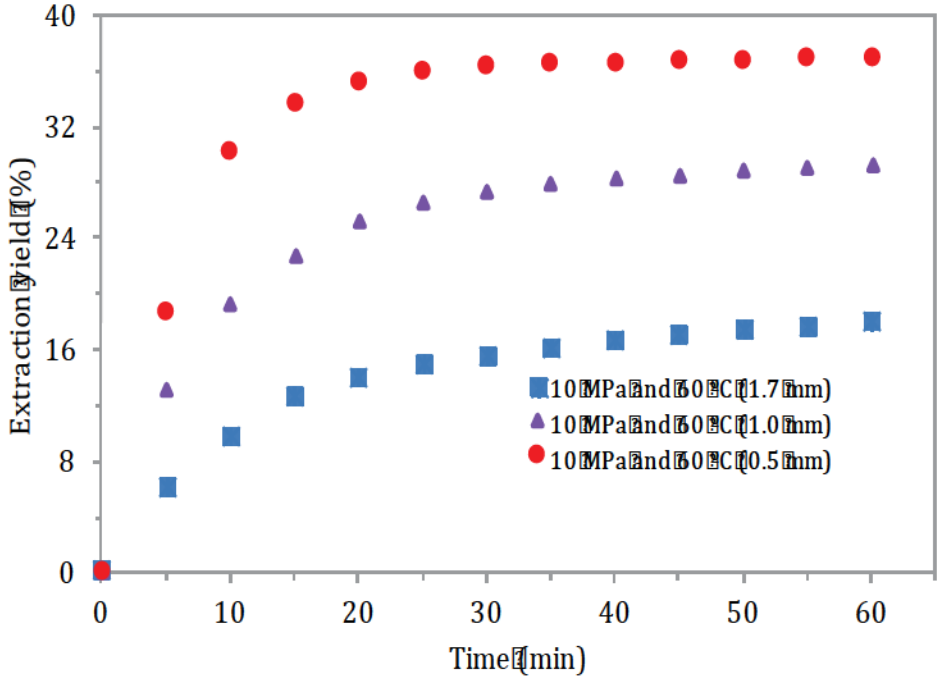


FIGURE 11 EXPERIMENTAL OVERALL EXTRACTION CURVES COMPARING THE RESULTS OF THREE DIFFERENT AVERAGE PARTICLE DIAMETER (PS = 0.5, 1.0 and 1.7 mm) OF BARU SEEDS USING PROPANE AS SOLVENT, AT 10 MPa AND 60 ºC.



4.1.2 Extraction with $scCO_2$ + ethanol

Preliminary tests showed that $scCO_2$ was not efficient to recovery baru seeds oil up to conditions of 25 MPa and 40 ºC (extraction yield around 6%), thus the addition of ethanol as co-solvent was considered to enhance the extraction of baru seeds oil (data showed in Table 12). For the experimental design using an average PS of 1.7 mm and an ethanol to raw material (solids) mass ratio of 1:1 (runs 18 to 22) both factors were significant ($p \leq 0.05$) and it can be observed that higher yields were obtained at higher pressure (25 MPa) and temperatures of 40 and 80 ºC (15.47 and 15.24%, respectively), which were statistically equals ($p \leq 0.05$). This result can be explained by the higher density of CO_2 (879.49 kg m^{-3}) under conditions of 25 MPa and 40 °C, which increase the solvent power improving the solubility of the system (MEZZOMO; MARTÍNEZ; FERREIRA, 2009; PAVLIĆ et al., 2017). Furthermore, although the CO_2 is non-polar the addition of a polar solvent miscible with it produces a solvent mixture with different characteristic recovering other different compounds as well as the oil (BITENCOURT; CABRAL; MEIRELLES, 2016). The interesting combination between carbon dioxide and ethanol under supercritical conditions gives a “new polarity” that has been studied and used for recovering other kinds of compounds, generating extracts with different properties (RAVEENDRAN; IKUSHIMA; WALLEN, 2005; ZEKOVIĆ et al., 2015).

Figure 12 and 13 present the overall extraction curves of baru seeds extraction with $scCO_2$ + EtOH system used in the experimental design and at the additional conditions. Figure 12 depicts the kinetic results at PS = 1.7 mm and ethanol to mass ratio of 1:1. The results show that the baru seed oil extraction was favored adding the ethanol as a co-solvent into the system (mass ratio of 1:1). The ethanol mixed to the raw material is carried into the particles by the $scCO_2$ promoting the effective contact and increasing the oil solvation when compared to pure $scCO_2$ (DOS SANTOS et al., 2016).

Increasing the temperature from 40 ^{o}C to 80 °C, at 15 MPa, the extraction rate and the extraction yield increased. For the same changes in the temperatures at 25 MPa, the extraction rate was much higher at 80 °C than 40 °C, but the extraction yields at 60 min were similar for these both conditions. It is possible that extending

the extraction time at 25 MPa and 80 °C higher amount of oil can be recovered because the $scCO_2$ without co-solvent is capable of solvate the oil molecules but at slower extraction rates, demanding larger amounts of $scCO_2$ and larger extraction time to recover the oil from the baru seed with a PS of 1.7 mm.

Most of the overall extraction curves in Figure 12 present an unconventional characteristic when compare to extraction using pure supercritical fluids or even using co-solvent in line. The three characteristic extraction steps, named CER, FER and diffusional steps are observed only for the condition at 25 MPa and 40 ^{o}C. For all other conditions the initial extraction rates increase and the oil recovery is suddenly stopped. This is indicating that in the very first minutes the process of extraction is driven by the interaction between ethanol and solute oil molecules and thus the extraction diminished (even stopped) as the ethanol concentration into the extractor decreased. According to Takemoto and Okada (TAKEMOTO; OKADA, 2001) the total lipids in baru seed is close to 40%, and using particles with 1.7 mm average diameter employing Soxhlet, $scCO_2$ and $scCO_2$+EtOH methods the performance was far from the ideal. However, from the results obtained in the present work it is possible to notice the potential of $scCO_2$ + ethanol to recovery the oil from baru seeds.

