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Passion fruit seed oil: extraction and subsequent transesterification reaction

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SUMMARY: This work aims to remove the oil from passion fruit seeds using ethanol as solvent and then to carry out the transesterification of the product from the extraction step (oil + ethanol). The effects of operational variables in the ultrasound-assisted extraction (UAE) were evaluated and traditional extraction was performed for comparison. The extraction product was directed to the reaction step using an enzymatic catalyst. UAE provided oil yield from 12.32 to 21.76%, and the maximum value (73.7% of the traditional extraction yield) was obtained at 60 °C and 50 min using a solvent-to-seed ratio of 4. Oil removal was favored by increases in the investigated variables. γ-tocopherol, δ-tocopherol and a high concentration of polyunsaturated fatty acids were identified in the oils. The oil obtained by UAE presented higher phytosterol contents. From the reaction step, samples were obtained with higher concentrations of ethyl esters, in addition to emulsifiers (diglycerides and monoglycerides).

KEYWORDS*: Emulsifiers; Ethanol; Ethyl esters; Oil yield; Ultrasound*

RESUMEN: ***Aceite de semilla de maracuyá: extracción y posterior reacción de transesterificación.*** Este trabajo tiene como objetivo la extracción del aceite de la semilla de maracuyá utilizando etanol como disolvente y posteriormente llevar a cabo la transesterificación del producto obtenido (aceite + etanol). Se evaluaron los efectos de las variables operativas en la extracción asistida por ultrasonido (EAU) y se realizó la extracción clásica a efectos comparativos. El producto de extracción se dirigió a la etapa de reacción usando catalizador enzimático. EAU proporcionó un rendimiento de aceite de 12,32 a 21,76%, y el valor máximo (73,7% del rendimiento de extracción clásico) se obtuvo a 60 °C durante 50 min usando una relación de solvente a semilla de 4. La extracción de aceite se favorece con el aumento de las variables investigadas. Se identificaron en los aceites γ- y δ-tocoferol y una alta concentración de ácidos grasos poliinsaturados. El aceite obtenido por los EAU presentó un mayor contenido de fitosteroles. De la etapa de reacción, se obtuvieron muestras con concentraciones más altas de ésteres etílicos, además de emulsionantes (diglicéridos y monoglicéridos).

PALABRAS CLAVE*: Emulsionantes; Ésteres etílicos; Etanol; Rendimiento de aceite; Ultrasonido*

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1. INTRODUCTION

Passion fruit is a tropical fruit which is popular worldwide and is commonly used in juice production, with Brazil accounting for ~80% of world production ("Embrapa", 2017). Bark and seeds are the main agro-industrial residues from the fruit-crushing process to obtain the juice and they cause problems for the industry due to the waste generated, whose volume amounts to many tons (Oliveira *et al.*, 2013). Passion fruit seeds represent ~12% of the fruit and can be considered good sources of oils, besides containing high amounts of fiber, which supports their use as a complementary source of these nutrients in the diet (Chau and Huang, 2004).

Passion fruit seeds have 18 to 30% oil in their composition (Malacrida and Jorge, 2012; Piombo *et al*., 2006; Santana *et al*., 2017). High levels of linoleic acid can be found in this oil (Malacrida and Jorge, 2012; Oliveira *et al*., 2013), in addition to phytosteroids and tocopherols, which give the oil high antioxidant activity (Lee *et al*., 2015; Pereira *et al.*, 2017; Santana *et al*., 2017). In relation to the tocopherols present in the oil of passion fruit seeds, α-tocopherol, γ-tocopherol, and δ-tocopherol have been identified (Pereira *et al*., 2017; Pereira *et al*., 2018). In addition, Silva and Jorge (2017) reported the presence of phytosterols in this oil, highlighting β-sitosterol in higher concentrations.

Ultrasound-assisted extraction (UAE) stands out as a technique for removing oil from oilseeds since it is considered cheap, simple, efficient (Khoei and Chekin, 2016), and selective when compared to traditional extraction (Luque-García and Luque de Castro, 2003), besides being conducted with less contact time and demanding lower consumption of solvent (Perrier *et al*., 2017). These characteristics are attributed to the cavitation process, which causes changes in the cell membrane of the sample, aiding in the process of extracting the target compound from the intracellular to the extracellular media (Koubaa *et al*., 2016). Zhong *et al*., (2018) evaluated the surface morphology of samples after extraction of the oil by ultrasound and observed drastic changes in the surface of the vegetal tissue, since the ultrasonic waves that pass through the solvent create cavities, which, when they come into contact with the surface of the solid, cause the formation of pores that facilitate the entrance of the solvent.

