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Sunflower seed oil: enzymatic aqueous extraction, oil recovery by green solvent and chemical composition

Aceite de semilla de girasol: extracción acuosa enzimática, recuperación de aceite mediante solvente verde y composición química

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Abstract
The combination of Alcalase® 2.4L FG (ALC) and Celluclast® 1.5L (CEL) enzymes in the sunflower seed oil extraction were evaluated, using ethyl acetate in the oil recovery step. The influence of the enzyme on the oil composition (minor compounds and fatty acid profile) was determined. Soxhlet extraction with ethyl acetate was also performed, as well as extraction with each enzyme isolated. Oil yield (Yo) after 4 cycles with the reuse of ALC and CEL enzymes was verified. The CEL/ALC extraction obtained the highest Yo (43.11 wt%) among the evaluated enzymatic extractions, which represents 76.65% of extraction efficiency. Oleic and linoleic acids constitute a significant portion of the oil's composition, totaling about 87% of the fatty acids. In the phytosterols composition, β-sitosterol represented the highest value and α-tocopherol showed a higher amount in extractions with the combination of enzymes. Squalene concentration was similar in the obtained oils. The reuse of ALC and CEL enzymes proved to be viable, as after the 4 cycles the Yo reduced about 9.50% and 11.33%, respectively.
Keywords: fatty acids, squalene, Alcalase® 2.4L FG, Celluclast® 1.5L.

Resumen
Se evaluó la combinación de enzimas Alcalase® 2.4L FG (ALC) y Celluclast® 1.5L (CEL) en la extraction de aceite de semilla de girasol, utilizando acetato de etilo en el paso de recuperación del aceite. Se determinó la influencia de la enzima en la composición del aceite (compuestos minoritarios y perfil de ácidos grasos). También se realizaron extracciones con Soxhlet utilizando acetato de etilo, así como extracciones con cada enzima por separado. Se verificó el rendimiento del aceite (Yo) después de 4 ciclos con la reutilización de las enzimas ALC y CEL. La extracción CEL/ALC obtuvo el mayor Yo (43.11% en peso) entre las extracciones enzimáticas evaluadas, lo que representa el 76.65% de la eficiencia de extracción. Los ácidos oleico y linoleico constituyen una parte significativa de la composición del aceite, totalizando aproximadamente el 87% de los ácidos grasos. En la composición de fitoesteroles, es β-sitosterol presentó el valor más alto y el α-tocoferol mostró una mayor cantidad en las extracciones con la combinación de enzimas. La concentración de escualeno fue similar en los aceites obtendos. La reutilización de las enzimas ALC y CEL demostró ser viable, ya que después de los 4 ciclos, el Yo se redujo aproximadamente 9.50% y 11.33%, respectivamente.
Palabras clave: ácidos grasos, escualeno, Alcalase® 2.4L FG, Celluclast® 1.5L.

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1 Introduction

The search for healthy foods that have nutritional effects has been the focus of interest for decades (Huesca-Toral *et al*., 2005), and in this sense, edible oils are an important raw material in the food industry, with considerable nutritional relevance (Núñez-Gastélum, 2023). The fourth most significant oilseed for the manufacture of vegetable oils is sunflower (*Helianthus annuus* L.) (FAO, 2022), since the seed has ~44 wt% oil (Pilorge, 2020). Sunflower oil is mainly composed of linoleic (omega 6) and oleic (omega 9) acids, totaling ~56% of its composition (Aquino *et al*., 2022). Vegetable oils with high concentrations of these fatty acids can help lower LDL cholesterol, resulting in a decreased risk of cardiovascular illnesses (Vijayakumar *et al*., 2016). In addition, sunflower oil has natural antioxidants in its composition, such as tocopherols, phytosterols and vitamins (Wang *et al*., 2018).

Enzymatic aqueous extraction (EAE), which is thought to be a green extraction method, breaks down the oleaginous cell wall in an aqueous media to liberate the oil (Cheng *et al*., 2019). Cell wall is composed of cellulose, hemicellulose, protein, and pectin (Mwaurah *et al*., 2020). The enzymes cellulases, hemicellulases, proteases and pectinases are commonly applied, and according to the structure of the oleaginous cell wall, a combination of these enzymes can be used (Liu *et al*., 2016). The use of two or more enzymes aims to hydrolyze different components that are part of the composition of the plant wall, and the increase in oil yield was reported when applied (Zeng *et al*., 2022; Peng *et al*., 2019; Tirgarian *et al*., 2019; Meng *et al*., 2018; Campbell *et al*., 2016).