FIGURE 12 – EXPERIMENTAL OVERALL EXTRACION CURVES OF BARU SEEDS USING $scCO_2$ + EtOH AS SOLVENT, AND ETHANOL TO SOLIDS MASS RATIO OF (1:1), PS = 1.7mm AND DIFFERENT CONDITIONS OF PRESSURE AND TEMPERATURE.

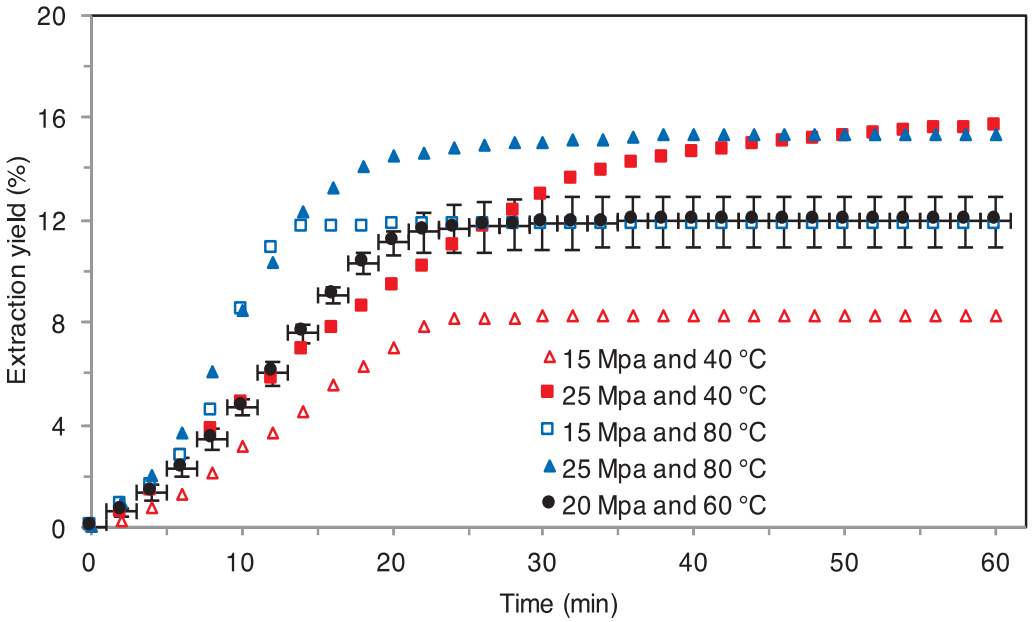

Extraction yield (%)
Time (min)
0
4
8
12
16
20
0
10
20
30
40
50
60
15 Mpa and 40 °C
25 Mpa and 40 °C
15 Mpa and 80 °C
25 Mpa and 80 °C
20 Mpa and 60 °C

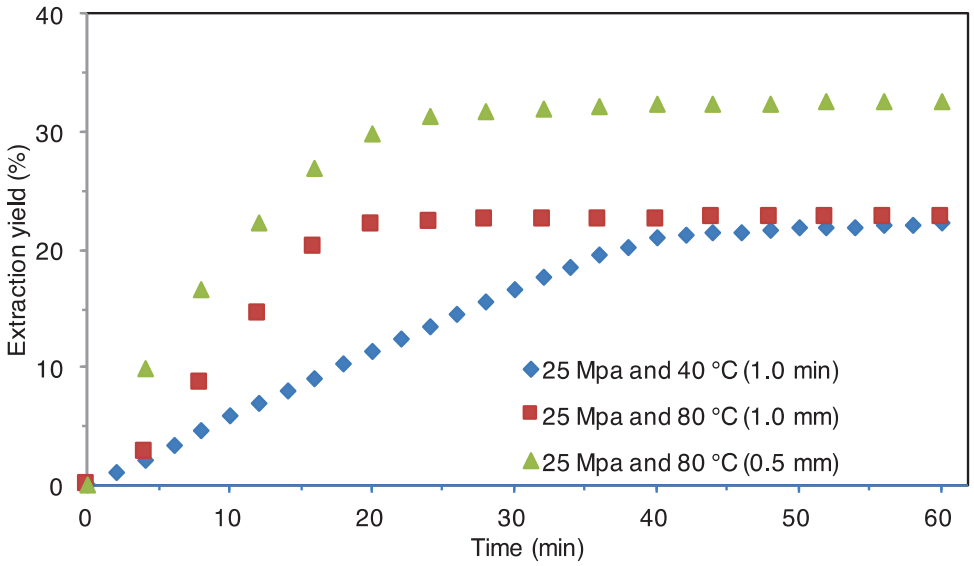
TABLE 12 - EXPERIMENTAL CONDITIONS AND RESULT FOR BARU SEED OIL EXTRACION YIELD USING $scCO_2$+EtOH AS SOLVENT.*

Run[c]	Solvent	P (MPa)	T (°C)	PS (mm)	Density**	Solubility #	q (g/min)	CT (min)	Extraction yield (wt%)
18	CO_2+EtOH	15 (- 1)	80 (+1)	1.7	427.15	0.0698	1.87 ± 0.16	10	11.71[d]
19	CO_2+EtOH	15 (- 1)	40 (- 1)	1.7	780.23	0.0585	1.98 ± 0.12	10	8.19[d]
20[c]	CO_2+EtOH	25 (+1)	80 (-1)	1.7	686.22	0.1050 ± 0.0067	1.97 ± 0.05	10	15.24 ± 0.01[d]
21[c]	CO_2+EtOH	25 (+1)	40 (-1)	1.7	879.49	0.0518	2.02 ± 0.14	10	15.47 ± 0.16[d]
22[c]	CO_2+EtOH	20 (0)	60 (0)	1.7	723.68	0.0689 ± 0.0030	1.98 ± 0.03	10	11.91 ± 0.82[d]
	Additional experiments								
23	CO_2+EtOH (2:1)	25	40	1.7	879.49	0.1266	2.05 ± 0.03	10	16.77[e]
24	CO_2+EtOH (3:1)	25	40	1.7	879.49	0.0256	2.06 ± 0.03	10	17.29[f]
25	CO_2+EtOH	25	40	1.0	879.49	0.0692	1.98 ± 0.03	10	22.15[d]
26	CO_2+EtOH	25	80	1.0	686.22	0.1389	1.99 ± 0.02	10	22.62[d]
27	CO_2+EtOH	25	40	1.0	879.49	0.2713	1.99 ± 0.02	30	24.35[d]
28	CO_2+EtOH	25	40	1.0	879.49	0.1548	1.99 ± 0.02	60	23.97[d]
29	CO_2+EtOH	25	40	0.5	879.49	0.1057	2.01 ± 2.05	10	23.50[d]
30	CO_2+EtOH	25	80	0.5	6.86.2	0.2713	1.97 ± 0.06	10	32.62[d]