A variety of studies available in the literature report the achievement of higher oil yields with the use of the ultrasound-assisted process for a wide range of plant matrices. For example, Rodrigues *et al.,* (2017), and Stevanato and Silva (2019) reported that the use of ultrasound allowed increases of ~40 and ~70% in the oil yield of macauba pulp and radish seeds, respectively, when compared to the yield obtained without ultrasound.

Oil extraction from the passion fruit seeds using ultrasound is reported in previous works (Lee *et al.*, 2015; Oliveira *et al*., 2013; Oliveira *et al*., 2014; Oliveira *et al*., 2016; Pereira *et al*., 2017; Pereira *et al*., 2018); however, the evaluation of the effect of the process variables performed in these works is limited and suggests that a detailed study with the aim of optimizing the extraction process is required.

Keeping in line with the efficiency of the extraction technique, environmental concerns, health, and safety, the use of ethanol has gained interest because it is a biodegradable solvent recognized as GRAS (generally recognized as safe) due to its low toxicity and lower volatility. In addition, this alcohol is obtained from renewable sources and is readily available (Neto *et al*., 2018; Plotka-Wasylka *et al.*, 2017).

Extraction with ethanol is an appropriate method when the oil obtained is to be used for the synthesis of higher added value products such as fatty acid esters, di- and monoglycerides. Considering that ethanol acts as a solvent for oil extraction and a reagent in the transesterification reaction, the sequential process can be applied. In such a process, the product from the extraction step (oil + solvent) is directed to the reaction step, without the need for solvent removal or oil treatment steps (Stevanato and Silva, 2019).

The objective of this work was to determine the maximum oil yield that can be obtained from passion fruit seeds using UAE with ethanol as solvent, and to use the extraction product (oil + ethanol) in its maximum condition for the reaction step, thereby simulating a sequential process. The effects of the main variables affecting the extraction were evaluated (temperature, sample/solvent ratio, and time) by means of an experimental Box–Behnken design, as well as the effect of ultrasound on the removal of oil. The maximum yield obtained by UAE and the composition of the oil obtained under these conditions were compared with those obtained from

traditional extraction. In the product (oil + ethanol) from UAE (maximum oil yield) an enzymatic catalyst was added and the transesterification reaction was conducted, with subsequent determination of the composition of the product obtained.

2. MATERIALS AND METHODS

2.1. Materials

In the present work, ethanol (99.75%, JT Baker) and *n*-hexano (98.5%, Anidrol) were used as solvents and the oil was obtained from the seeds of passion fruit (purchased in the local market of Umuarama, PR). Methyl heptadecanoate (> 99%, Sigma-Aldrich), 5α-cholestane (> 99%, Sigma-Aldrich), a FAME (fatty acid methyl esters) mixture (Supelco), heptane and the derivatization reagent Boron trifluoride-methanol solution (14%, Sigma Aldrich) were used for the characterization of the oil, and *N,O* bis (trimethylsilyl) trifluoroacetamide (1% TMCS, Fluka). Novozym® 435 lipase (*Candida Antarctica* immobilized), heptane (Anidrol) and methyl heptadecanoate (Sigma-Aldrich, 99.9% purity) were used to determine the ethyl ester contents in the samples, and *N*-Methyl-*N*- (trimethylsilyl) trifluoroacetamide (MSTFA) was used to determine the mono-, di- and triglyceride contents.

2.2. Preparation of the raw material

The fruits were cut in half in the horizontal direction, and their seeds were removed manually. The seeds were then washed under running water, the surface water was removed, and the seeds were put in the oven (Marconi, MA 035) at 70 ºC to obtain samples with a moisture content of 2.34 ± 0.69%. After drying, the aryl was removed completely and the material was triturated and passed through granulometric grading to separate the 0.638-mm fraction for the experiments.

2.3. Oil extraction

UAE was conducted using ethanol as a solvent. In each experimental run, the flask containing the passion fruit seeds and ethanol was placed in the center of an ultrasound bath (Ultronique, Q 5.9/40A), previously heated to the experimental temperature, and ultrasonic irradiation was immediately initiated (power of 165 W and frequency of 25 kHz). To avoid the loss in solvent, the flask was connected to the condenser which was held at 10 °C.