The advantages that EAE presents compared to conventional solvent extraction refer to the lower energy consumption due to the use of mild temperatures and the non-use of toxic organic solvents, which are less harmful to the environment and health (Mwaurah *et al*., 2020). The reuse of enzymes favors the increase in the economic viability of the process, since the cost of the enzyme can reach 30% of the cost of the materials used in the technique (Cheng *et al*., 2019). Nguyen *et al*. (2020) and Talekar *et al*. (2020) reported the recycling of free protease and immobilized protease enzymes for up to 10 cycles with a loss of 21 and 26% of oil yield, respectively.

In addition, due to the fact that the extraction takes place in an aqueous medium and due to the presence of proteins in the composition of the oilseeds, the oil extracted from EAE is usually emulsified (Liu *et al*., 2016). To recover this oil, it is necessary to destabilize the emulsion, for which a solvent can be added (Mwaurah *et al*., 2020) and to have an ecofriendly process, ideally the solvents used should be considered green and alternatives to n-hexane, commonly used, should be considered. Vegetable oils may be efficiently extracted using ethyl acetate (Stevanato *et al*., 2022; Gasparetto *et al*., 2022; Sitepu *et al*., 2022; Gao *et al*., 2019) and its potential is due to factors such as human safety and lower environmental impact, in addition to providing oil with yield and quality parameters comparable to n-hexane. This ester also stands out for having high neutral lipids extraction selectivity (Lu *et al*., 2015) and versatility, since it can be used in the oil extraction as a solvent and in the interesterification reaction to produce alkyl esters as a reagent, whose stoichiometric molar ratio of ethyl acetate to oil is 3:1 (Stevanato *et al*., 2022). In this way, the EAE product can be directed to the reaction step, enabling the establishment of an integrated production process.

The present study aims to evaluate the combination of enzymes Alcalase® 2.4L FG and Celluclast® 1.5L to obtain oil from sunflower seeds, using ethyl acetate in the oil recovery step and compare with the results with the isolated use of the mentioned enzymes. Soxhlet extraction was conducted to evaluate extraction process yield. The influence of the enzyme in the composition of the oils was obtained and the reuse of enzymes in the extraction process was determined.

2 Materials and methods

2.1 Materials

Sunflower seeds (without shells) were purchased in local commerce in the city of Umuarama/PR - Brazil. Celluclast® 1.5L (celulase from *Trichoderma reesei*, enzyme activity of 700 Endoglucanase Units) per g) and Alcalase® 2.4L FG (alkaline serine endopeptidase from *Bacillus licheniformis*, enzyme activity of 2.4 Anson Units per g), were donated by LNF Latino Americana. In the conventional extraction and oil recovery in the enzymatic extraction, ethyl acetate (Exodus, 99.5%) was used.

In determining the fatty acid profile and minor compound content, were used: methanol (Panreac), potassium hydroxide (Anidrol), sulfuric acid (Anidrol), heptane (Exodus), N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA with 1% TMCS) (Sigma-Aldrich) and 5α-cholestane (Sigma-Aldrich, >99%).

2.2 Oil extraction

The experimental procedure and parameters used in the EAE (enzyme concentration, pH, temperature and time) are described in Aquino *et al*. (2019) and Aquino *et al*. (2022), for the enzymes Celluclast® 1.5L (CEL)

Table 1. Experimental conditions for conducting enzymatic aqueous extraction.

Codification	Enzyme concentration, temperature, pH and extraction time	
CEL	Celluclast, 1% (v/w), 60 °C, pH 4.5 and 5 h	
ALC	Alcalase, 9% (v/w), 40 °C, pH 8.0 and 4 h	
CEL/ALC	Celluclast	Alcalase
	1% (v/w), 60 °C, pH 4.5 and 5 h	9% (v/w), 40°C, pH 8.0 and 4 h
ALC/CEL	Alcalase	Celluclast
	9% (v/w), 40 °C, pH 8.0 and 4 h	1% (v/w), 60 °C, pH 4.5 and 5 h

and Alcalase® 2.4L FG (ALC), respectively. Table 1 presents parameter values for each enzyme, in which 10 g of crushed sunflower seed (mean diameter of 0.711 mm) and 40 mL of distilled water (mass ratio of 1:5 g/g) were used in each assay (performed in triplicate). In the extractions with the subsequent combination of enzymes (CEL/ALC and ALC/CEL), a mass ratio of 1:5 of crushed sunflower seed and water was used. The first enzyme was added and the conditions for this enzyme were used as described in this Table 1. After the extraction time with the first enzyme, the pH of the medium was adjusted and the second enzyme was added and its conditions of pH, temperature and extraction time were used.