*Factors and levels, and 2^2 experimental design with independent variables. Average particle diameter (PS).
** Obtained from the NIST database.
[a] Mass of oil extract by the mass of dried matrix.
[c] Average and standard deviation values of three extractions.
[d] Ethanol to solids mass ratio of (1:1).
[e] Ethanol to solids mass ratio of (2:1).
[f] Ethanol to solids mass ratio of (3:1).
Apparent solubility (g extract/g CO_2).
q solvent flow rate.
U ultrasound.
CT Confinement time.

As mentioned before, the experimental design was expanded with 16 additional experiments to provide more information about the extraction using $scCO_2$ + ethanol and better understand the phenomena involved in such process. These experiments were conducted varying the ethanol to raw material mass ratio, average particle diameter (PS), static extraction time (confinement time) and temperatures of 40 and 80 ^{o}C. For sake of comparison, three of these conditions are presented and compared in Figure 13.

FIGURE 13 – COMPARISON OF ADDITIONAL OVERALL CURVES OF BARU SEEDS WITH $scCO_2$+EtOH AT DIFFERENT PARTICLE SIZE (0.5 mm AND 1.0 mm) AND MASS RATIO OF 1:1 AND 25 PMa, AND AT 40 ºC AND 80 ºC.



As observed for the extraction with propane, the particle size (PS) plays a vital role in extracting the oil from baru seeds, as demonstrated using 1.7, 1.0 and 0.5 mm PS. From Figure 13, it has been inferred that the amount of oil extracted was determined by the decrease in the particle size reaching yield up to 32.62% at 25 MPa and 80 ^{o}C with PS = 0.5 mm. This expected behavior corresponds to the characterization of particle size showed in Table 9, where the true density didn't change significantly with different particle diameter, in contrast to the apparent density that greatly influenced the porosity of the sample as shown by other studies (FERNÁNDEZ et al., 2015; MASSAROLO et al., 2017). The enhanced in the oil recovery and extraction rate as the particle size decreased is caused by the increase in oil availability due to the enhancement in particle surface area according to the

particle size reduction. Furthermore, this positive tendency was observed in the kinetic curves presented in Figure 13, where it could be observed that the particle size has a great impact on extraction, because their CER, influenced by the solubility, increases when the particle size is reduced. The kinetic curves showed a small difference in the extraction time under 25 MPa and 80 ºC. For 1.7 and 1.0 mm PS the extraction stabilized up to 20 min, while the curve using 0.5 mm PS stabilized at 25 min (Figure 13). On the other hand, the ethanol to seeds mass ratio (1:1, 2:1 and 3:1) did not significantly affect the extraction yield, as demonstrated in Table 12 (runs 21, 23 and 24) and Figure 14.

The time of static extraction was analyzed over the extraction yield at the end of extraction, and as it can be observed from Table 12 (runs 25, 27 and 28) the extraction of 30 min can be used to assure the adequate equilibration and oil solubilization in the solvent mixture $scCO_2$+EtOH as show the Figure 15 and 16.

Maximum of oil extraction from baru seeds achieved in this work were 46.18%, acquired with 0.5 mm particle size and ethanol as solvent, and 39.76% using hexane, both in Soxhlet extractions. The latter result indicates that the extraction with 0.5 mm PS recovers all the oil of the amend, an amount that was described in other baru study (TAKEMOTO; OKADA, 2001).

FIGURE 14 - ETHANOL TO SOLIDS (RAW MATERIAL) MASS RATIO (0:1, 1:1, 2:1 AND 3:1). 1.7 PS: 25 MPa AND 40 °C

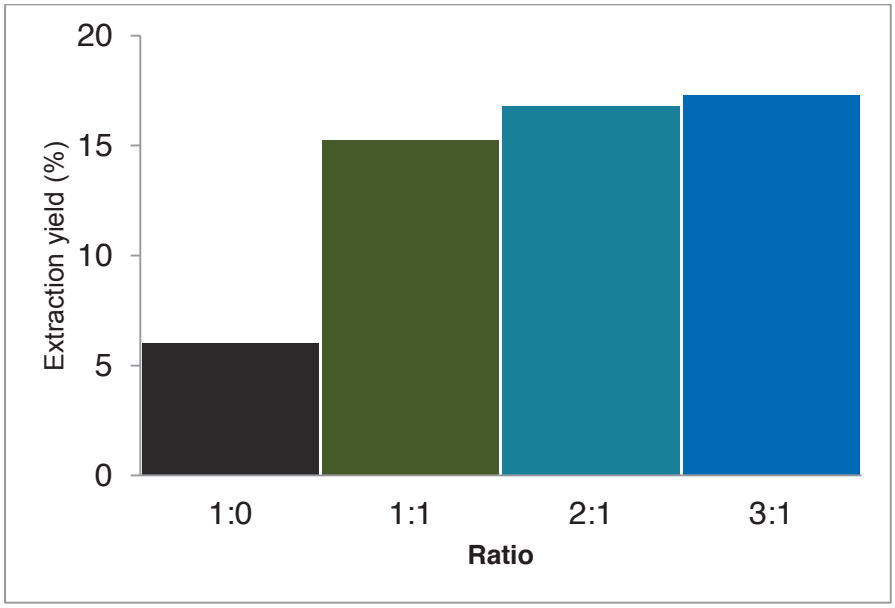


FIGURE 15 – EXPERIMENTAL KINETIC CURVES FOR COMPRESSED PROPANE USING 10, 30 AND 60 MINUTES OF CONFINEMENT TIME WITH PS = 1.7 mm.