In order to determine the effect of the process variables and the experimental conditions that maximize the removal of the oil from the seeds, the following levels for each variable were evaluated: (a) times (X_1) of 10, 30, and 50 min; (b) temperatures (X_2) of 30, 45, and 60 ºC; and solvent-to- seed ratios (X_3) of 2, 3, and 4 ($mL \cdot g^{-1}$), which were combined by means of a Box–Behnken design. The experimental range for process variables was defined based on previous studies: time (Pereira *et al.*, 2017), temperature (Rodrigues *et al*., 2017; Rosa *et al*., 2019; Xu *et al*., 2016) and solvent-to-seed ratios (Oliveira *et al.* 2014).

Analysis of variance was carried out to evaluate the effect of the variables on the oil yield with a 95% confidence interval using Statistica 8.0 software (StatSoft™, Inc.). In addition, the experimental data were fitted to a second-order polynomial model, which was used to determine the condition of maximum oil extraction. In this condition, the effect of ultrasound on the removal of oil from passion fruit seeds was evaluated, with extractions performed without ultrasound in an orbital shaker (Marconi, MA 839/A) at 40 rpm as described above.

For comparative purposes, the traditional extraction was performed in a Soxhlet extractor using the *n*-hexane a at a sample ratio of 30 ($mL \cdot g^{-1}$) for 480 min.

For each extraction method used, after the duration of the experimental run, the seeds were separated and the solvent was removed until constant weight was achieved, thereby obtaining the oil. The oil yield was determined as the ratio between the mass of oil extracted and the mass of seeds used.

2.4. Oil characterization

The methodology reported by Gonzalez *et al*., (2013) was used in the preparation of the samples to determine the fatty acid composition. In this methodology, the samples were derivatized with BF3-methanol and subsequently methyl heptadecanoate diluted in heptane was added to quantify the compounds. The analysis was performed in a gas chromatograph (GC–MS QP2010 SE, Shimadzu) equipped with Rtx – Wax (Shimadzu, 30 m x 0.32 mm id x 0.25 μm) capillary column and flame ionization detector, using the

chromatographic conditions reported by Stevanato and Silva (2019). The components present in the samples were identified through comparison with authentic standards of fatty acid methyl esters.

In the determination of phytosterol and tocopherol contents, 5α-cholestane as internal standard and thereafter 40 μL of *N,O*-bis (trimethylsilyl) trifluoroacetamide were added to 40 mg of sample. The samples were maintained for 60 min at 60 °C and after dilution in heptane to obtain a volume of 1000 μL of solution, they were analyzed on an GC–MS QP2010 SE (Shimadzu) with SH-RTx-5MS column (Shimadzu, 5% phenyl–methylsiloxane, 30 m × 0.25 mm id x 0.25 μm). The column temperature was initially programmed at 50 °C, increasing to 230 °C at a rate of 10 °C·min^{-1} and then to 280 °C at a rate of 15 °C·min^{-1}, which was held for 22 min. Helium was used as the carrier gas at a 1.0 mL·min^{-1} flow rate with split ratio of 10 and the amount of injected sample was 1 μL. Mass spectra were recorded at 70 eV with mass range from m/z 50 to 550 amu. Compounds were identified through the comparison of spectrum data to those presented in the NIST14.lb and NIST14.lbs spectral libraries.

2.5. Sequential reaction

The UAE product (oil + ethanol) was added again in an Erlenmeyer flask and received the enzymatic catalyst in the proportion of 40% (relative to oil mass) as defined by Stevanato and Silva (2019). The enzyme was activated for 1 h at 40 °C prior to use. The flask was incubated in orbital agitation (Marconi MA 830/A) at 150 rpm by applying reaction times of 12 and 24 h. The experiments were carried out under the temperature of maximum oil yield determined for the UAE (60 ºC). At the end of each reaction, the enzymes and seeds were separated by filtration and the excess ethanol in the samples was evaporated until reaching constant weight.