After the extraction was completed, the flasks were stored at 4 °C (Consul, 340) overnight. Then, 10 mL of ethyl acetate were added for each 50 mL of extraction medium and centrifugation (Metroterm, Model MTD III PLUS) was performed at 2700 rpm for 15 min. The sample was separated into three phases: organic, aqueous and solid. With the use of a pasteur pipette, the organic phase was recovered. The solvent was again added to the flask with the aqueous and solid phases, three times. The solvent of the organic phase was recovered in a rotary evaporator (Marconi/MA120).

Soxhlet extraction was performed using ethyl acetate as solvent at its boiling temperature (~80 °C) and solvent to sample ratio of 30 mL g^{-1} for 480 min (Stevanato & Silva, 2019).

The organic solvent, enzymatic and Soxhlet extraction, was eliminated by evaporation in oven with forced air circulation (Marconi, MA035), at 80°C until reaching constant weight.

The ratio of the mass of the extracted oil to the mass of the sunflower seeds utilized in the extraction was used to calculate the oil yield (Yo). The extraction process yield (EPY) was calculated by the ratio between the yield of EAE and the yield of Soxhlet extraction.

2.3 *Oil characterization*

The oil characterization analyzes were carried out in a gas chromatograph coupled to a mass spectrophotometer (GC-MS) (Shimadzu, CGMS-QP2010 SE, Tokyo, Japan) as reported by Stevanato and Silva (2019) Helium was used as carrier gas at a flow rate of 1 mL min^{-1}. The identification of compounds was based on comparisons of their mass spectra with the NIST database.

To determine the fatty acid profile of the samples, the following heating schedule was used: initial temperature of 120°C; then ramp to 180 °C at 10 °C min^{-1}; then to 240 °C at 6 °C min^{-1} (held for 2 min). Fatty acid methyl esters (FAMEs) were obtained and the quantification of these compounds was performed by area normalization, determining the percentage of individual peak areas in relation to the total area. For quantification of minor compounds, the sample was derivatized with BSTFA and 5α-cholestane was added to the mixture as an internal standard for quantification of compounds. Compounds were separated using the following temperature gradient: initial oven temperature was 160 °C, then increased to 230 °C at 10 °C min^{-1}, then to 280 °C at 15 °C min^{-1} and held constant for 16 min.

The determinations free fatty acids (method Ca 5a-40) and of water content (Bc 2-49) and were carried out according to American Oil Chemists' Society (AOCS, 1998) and (AOCS, 1993), respectively.

2.4 *Enzyme reusability experiments*

The recycle of CEL and ALC enzymes were evaluated, in triplicate, in relation to YO. The first extraction cycle was conducted as described in item 2.1 and under the conditions shown in Table 1. After extraction, the aqueous phase containing the enzyme was separated by centrifugation (2700 rpm for 15 min), filtered and transferred to an Erlenmeyer flask, where sunflower seeds were added (mass ratio 1:5 g/g seed to medium). Next, the pH was adjusted and the flasks were incubated in the shaker. The organic phase was collected according to the previously described methodology for determining the YO.

2.5 *Data analysis*

The comparison of the mean values obtained was conducted by analysis of variance (ANOVA) and Tukey's test with a significance level of 5% (α=0.05),

Table 2. Results of oil yield (YO) and extraction process yield (EPY) obtained from enzymatic aqueous extraction (EAE) and Soxhlet extraction.

Extraction method		YO (wt%)	EPY (%)
EAE[1]	CEL	35.50 ± 0.07^{e}	63.13
	ALC	38.22 ± 0.50^{d}	67.97
	CEL/ALC	43.11 ± 1.21^{b}	76.66
	ALC/CEL	41.24 ± 0.23^{c}	73.34
Soxhlet		56.23 ± 0.11^{a}	100

[1] as Table 1.

using the Statistica® 8.0 software. The results were tabulated in what means followed by the same letters (on the same line) do not differ statistically (p>0.05).