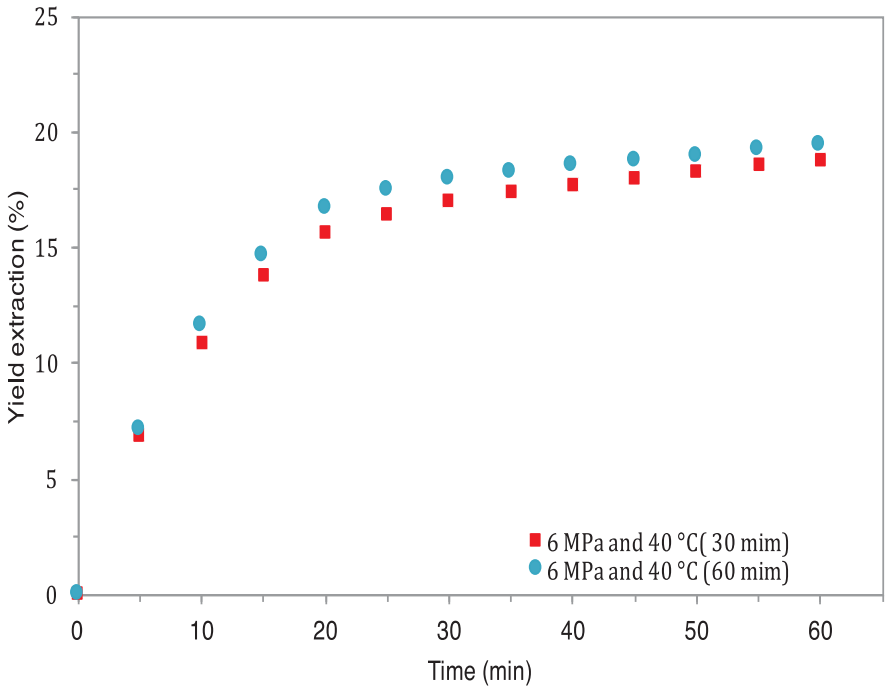
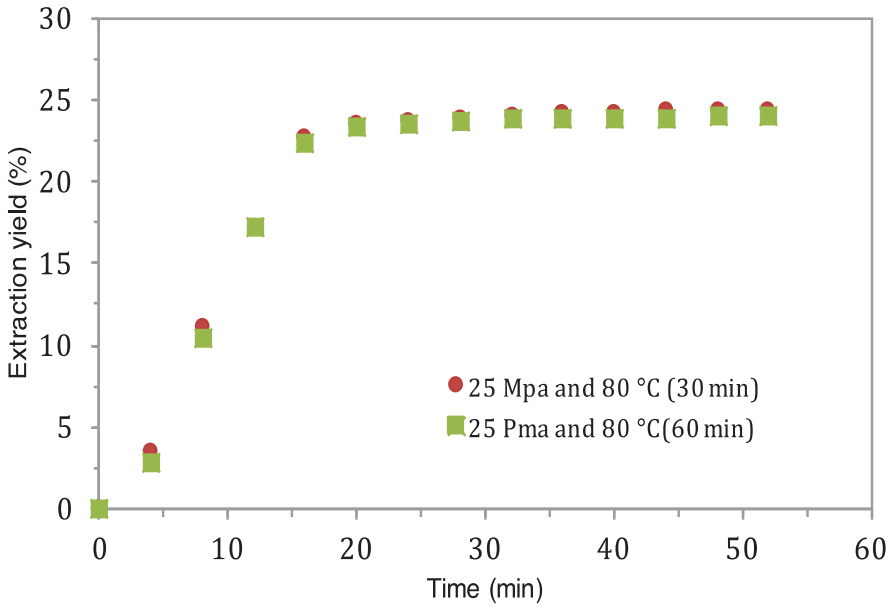


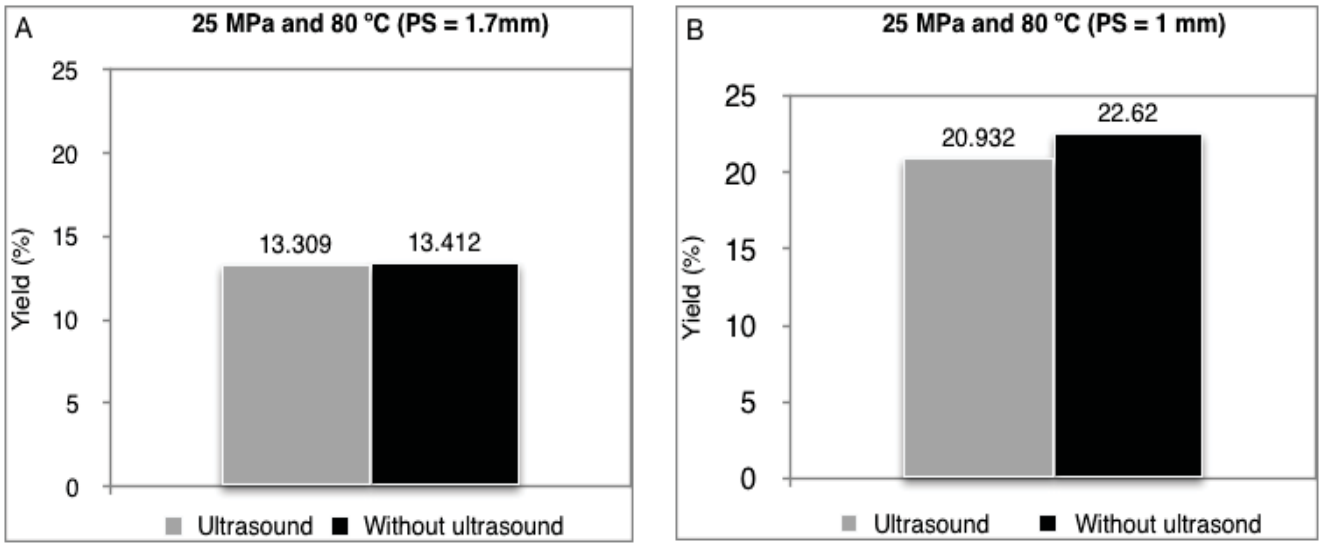
FIGURE 16 - EXPERIMENTAL KINETIC CURVES FOR $scCO_2$+EtOH USING 10, 30 AND 60 MINUTES OF CONFINEMENT TIME WITH PS = 1.0 mm.