2.6. Characterization of the reaction product

The analysis of the chemical composition of the reaction product was carried out in a gas chromatograph (Shimadzu, GC-2010 Plus) equipped with a flame ionization detector and capillary columns SH-Rtx-Wax™ (Shimadzu, 30 m × 0.32 mm × 0.25 μm) and ZB-5HT inferno™ (Zebron, 10 m × 0.32 mm × 0.10 μm) to determine the content of methyl esters and acylglycerols, respectively. The chromatographic conditions for both analyses were previously established (Stevanato and Silva, 2019).

To quantify the content ethyl esters, the injected sample was prepared with 100 μL of a solution of oil in heptane (C = 30 mg·mL^{-1}), 80 μL of internal standard (methyl heptadecanoate, C = 12.5 mg·mL^{-1}) and 820 μL of heptane. For simultaneous determination of the contents of glycerol, mono-, di- and triglycerides, MSTFA was used as a silylating agent. Glycerol, monolein, diolein and triolein were used as external standard to construct the calibration curve ($R^2 > 0.99$).

The free fatty acid (FFA) content in the reaction product was analyzed in a gas chromatograph (Shimadzu, GC-2010 Plus) and the samples were prepared and analyzed according to the methodology for determining phytosterols and tocopherols as described in section 2.4, using methyl heptadecanoate as an internal standard.

2.7. Analysis of data

The data were subjected to ANOVA using Excel® 2010 software and the Tukey test (p=0.05) to evaluate differences between the results. The experiments and analyses were performed in duplicate, so four observations were used to calculate each mean value.

3. RESULTS AND DISCUSSION

3.1. Ultrasound-assisted extraction

3.1.1. Effect of experimental variables

The experimental results for the oil yield obtained from passion fruit seeds in each experimental run of the experimental design are presented in Table 1. Table 2 shows the effect of the independent variables on oil yield, considering the linear, quadratic, and interaction effects among the variables.

Equation 1 shows the correlation between oil yield and the experimental variables obtained from regression analysis data (Table 2). From the F-test, the validation of the predictive equation was verified, with values of 101.15 and 3.73 for F_{CAL} and F_{TAB}, respectively. Thus, the equation was capable of representing the experimental data for the range of variables investigated, because $F_{CALC} > F_{TAB}$ (calculated from the ANOVA table and tabulated, respectively).

$$\text{Oil yield (\%)} = 18.06 + 1.62X_1 + 2.44X_2 + 2.03X_3 - 0.86X_1^2 - 0.93X_3^2 - 0.42X_1X_2 - 0.32X_3X_2 \quad (1)$$

From the data presented in Table 1, it can be verified that high yields (20.82 and 21.12%) were obtained at the highest level (60 ºC) of the temperature variables (runs 4 and 12), which corroborates the effect of this variable presented in Table 2, which indicates that in the experimental range evaluated, the oil removal from passion fruit seeds was favored by the increase in temperature.

Baümler *et al.,* (2016) determined the equilibrium constant (K) of the oil extraction of sunflower collets and reported a decrease in this parameter with increasing temperature and a greater oil extraction capacity. According to Stamenković *et al.*, (2018) and Zhong *et al*., (2018) the increase in temperature may cause increased solubility of the oil in the solvent and diffusivity of the oil from the sample to the solvent, since the properties of ethanol are drastically affected by the increase in temperature. According to Granero *et al*., (2014), the increase in temperature from 20 to 50 °C results in a reduction in the density and dynamic viscosity of ethanol from 789.48 to 763.22 kg·m^{-3} and 1.24 to 0.72 mPa s, respectively. Pereira *et al*., (2017) reported higher solubility of passion fruit oil in ethanol with increasing temperature and obtained better oil extraction at 60 ºC.

Table 1. Experimental conditions applied and oil yield obtained in experiments to assess the effects of the operating variables using a Box-Behnken design.

Run	X_1[1]	X_2[2]	X_3[3]	Oil yield (%)
1	10	30	1:3	12.69
2	50	30	1:3	16.39
3	10	60	1:3	18.79
4	50	60	1:3	20.82
5	10	45	1:2	12.75
6	50	45	1:2	16.06
7	10	45	1:4	16.16
8	50	45	1:4	20.09
9	30	30	1:2	12.32
10	30	60	1:2	17.48
11	30	30	1:4	17.37
12	30	60	1:4	21.25
13-16	30	45	1:3	18.06[4] ±0.08

[1]X_1: time (min); [2]X_2: temperature (ºC); [3]X_3: solvent to seed ratio (mL·g^{-1}); [4]Average of four experiments.