3 Results and discussion

3.1 Oil yield

Table 2 presents the oil yield results of EAEs and Soxhlet extraction. The extraction with the CEL/ALC combination obtained the highest YO among the evaluated EAEs, which corresponds to the extraction process yield (EPY) of 76.65%. Ribeiro *et al*. (2016) carried out the extraction of sunflower oil with the combination of cellulase and pectinase enzymes and added chloroform for oil recovery, so the EPY was 66.4%. Campbell *et al*. (2016) extracted oil from sunflower seeds with the enzyme cellulase and obtained an EPY of 39% with the addition of 3% (m/m) of the surfactant sodium dodecyl sulfate in the extraction medium to help with oil recovery. Moradi and Rahimi (2018) reported an EPY of 77.4% for EAE with a combination of pectinase and cellulase in sunflower oil extraction.

The combination of CEL/ALC enzymes favored oil extraction by 21.4% when compared to the use of CEL. This fact suggests that the hydrolysis of cellulose allowed the oil bodies that were between the cellulose chains to be released for later protease to release oil degrading the proteins around the oil bodies (Campbell *et al*., 2016). However, oil extraction by the combination of ALC/CEL had the lowest YO compared to CEL/ALC. In this case, the oil bodies that were released after the action CEL enzyme did not have their proteins hydrolyze because the protease enzyme was add before CEL enzyme.

The use of ALC resulted in a 7.6% increase in YO compared to CEL extraction. Cellulase acts in the hydrolysis of cellulose present in the cell wall, while Alcalase, as a protease, hydrolyses the protein network around the oleosome, also called the oil body (Pérez-Salazar *et al*., 2022; Becerra *et al*., 2021). The higher YO with Alcalase can be explained because the proteins are part of the barrier around the oil body responsible for hindering the release and coalescence of the oil (Meng *et al*., 2018; Oliveira Filho & Egea, 2020).

Weng *et al*. (2022) verified the influence of cellulase, pectinase and protease enzymes on the yield of *Torreya grandis* oil extraction and the extraction oil yield with protease reached 58% while with the other enzymes it was around 50%. Liu *et al*. (2019) evaluated the action of the enzymes protease, cellulase, pectinase and hemilcellulase in the extraction of oil from *Sapindus mukorossi* and obtained the highest YO with protease (~65%) followed by cellulase (~61%), while the other enzymes had yield below 50%. In the extraction of pecan oil, Polmann *et al*. (2019) obtained YO with Alcalase® 2.4L FG enzyme (47.3%) greater than with Celluclast® 1.5L enzyme (23.8%), which was also reported by Wang *et al*. (2019) in the extraction of oil from rice germ. Despite the different oleaginous matrices used in the reported works, the protease enzyme proved to be more efficient compared to cellulase. This fact, as already described, may be related to the hydrolysis of the proteins that are surround the body of oil, which results in a less compact structure and a higher release of the oil.

3.2 Oil characterization

Table 3 reports the fatty acid profile and minor component content (phytosterols, tocopherol and squalene) of sunflower seed oil obtained from EAEs and Soxhlet.

Nine fatty acids were identified in the composition of sunflower seed oil, among which, predominantly unsaturated fatty acids (~87.0%), oleic (46.37-47.41%) and linoleic (38.28-39.67%). This fatty acid composition has been reported to be beneficial in preventing cancers and coronary heart disease (Orsavova *et al*., 2015). In addition, higher oleic acid content relative to linoleic acid exhibits greater oxidation stability (Belingheri *et al*., 2015), allowing a greater range of processing for this oil, extending its storage time and preparation of foods containing these lipids (Mohammadi *et al*., 2016). The proportion of these compounds in the oil from the investigated extractions was similar to that reported for rice germ

Table 3. Fatty acid profile and minor compound content of the oil obtained from enzymatic aqueous extraction and Soxhlet extraction.