Following the objective of increasing the extraction yield was adding ultrasound-assisted extraction (UAE) with ethanol before the supercritical fluid extraction. In pre-treatment the same quantity of sample and co-solvent (1:1) by $scCO_2$+EtOH extraction, was used. The ultrasound-assisted extraction used 25 MPa and 80 ºC in an ultrasonic bath (Eco-sonics, Q 5.9/37A, Brazil), with 37 kHz

frequency and 165 W (CORREA et al., 2017). During the 20 min of the sonication pre-treatment, the temperature at 20 ºC, was controlled. After that, the sample in the extraction vessel of the SFE equipment was placed. The yield extraction does not represent any change in the total process as exhibited in the Figure 17.

FIGURE 17 - ULTRASOUND-ASSISTANT AS PRETREATMENT WITH $scCO_2$+ETOH USING PS =1.0 AND 1.7 mm.



4.2 PHYSICOCHEMICAL ANALYSES OF THE OIL SEED

Physicochemical properties of each assay, such as, free fatty acid content (FFA), density and refractive index were analyzed and are presented in Table 13. It showed that baru seed oil from the lowest condition (2 MPa/20 ºC) of compressed propane presented lower values in FFA, RI and density with respect to the other extracts. In general, the baru oilseed density was higher by using Soxhlet (ethanol and hexane) and $scCO_2$+EtOH, while the extracts using propane as solvent showed lower density. In the RI analysis the same tendency was observed. Thus, these results may be due to the non-polarity of the propane and the compounds that were extracted. This difference between the oils is visually noted from Figure 18. Other quality parameters such as the saponification value and iodine value were calculated using the FFA content as reference, in which all the result exhibit similar values using other extraction methods as described in the literature (MARQUES et al., 2015; MATHIARASI; PARTHA, 2016; PINELI et al., 2015).

FIGURE 18 - BARU SEED OIL EXTRACTION (A: $scCO_2$+EtOH, B: SOXHLET WITH ETHANOL, C: SOXHLET WITH HEXANE, D: COMPRESSED PROPANE).

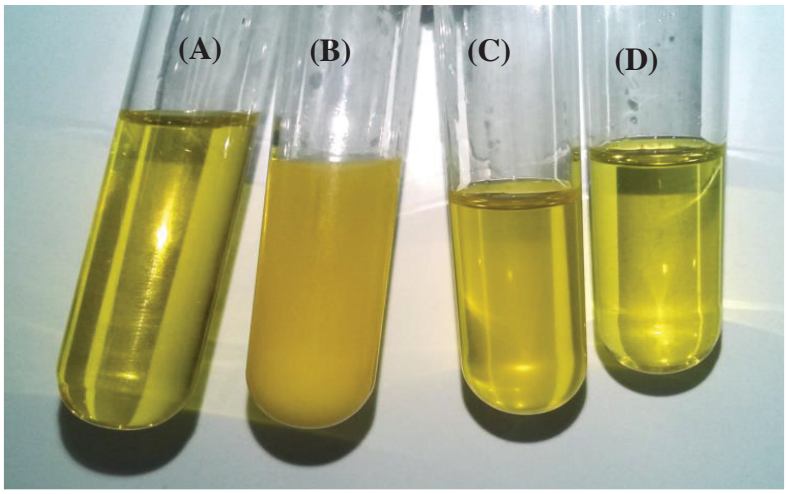


TABLE 13 – PHYSICOCHEMICAL PARAMETERS VALUES AND ERROS OF OIL SEED BARU.

Run	Extraction methods	Solvent	Free fatty acid (%)**	Refraction index	Saponification value (mg KOH g^{-1})	Iodine value	Density ($kg.m^{-3}$)
1	Soxhlet	Hexane	0.879 ± 0.032	1.4705	209.59	94.21	914.87
2	Soxhlet	Ethanol	1.305 ± 0.159	1.4710	209.59	93.53	929.61
7	2 MPa/60 °C	Propane	0.802 ± 0.030	1.4690	209.59	91.08	910.54
8	2 MPa/20 °C	Propane	0.682 ± 0.068	1.4680	209.59	92.32	907.10
9	10 MPa/60 °C	Propane	0.803 ± 0.030	1.4690	209.59	94.47	910.13
10	10 MPa/20 °C	Propane	0.622 ± 0.051	1.4690	209.59	93.29	909.55
11	6 MPa/40 °C	Propane	0.707 ± 0.117	1.4690*	209.59	94.29	910.54
18	15 MPa/80 °C	CO_2+EtOH	0.757 ± 0.159	1.4697	209.59	93.71	908.58
19	15 MPa/40 °C	CO_2+EtOH	0.857 ± 0.015	1.4695	209.59	99.25	911.67
20	25 MPa/80 °C	CO_2+EtOH	0.721 ± 0.020	1.4697	209.59	93.93	912.95
21	25 MPa/40 °C	CO_2+EtOH	0.756 ± 0.030	1.4697	209.59	94.17	912.67
22	20 MPa/60 °C	CO_2+EtOH	0.694 ± 0.046	1.4697	209.59	94.29	912.50

*Average value of three extractions (standard deviation ± 0.00007)
** Average and standard deviation values of three extractions.