Table 2. Model coefficients and *p*-value of the model for the extraction of passion fruit seed oil by UAE.

Variables	Effect [a]	*p*-value [b]	Coefficient [c]
Mean/Interaction	16.85	< 0.0001	18.06
X_1 (L)	3.24	0.0003	1.62
X_1 (Q)	0.86	0.0026	-0.86
X_2 (L)	4.89	0.0001	2.44
X_2 (Q)	0.02	0.6470	-0.02
X_3 (L)	4.06	0.0002	2.03
X_3 (Q)	0.93	0.0023	-0.93
X_1 x X_2	-0.83	0.0103	-0.42
X_1 x X_3	0.31	0.0685	0.15
X_2 x X_3	-0.64	0.0175	-0.32

[a] Effect of the independent variable on the dependent variable; [b] statistical significance $p < 0.05$; [c] coefficients of second-order polynomial model (Equations 1 and 2).

The increase in oil yield with the increase in the solvent to seed ratio is due to the higher dissolution capacity, which leads to changes in the thermodynamic and mass transfer properties of the extraction process. Stamenković *et al*., (2018) performed thermodynamic analyses of oil extraction of white mustard (*Sinapis alba* L.) seeds, respectively, and found that the spontaneity of the process was favored by increases in the volume of solvent used. Meziane and Kadi (2008) and Toda *et al.,* (2016) reported higher values for the mass transfer coefficients with the use of higher amounts of solvent.

In addition, during ultrasonic treatment, the collapse between the cavitation bubbles generated high-speed solvent microjets that caused damage to the plant matrix cell wall, increasing the contact area between the matrix and solvent and, consequently, increasing the efficiency of oil extraction (Vinatoru *et al*., 2017). Other authors have reported an effect similar to that observed in this study for the experimental interval evaluated; for example, Mabayo *et al*., (2018) reported that an increase in the ratio of *n*-hexane to rubber seeds from 2 to 4 (mL·g^{-1}) led to an increase in the oil yield from 21.3 to 30.7%, respectively.

On analyzing the interaction between the variables solvent-to-seed ratio and time, it was verified that they had no effect on oil yield. This can be explained on the basis of the results of Meziane and Kadi (2008), who determined the mass transfer coefficients for the extraction stages characterized by washing and diffusion, respectively, using a different solvent-

to-sample ratio and obtaining higher mass transfer coefficients with increasing amounts of solvent used, with higher coefficients in the initial stage.

Extraction occurs in two phases: the first corresponds to rapid extraction, with the removal of the surface constituents of the particle, followed by a slower second phase called diffusion, in which the inner components of the matrix are removed (Chanioti and Tzia, 2018; Stamenković *et al*., 2018; Toda *et al*., 2016). In the initial stage, there is a greater extraction of the oil. This effect is observed in the comparison of the results presented in Table 1 for runs 5–6 and 7–8, for example, where it is possible to observe the removal of ~80% of the oil obtained in 50 min (runs 6 and 8) in an extraction time of only 10 min (runs 5 and 7). However, the use of longer extraction times contributes to further rupture of the cell walls, resulting in greater penetration of the solvent (Balachandran *et al.,* 2006; Kazemi *et al.,* 2016) and consequently an increase in oil yield ($p < 0.05$).

Other studies report the best removal of the oil by subjecting the sample to a longer ultrasound treatment time, among which we can mention the works of Mello *et al*., (2015) and Rodrigues *et al*., (2017), who obtained higher oil yields with increases in the time of treatment with ultrasound from 20 to 60 min.

The effect of time is more pronounced at lower temperatures, as can be seen in the comparison of the data from runs 1–2 and 3–4, since the equilibrium is reached more rapidly with the rise in temperature.

3.1.2. Maximizing oil yield

From the predictive equation, Equation 1, the maximum oil yield that could be obtained within the experimental range evaluated was 21.76% at 50 min and 60 °C with a solvent-to-seed ratio of 4 ($mL \cdot g^{-1}$). Additional experiments were performed in this predicted experimental condition, in triplicate, providing an oil yield of 21.39 ± 0.7%.