Properties		Enzymatic aqueous extraction[1]				Soxhlet
		CEL	ALC	CEL/ALC	ALC/CEL	
Fatty acids	Myristic	0.08±0.00^{a}	0.06±0.00^{b}	0.06±0.00^{b}	0.07±0.00^{b}	0.08±0.00^{a}
	Palmitic	6.36±0.02^{b}	5.72±0.07^{d}	6.80±0.04^{a}	6.88±0.01^{a}	6.10±0.06^{c}
	Palmitoleic	0.08±0.00^{a}	0.08±0.04^{a}	0.09±0.01^{a}	0.08±0.01^{a}	0.10±0.00^{a}
	Stearic	4.55±0.02^{c}	4.46±0.01^{c}	5.04±0.04^{b}	5.16±0.02^{a}	4.22±0.05^{d}
	Oleic	47.13±0.13^{a}	47.22±0.73^{a}	46.37±0.04^{a}	46.47±0.05^{a}	47.41±0.11^{a}
	trans-Vaccenic	0.77±0.02^{a}	0.50±0.01^{d}	0.73±0.01^{ab}	0.64±0.01^{c}	0.67±0.03^{bc}
	Linoleic	39.13±0.21^{ab}	39.67±0.73^{a}	38.63±0.08^{ab}	38.28±0.06^{b}	39.21±0.05^{ab}
	Arachidic	0.33±0.05^{a}	0.36±0.03^{a}	0.34±0.02^{a}	0.40±0.06^{a}	0.35±0.01^{a}
	Behenic	1.09±0.01^{d}	1.20±0.00^{c}	1.27±0.02^{b}	1.33±0.00^{a}	1.08±0.01^{d}
	Not identified	0.49±0.01	0.77±0.00	0.68±0.01	0.68±0.08	0.79±0.04
Phytosterols (mg per 100 g)	Campesterol	30.91±1.15^{b}	28.01±0.77^{bc}	27.05±1.06^{c}	30.14±0.49^{bc}	34.60±0.02^{a}
	Stigmasterol	44.00±0.94^{ab}	36.50±0.01^{c}	40.25±1.70^{bc}	45.19±0.02^{a}	41.98±1.27^{ab}
	β-Sitosterol	185.43±1.66^{a}	164.96±4.04^{b}	161.18±2.65^{b}	177.42±3.00^{a}	184.09±0.76^{a}
	Total	260.35±3.75^{a}	229.47±4.80^{b}	227.48±1.31^{b}	252.75±3.51^{a}	260.68±2.05^{a}
Tocopherol (mg per 100 g)	α-Tocopherol	53.20±0.09^{d}	54.09±1.08^{d}	93.54±1.16^{a}	64.79±0.59^{c}	72.89±1.34^{b}
Squalene (mg per 100 g)		80.81±1.91^{b}	83.55±1.75^{ab}	82.67±0.26^{b}	89.17±1.79^{a}	81.61±1.58^{b}

[1] as Table 1.

oil obtained from aqueous extraction with enzymes alcalase/cellulase (1:1 w/w) (Wang *et al*., 2019).

The fatty acid profile of the oils showed differences for the extractions in terms of linoleic acid, where the obtainment of this compound with ALC was ~3.7% higher than that resulting from the extraction of ALC/CEL. However, in relation to behenic acid, reported as a natural pancreatic lipase inhibitor (Kojima *et al*., 2010), the combination of ALC/CEL provided the highest result for this compound, ~22.0% higher in relation to CEL and in relation to that obtained by Soxhlet extraction, suggesting that the aqueous enzymatic extraction method is more suitable for obtaining sunflower oil in terms of their functional composition. The difference in the value fatty acid composition can be attributed to the synergy of the combination of enzymes in the extraction conditions, since the process was carried out under optimal working temperatures enzymes (40 °C and 60 °C), relatively milder than Soxhlet, performed at ~80 °C.

The highest levels of total phytosterols were obtained with CEL and ALC/CEL, reaching levels similar to the conventional Soxhlet technique. Regarding α-tocopherol, the enzymatic combination CEL/ALC was superior to the other investigated strategies, including Soxhlet extraction. Furthermore, it was verified that for the extraction of α-tocopherol, the combination of enzymes was more efficient compared to the isolated use. The difference in the performance of the enzymes in the recovery of phytosterols and α-tocopherol can be explained by the fact that these substances are located in different parts of the plant cell. Phytosterols are components of the cell membrane, while tocopherols are located in plastids (Velasco & Ruiz-Méndez, 2015). The highest levels of total phytosterols were obtained with CEL and ALC/CEL, reaching levels similar to the conventional Soxhlet technique. Regarding α-tocopherol, the enzymatic combination CEL/ALC was superior to the other investigated strategies, including Soxhlet extraction. Furthermore, it was verified that for the extraction of α-tocopherol, the combination of enzymes was more efficient compared to the isolated use. The difference in the performance of the enzymes in the recovery of phytosterols and α-tocopherol can be explained by the fact that these substances are located in different parts of the plant cell. Phytosterols are components of the cell membrane, while tocopherols are located in plastids (Velasco & Ruiz-Méndez, 2015).