4.3 FATTY ACID PROFILE AND TOCOPHEROLS

Table 14 presents the quantification of fatty acids profile for the baru seed oil samples extracted using compressed propane, $scCO_2$+EtOH, hexane and ethanol analyzed by gas chromatography. According to the results, the major fatty acid of all baru seed samples were oleic acid (ranged from 50.52 to 53.35%) and linolenic acid (23.34 to 24.59%), independent of the solvent used. The evidence therefore suggests that due to the polarity, there is a small difference between baru seed oil in terms of the C18:Y (where Y: 1,2,3, named oleic acids) fatty acid employing hexane and propane in contrast to ethanol and $scCO_2$+EtOH. Similar results, using carbon dioxide, for the fatty acid profile of other kind of oilseed were found by Çelik (CELIK; GURU, 2015), when using propane of solvent for extraction and by Pessoa et al. (PESSOA et al., 2015). The results presented by those authors showed a predominance of oleic acids. Furthermore, the low average of saturated FA (12%) with respect to the high average of monounsaturated and polyunsaturated FA content (86%) in baru seed oil can be the main feature attributed to reduction of cardiovascular diseases risk, as previous studies regarding the baru oil suggest (BENTO et al., 2014).

On the other hand, higher amount of tocopherol was obtained in the present work when compared to the results reported in the literature, as is showed in Table 7. The best result was obtained using $scCO_2$+EtOH as solvent (10.91 and 11.44 mg/100 g). In the case of the oil extracted using propane as solvent, tocopherol content was ranged from 7.89 to 9.54 mg/100 g. And these results may be due to the selectivity of the different solvents.

TABLE 14 - FATTY ACID COMPOSITION OF BARU SEED OIL BY DIFFERENT METHODS OF EXTRACION.

Fatty acids	Propane					$scCO_2$ + EtOH				Hexane	Ethanol
	2 MPa		10 MPa		60 MPa	15 MPa		25 MPa			
	20 °C	60 °C	20 °C	60 °C	40 °C	40 °C	80 °C	40 °C	80 °C		
Palmitic (C16)	5.45	5.54	5.44	5.47	5.47	5.71	5.52	5.56	5.56	5.52	5.72
Stearic (C18:0)	5.16	5.21	5.14	5.16	5.18	5.14	5.07	5.08	5.16	5.12	5.07
Oleic (C18:1)	50.69	50.12	50.78	51.67	51.75	53.35	52.3	52.41	52.46	50.52	52.17
Linoleic (C18:2)	23.8	23.50	24.21	24.10	24.09	24.59	24.11	24.21	23.92	23.66	24.35
Linolenic (C18:3)	2.87	2.78	2.94	3.17	3.08	4.12	2.75	2.74	2.74	3.48	2.74
Arachidic (C20:0)	1.57	2.74	2.14	1.37	1.36	2.77	1.31	1.29	1.31	4.23	1.35
Arachidonic (C20:4)	4.42	4.35	4.27	4.29	4.35	ND	4.16	4.11	4.22	2.81	4.14
Docosadienoic (C22:2)	0.59	0.39	0.41	ND	ND	ND	ND	ND	ND	ND	ND
Nervonic (C24:1)	4.74	4.66	4.67	4.77	4.72	4.33	4.77	4.58	4.63	4.66	4.46
Palmitic (C16)	5.45	5.54	5.44	5.47	5.47	5.71	5.52	5.56	5.56	5.52	5.72

ND:non-detected

4.4 TOTAL PHENOLIC CONTENT (TPC) AND ANTIOXIDANT ACTIVITY (AA).

The obtained TPC values of baru seed oil are between 680 and 1,386 mg GAE/100g (Table 15). These results indicated that the highest pressure combined to highest temperature leads to obtain oils with lowest TPC values. This was observed at extracts obtained with propane and with CO_2 plus ethanol, respectively 680 and 685 mg GAE/100g. In contrast, in extractions with propane, when was combined the lowest pressure (2 MPa) with highest temperature (60 °C) the highest TPC value of all oil was obtained (1,386 mg GAE/100 g). The same result was not observed to the extracts obtained with CO_2 plus ethanol, the lowest pressure (15 MPa) showed effect to obtain highest TPC value (960 mg GAE/100 g), indifferent of temperature (40 or 80 °C). However, comparing results of Soxhlet extraction the values of TPC are similar and near to the highest value of TPC obtained. The seed oil extraction obtained in this study was observed for the process under highest temperature (60 ºC) and lower pressure (2 MPa) condition using propane as solvent. Moreover, the TPC baru seed oil values obtained in Lemos et al. (LEMOS et al., 2012) presented low values when compared with our research.

TABLE 15 - TOCOPHEROL TOTAL, TOTAL PHENOLIC CONTENT (TPC) AND ANTIOXIDANT ACTIVITIES (AA) BY ABST METHODS FOR THE EXTRACTS.

Run	Extraction methods	Solvent	Tocopherol total (mg/100 g)	TPC (mgGAE/ 100 g extract)	ABTS (μM de trolox/g)
1	Soxhlet	Hexane	9.45	1260 ± 0.03	9.98 ± 0.03
2	Soxhlet	Ethanol	8.85	1118 ± 0.76	23.20 ± 0.07
7	2 MPa/60 °C	Propane	8.85	1386 ± 1.41	22.18 ± 7.47
8	2 MPa/20 °C	Propane	9.04	810 ± 0.35	16.82 ± 0.00
9	10 MPa/60 °C	Propane	9.54	685 ± 1.41	11.72 ± 2.46
10	10 MPa/20 °C	Propane	7.86	885 ± 0.71	96.57 ± 10.19
11	6 MPa/40 °C	Propane	7.89	1110 ± 1.06	71.04 ± 0.45
18	15 MPa/80 °C	CO_2+EtOH	7.99	960 ± 1.06	33.70 ± 0.00
19	15 MPa/40 °C	CO_2+EtOH	11.44	960 ± 0.35	34.01 ± 0.16
20	25 MPa/80 °C	CO_2+EtOH	10.91	685 ± 0.50	13.97 ± 0.00
21	25 MPa/40 °C	CO_2+EtOH	6.27	860 ± 1.06	20.42 ± 0.06
22	20 MPa/60 °C	CO_2+EtOH	9.44	810 ± 0.35	24.49 ± 4.89