The oil yield obtained is close to those reported in other studies that evaluated oil removal from passion fruit seeds using UAE; however, it is worth noting the lower volume of solvent used in this work. Oliveira *et al*., (2013) obtained an oil yield of 19.5% through extraction conducted for 60 min using an ethanol-to-seed ratio of 6 ($mL \cdot g^{-1}$). Pereira *et al*., (2017) reported an oil yield of 20.96% in extraction conducted for 60 min with an ethanol-to-seed ratio of 50 ($mL \cdot g^{-1}$).

The results obtained by Liu *et al*., (2009) and Oliveira *et al*., (2016) showed higher yields of 25.98 and 27%, respectively, with supercritical CO_2 extraction; however, they used high operating pressures (25 MPa) and longer extraction times (150 and 180 min).

3.2. Effect of ultrasound on extraction

Extraction performed without the use of ultrasound, provided an oil yield of 15.82 ± 0.8% under the conditions of maximum oil yield obtained by Equation 1, which represents 75% of the yield obtained with ultrasound. The advantages and characteristics of ultrasound have already been discussed and justify the result obtained.

3.3. Comparison between UAE and classical extraction

Table 3 shows the results of the comparison between traditional extraction and UAE in terms of oil yield, fatty acid composition, and phytosterol and tocopherol contents.

In the traditional extraction (in Soxhlet), the passion fruit seeds used in this work presented oil yields of 29.02 ± 1.61%. This result is close to that reported in the works of Oliveira *et al*., (2013), Pereira *et al*., (2017) and Santana *et al*., (2017), who presented oil yields of 26.4, 28.33, and 28.87%, respectively.

UAE provided 73.7% of the yield obtained from traditional extraction; however, with this method the oil is obtained in a shorter time and mainly using a smaller volume of solvent, since a solvent-to-seed ratio of 4 ($mL \cdot g^{-1}$) is required, while in traditional extraction a solvent-to-seed ratio of 30 ($mL \cdot g^{-1}$) is used. In UAE, the cavitation of the medium favors mass transfer and thus a smaller volume of solvent is necessary; whereas in the classic method the extraction process is governed by mass diffusion, requiring a high consumption of solvent (Menezes *et al.*, 2018).

The results presented in Table 3 show that there is no difference between the fatty acid compositions of the oils obtained with the techniques used. Passion fruit seed oil contains a high concentration of PUFAs (59.28 to 61.19), with linoleic acid forming the major part (59.13 to 61.07%). Another acid that stands out is oleic acid (17.45%). Other studies have also reported that these were the main fatty acids found in passion fruit seeds (Pereira *et al*., 2018; Silva and Jorge, 2017).

Table 3. The fatty acid profile of the passion fruit seed oil.

Parameter evaluated		Extraction method	
		CE	UAE[1]
Oil yield (%)		29.02±1.61[a]	21.39±0.7[b]
Fatty acid (g per 100 g of oil)	Palmitic	12.25±0.15[a]	12.31±0.01[a]
	Palmitoleic	0.17±0.05[a]	0.19±0.05[a]
	Stearic	2.48±0.04[a]	2.44±0.14[a]
	Oleic	17.45±1.25[a]	17.03±0.28[a]
	Linoleic	59.13±1.39[a]	61.07±0.98[a]
	Linolenic	0.15±0.04[a]	0.12±0.03[a]
	SFA	14.73	14.75
	MUFA	17.62	17.22
	PUFA	59.28	61.19
Phytosterol (mg per 100 g of oil)	Stigmasterol	74.29±2.17	75.67±0.05
	Campesterol	19.20±1.42	21.55±0.30
	β-Sitosterol	54.26±4.91	69.11±3.12
	Total	147.75±4.16[a]	166.34±3.36[b]
Tocopherol (mg per 100 g oil)	γ-tocopherol	7.94±1.20	7.63±1.30
	δ-tocopherol	26.00±4.83	37.02±5.36
	Total	33.94±6.03[a]	44.65±6.65[a]

[1]obtained in the condition of maximum oil yield in oil: 50 min, 60 °C and solvent-to-seed ratio of 4 ($mL \cdot g^{-1}$). (CE) Conventional extraction, (UAE) Ultrasound-assisted extraction (UAE) (SFA) Saturated fatty acids, (MUFA), Monounsaturated fatty acids and (PUFA) polyunsaturated fatty acids. Experiments and analyses conducted in duplicate. Means followed by same letters (on each line) did not differ statistically ($p > 0.05$) by the Tukey test.