The squalene content was little influenced by the extraction method and reached a content of up to 89.17 mg per 100 g with the ALC/CEL combination. This active compound is a precursor of phytosterols with nutraceutical properties, such as antioxidant and cardioprotective effect, which is present in the plasma incorporated into lipoproteins (Micera *et al*., 2020).

Free fatty acid and moisture content are part of the vegetable oils quality about stability oxidative. The percentage of free fatty acids in the CEL/ALC oil was 2.331% ± 0.007, with up to 4.0% being the allowed value for this parameter (CODEX STAN 210-1999). Silva *et al*. (2020) evaluated commercial sunflower oil and obtained a free fatty acid value

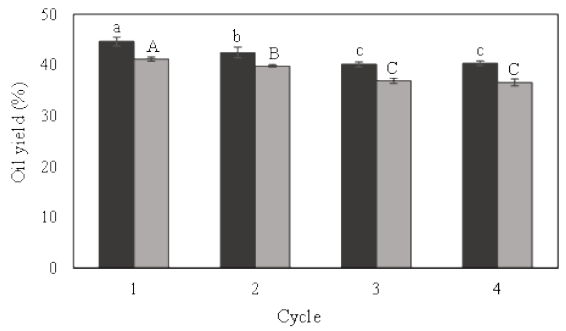

Figure 1. Recycle of the enzymes Alcalase® 2.4L (dark grey bars) and Celluclast® 1.5L (light grey bars) in the sunflower seed oil extraction. Values with different uppercase are significantly different ($p < 0.05$) for Alcalase® 2.4L and values with different lowercase are significantly different ($p < 0.05$) for Celluclast® 1.5L.

of 1.37%. In this case, the lower value compared to the present work is since the commercial oil is refined and it has gone through the neutralization step. In addition, the moisture content of the CEL/ALC oil was also evaluated, which was 0.078% ± 0.001. Saudodi *et al*. (2014) obtained 0.10% moisture from commercial sunflower oil. Pal *et al*., (2015), analyzed the moisture of crude and refined sunflower oil, both showed moisture below 0.10%.

3.3 Enzyme reusability

Figure 1 presents the results obtained (in relation to oil yield) obtained with the reuse of CEL and ALC enzymes, in which each cycle consisted of 5 and 4 h of extraction, respectively. The information in this figure shows that the enzymes showed activity during the four cycles that were tested, with an average reduction of 10% in oil yield.

The decrease in oil yield could be due to the loss of enzyme quantity due to the oil recovery step after each cycle (Chen & Diosady, 2003). In addition, as the aqueous phase containing the enzyme was reused, there may be a substance extracted in it that compromises the enzymatic activity (Liu *et al*., 2016). However, these situations can be remedied by adding more enzymes to compensate for the loss of enzyme and/or enzyme activity in each cycle (Peng *et al*., 2019).

Yusoff *et al*. (2016) reused aqueous phase from enzymatic extraction process of *Moringa oleifera* oil with a mixture Neutrase 0.8L and Celluclast 1.5L enzymes for a second cycle with duration 1 hour. Although, the free oil did not observe, but a thick layer of emulsion, which concluded that oil extraction occurred in the second cycle with the reuse of the enzyme mixture. Nguyen *et al*. (2020) reused the papain protease contained in the aqueous phase of *Sacha inchi* oil extraction in 10 cycles (5h each) and reported that the free oil yield decreased from the third cycle, from 28% to 26% and to 23% after the ten cycles.

Conclusions

The combination of CEL and ALC enzymes in the extraction of oil from sunflower seeds and the recovery of the oil from the aqueous medium with ethyl acetate as solvent has been reported. The aqueous extraction using CEL/ALC obtained the highest Yo which corresponds to the extraction process yield of 76.65%. In addition, the oil composition obtained by EAE was promising, indicating that this method can be used as an ecologically correct procedure for extracting sunflower seed oil, being an alternative to conventional solvent extraction methods.

The ALC and CEL enzymes can be reused for the aqueous enzymatic extraction of oil from sunflower seeds, because after 4 cycles with the reuse of the enzymes the yield was 40.37% ±0.43 and 36.56% ±0.68, respectively. Thus, with the reuse of the enzyme there may be a reduction in the amount of enzyme used in the extraction process and a probable increase in the economic viability of the process.

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