All the oil samples were tested for the antioxidant activity. The extract obtained at 10 MPa/20 °C showed the highest AA (96.57 μM de Trolox/g) employing ABTS methods. This result might be related to the uses of lower temperatures. In addition, the use of hexane in Soxhlet presented the lowest antioxidant activity. The results are possibly attributable to contact with the solvent at boiling point during the extraction and the non-polar characteristic of the solvent, where the quality and quantity of AA were injured. Moreover, at low pressure (15 MPa) and different temperature (40 and 80 °C) the highest AA and TPC were obtained employing $scCO_2$+EtOH as solvent. The unexpected result could be due to high pressure conditions where the AA and TPC capacity were reduced by small changes in the mirror compounds present in the extracts. At the same time, it is interesting to note that using higher operation conditions (temperature and pressure) the AA and TPC exhibit low results for the super and supercritical conditions.

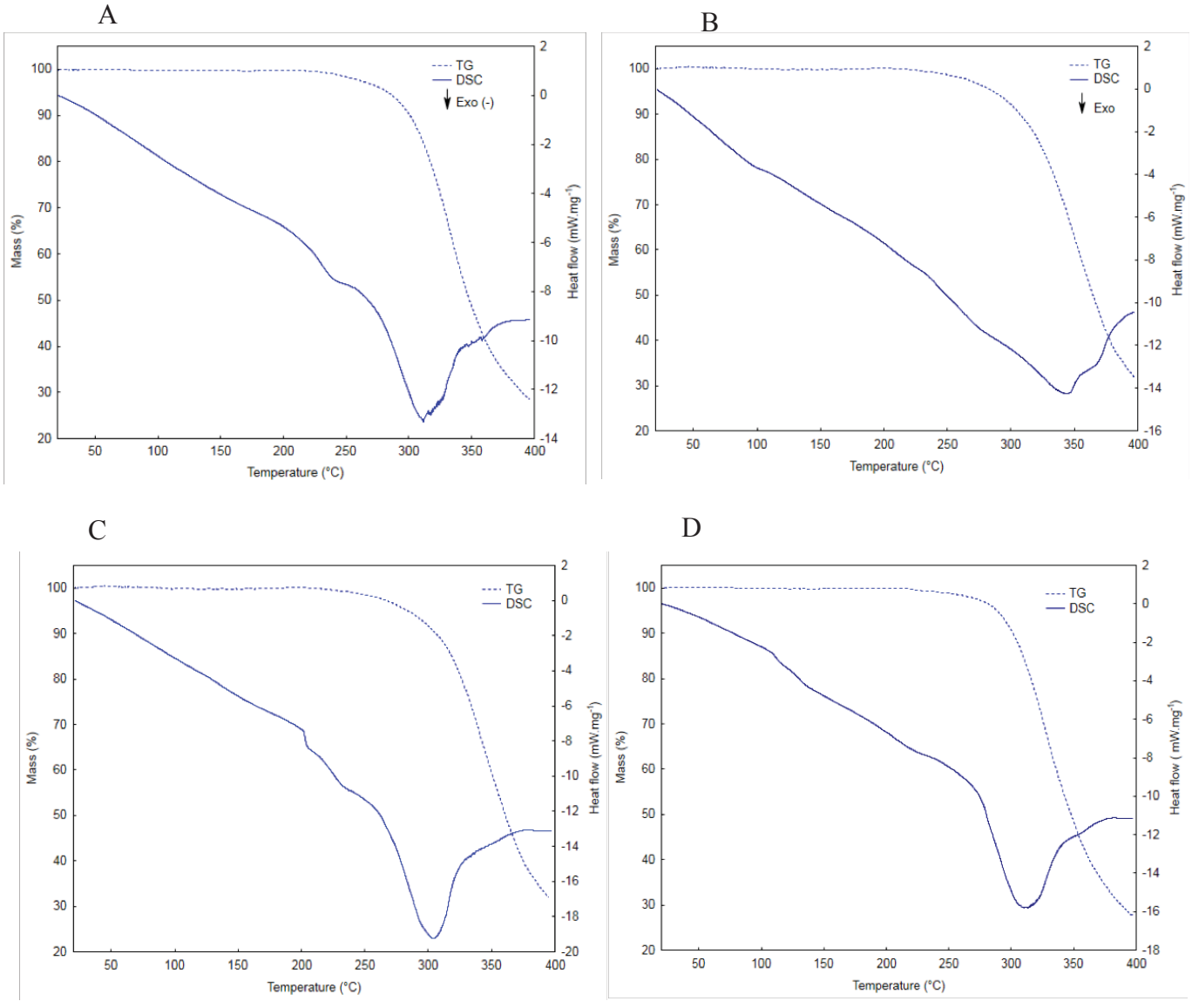
4.5 OXIDATIVE STABILITY OF BARU OIL

Figure 19 shows similar DSC and TG curves of baru oil as a function of temperature in oxygen atmosphere, indicating similar thermal decomposition profiles for all oil samples analyzed. TG curves indicated a progressive degradation of baru oil represented by tree main stages. In the first stage can be observed that the onset of mass loss (about 2%) occurs at 250 °C, followed by a second stage up to 300 °C, with 18% of mass loss, and by third and more intense in which mass loss is higher than 80% that occurred up to 400 °C, the highest temperature evaluated. The tree stages may represent degradation of mono, poly and, saturated fatty acid followed by the volatilization of products from polymerization and pyrolysis.