As can be seen in Table 3, stigmasterol and δ-tocopherol are the predominant phytosterols and tocopherols, respectively, in the composition of passion fruit seed oil.

In the extraction of tocopherols, the evaluated methods provided similar levels of these compounds. The values obtained (33.94 to 44.64 mg per 100 g of oil) were higher than those reported by Pereira *et al*., (2017) in the extraction using pressurized propane (9.87 to 11.14 mg per 100 g of oil) and UAE with ethanol (10.10 mg per 100 g of oil). However, they were lower than the value of 49.9 mg per 100 g of oil as reported by Malacrida and Jorge (2012) with the traditional extraction using petroleum ether.

The UAE provided an extract with a higher content in phytosterols of 166.64 mg per 100 g of oil. The content obtained in this study was lower than that obtained by Piombo *et al*., (2006) and Silva and Jorge (2017), who reported values of 209.74 and 274.67 mg per 100 g of oil, respectively.

The differences found in relation to the literature are related to the nature of the raw material used, as well as the solvent extractor and the efficiency of the extraction method used.

3.4. Sequential reaction

Table 4 shows the results of the chemical composition of the samples obtained after conducting the reaction step in 12 and 24 h. The analysis of the data must be carried out by observing which compound is of most interest in the product, which must be directed to the separation step aiming at the concentration of the target compound. It should be noted that in addition to the compounds identified (Table 4), these samples contain phospholipids, waxes and sugars (Baümler *et al.,* 2016), compounds identified in vegetable oils obtained using ethanol as a solvent.

From the data presented in Table 4, it appears that in 12 h of reaction the product obtained contains ethyl esters, diglycerides (DG) and monoglycerides (MG) in higher concentrations. The high triglyceride conversion in the reaction transesterification products

Table 4. Chemical composition of reaction products obtained from transesterification reaction of product of extraction step (oil + ethanol).

Reaction time (h)[1]	Component (wt%)					
	Ethyl esters	TG	DG	MG	GLY	FFA
12	42.30±0.52	4.82±0.05	22.26±0.27	21.80±0.30	0.314±0.01	0.063±0.001
24	55.58±0.26	0.35±0.01	6.71±0.05	25.63±0.06	0.549±0.01	0.055±0.004

[1]Experiments and analyses conducted in duplicate. (TG) Triglycerides, (DG) Diglycerides, (MG) Monoglycerides, (GLY) glycerol, (FFA) free fatty acid.

after 24 h of reaction was observed, obtaining a sample with a predominance in ethyl esters and MG under these conditions.

Ethyl esters can be directed to use as biofuels (Gonzalez *et al*., 2013; Stevanato and Silva, 2019), in compliance with current legislation. Bitonto *et al*., (2019) reported that these compounds are considered non-hazardous organic compounds, in addition to biofuels, and have industrial applications such as solvent, fragrances and cosmetics.

MG and DG have applications as emulsifiers in the food and pharmaceutical industry (Hartel *et al*., 2018). These emulsifiers are frequently used in bakery products, frozen desserts, and sauces/dressings (Nicholson *et al*., 2019). According to Ferreti *et al*., (2018) diglycerides are widely used for foods such as mayonnaise, salad dressings and in the confectionery industry, as they combine a hydrophilic head and a hydrophobic tail in the molecule that help hydrophilic and lipophilic substances to mix.

Thus, it was found that the sequential process provided a high-quality oil, eliminating the solvent removal step, which favors its application in different products and because it is a food residue, the oil obtained from the seeds Passion fruit can be applied to food products that require emulsifiers during their production process.

4. CONCLUSIONS

This study presents the use of UAE to obtain the oil of passion fruit seeds. Through the use of ethanol with solvent, it was verified that temperature was the variable with the greatest influence on the extraction, and high yields of oil (21.7%) were obtained using a low solvent volume (a solvent-to-seed ratio of 4 $mL \cdot g^{-1}$). High yields can be obtained at low extraction times (10 min), representing ~80% of the maximum yield obtained in 50 min. The extraction of the oil is favored by the application of ultrasound, with which it was possible to obtain 73.7% of the yield provided by traditional extraction. Oleic and linoleic acids represent ~80% of the oil composition. The evaluated techniques provided oil with a similar tocopherol content; however, higher levels were obtained through extraction by ultrasound. Conducting the sequential reaction to the extraction process provided products with different applications, eliminating the solvent removal step.

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