DSC curves reinforce the oil stages degradation, the end of first two stages are characterized by exothermic peaks at 250 °C, associated with formation of peroxides, and 300 °C, associated with decomposition of peroxides to further products, for all samples analyzed. However, a sensible deviation in the second stage was observed for the oil samples extracted by hexane (Soxhlet), which occurred after reaching 340 ºC. This may indicate a potential higher oxidative stability between samples analyzed. In a general way, all the baru seed oils studied can be used as frying oil and to be processed with temperature lower than 250 ºC. The results obtained to the behavior of oxidative stability of baru oil display similar

tendency to others studies of edible oil characterized by a mixture of fatty acid principally unsaturated (> 70 %) that is the case of baru oil as described in the section 3.3 (LIM et al., 2010; MICIĆ et al., 2015; TIMILSENA et al., 2017).

FIGURE 19 - ISOTHERMAL DSC & TG OXIDATION CURVES OF FOUR DIFFERENT BARU SEED OIL EXTRACTION (A: SOXHLET WITH ETHANOL, B: SOXHLET WITH HEXANE, C: $scCO_2$+EtOH,D: COMPRESSED PROPANE) IN AIR SYNTHETIC FLOW 50 mL/min.

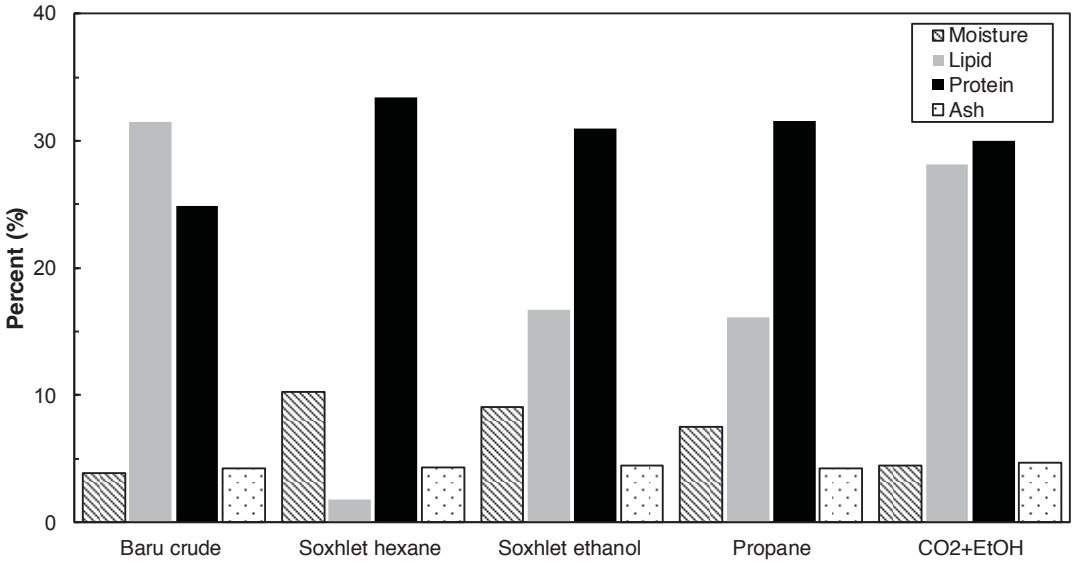


4.6 RESIDUE COMPOSITION ANALYSIS

After the extraction, the main components of the residual raw material were analyzed and are presented in Figure 20. The residue samples were obtained from the extractions with higher yields as described in the section 3.1., using 1.7 mm particle size distribution. Hence, it is interesting to note that using the residue from the Soxhlet method, the moisture content is higher than the residue with compressed solvents, especially using $scCO_2$+EtOH that was the lowest value among all the methods. This could be explained by the properties of the solvent because, at room temperature, the propane is a gas the residue was solvent-free by-product, and in this way, being fit for human consumption. The ash was constant throughout all the extractions. However, the lipids content also was evaluated in the baru seed residues from $scCO_2$+EtOH extraction and particles with 1.7 mm PS, in which a high quantity of oil was found (28.11%). This oil remained after the extraction. This result is because it still conserves lipids after the extraction and using 1.0 (18.73%) and 0.5 mm (9.46%) lipids in the residues were found. Using Soxhlet with ethanol and compressed propane (1.7 mm PS), it was fund around 16% of lipids in the residues, in contrast to hexane that any amount of lipids was recovered from the residues.

The protein is the second major component in baru seeds and after the extraction it is easy recovered in contrast to baru seed in nature (non-extracted baru seeds), as showed in Figure 20. The protein was just evaluated by each solvent because other study presented in the literature did not find significant differences in the results from different conditions (CORSO et al., 2010). In a general way, it can be seen that the residues obtained after the extractions with high pressure and compressed solvents as propane and $scCO_2$+EtOH, a high-value by-product can be released with low or even without traces of solvents and high percentage of protein and ash. Similar results were found in the literature for canola seed (KOUBAA; MHEMDI; VOROBIEV, 2016).

FIGURE 20 - CHARACTERISTICS OF THE BARU SEED RESIDUE AFTER THE EXTRACION (PS = 1.7 mm).



5 CONCLUSIONS

Subcritical and supercritical fluid extractions were studied as a green technology to recover oil. The effects of temperature, pressure, solvents, co-solvent, and manly the particular particle size were considered the major factors affecting extraction efficiencies. This work demonstrates that is possible to extract 92.70% of the baru seed oil using compressed propane at 10 MPa/60 ºC (higher conditions) and a particle diameter of 0.5 mm. On the other hand, 32.62% extraction yield was reached using 25 MPa/80 ºC and adding ethanol at (1:1) of mass ratio to the raw material, which means that 80% of the total oil was removed.

The results indicated that physicochemical analyses and total phenolic present similarly behavior between all the extractions and are agreement with other research. Higher tocopherol values were obtained in the present study when compared to results reported in the literature. For the fatty acids profile, high quantities of oleic and linolenic acid were found. To the end, the high quantity of available proteins and low traces of solvents was finding in the residues extraction.

This study indicated that the unconventional compressed propane and $scCO_2$+EtOH extraction processes can be performed to obtain highly potential nutritious oil from baru seeds with good antioxidant and antimicrobial activities, adding value to this agroindustry waste. The results demonstrate that baru seeds might be explored as a raw material to development of functional food ingredients and or for pharmaceutical applications.

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