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Effect of pectinase on the oil solvent extraction from different genotypes of sunflower (*Helianthus annuus* L.)

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ABSTRACT

Pectinase-assisted solvent extraction of oil and total tocopherol from sunflower hybrids with different structural characteristics was studied by means of laboratory tests carried out in a stirred batch extractor at 50 °C. From the oil yield results obtained for the two hybrids studied, black-hull (P20) and striped-hull hybrids (ACA 884), it could be observed that oil was extracted at different rates in both hybrids. Analyzing the performance data versus time in the control samples, ACA 884 oil was released more easily and remained invariant with time, whereas for P20 the extraction was slower and the maximum value was reached at 60 min. The fiber wall structure collapsed by the enzymatic action, improving oil release and allowing a faster extraction. The efficiency of the treatment was measured in terms of the percentage of increase in oil yield compared with the control samples. It was observed that in hybrid ACA 884 the mean increases were not significantly different ($p = 0.9295$) over time (4.12% d.b.). Hybrid P20 showed a significant increase in the percentage of oil yield ($p = 0.0001$) at 10 min (10.85% d.b.), and then it remained virtually constant over time (4.89% d.b.). The black-hull hybrid (P20) presented significant differences in fatty acid composition due to the enzymatic action. Pectinase treatment was highly effective in the tocopherol extraction from hybrid P20 ($p < 0.001$), obtaining a 32.3% increase on average. The maximum values were 662 and 220 μg g dried ground seed^{-1} at 60 and 120 min for black-hull and striped-hull hybrids, respectively. The enzymatic treatment effectively produced an increase in oil and tocopherol yields for the black-hull hybrid.

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1. Introduction

One of the main objectives in the oilseed industry is the optimization of the oil extraction process. Although there is abundant literature on the topic, the search for technologies that reduce energy consumption and that are safe for the environment and workplace continues.

Oil extraction is favoured by the application of pretreatments that break and/or degrade the cell wall structure, facilitating not only the oil release from the oilbodies, but also the access of the solvent. An understanding of the microstructure of the oilseed is of fundamental importance to understand the extraction process and break the major barrier for the oil extraction.

In the last decades, the use of enzymes in oil extraction was associated with the use of water as a non-specific solvent. The cell wall degradation caused by enzymes increases the release of oil by dissolving water-soluble components. Enzyme-assisted aqueous extraction also has a potential use in the simultaneous recovery of oil and protein (Rosenthal et al., 1996). The increase in oil yield and/or extraction rates have been reported for several seeds (Sosulski and Sosulski, 1993; Dominguez et al., 1994, 1995; Ramadan et al., 2008). As it is well known, the process of extracting oil from seeds depends on the type and structure of the seeds, so it is recommended to select an enzyme taking into account the structural characteristics of each oilseed, and the behavior of cell wall polysaccharides. Celulase and hemicelulase are the most suitable enzymes to degrade the cell wall. Pectinase is also effective because pectic substances are structural components of fruits and vegetables, and they are largely responsible for the coherence and integrity of the plant tissue (Sineiro et al., 1998). Although pectinase is mainly used to reduce the cloudiness and bitterness of fruit juices, there has been a number of reports on other uses, for example retting and degumming of fiber crops in the textile industry, production of quality paper, fermentation of coffee and tea, oil extractions, and treatment of pectic waste water (Kashyap et al., 2001; Ramadan et al., 2008).

At present, the incorporation of an enzymatic treatment in the oil industry has some disadvantages, such as: additional energy costs to assure an appropriate particle size of the milled material for the action of the enzyme to be effective, separation of the oil from the formed emulsion, drying the meal to adequate moisture

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levels for storage or subsequent extraction by solvent, and time involved in the soaking phase (Dominguez et al., 1995). Apart from being more expensive, aqueous extraction results in lower oil yields compared with solvent extraction. Dominguez et al. (1995) studied the enzyme-assisted hexane extraction of soya bean oil using a commercial cellulolytic formulation and a multiactivity complex consisting of cellulase, hemicellulose and other side-degrading activities. An increase in oil yield and phosphorus content with respect to the untreated samples was observed.

With regard to sunflower seeds, the effects of enzymatic treatments (protease, cellulose, hemicellulose, pectinase) on the quality of the extracted oil have also been studied. As with other oilseeds, an increase in oil yield, phosphorus and tocopherol content was observed for the enzyme-extracted oil with respect to that of control and solvent-extracted oils (Sineiro et al. 1998; Sajid Latif and Anwar, 2009). Sunflower hybrids that can potentially produce high concentrations of oil usually have kernels covered by a black hull, whereas those with a lower oil concentration have a striped hull. Most of the oil is accumulated in the kernel and only 3–5% is located in the hull (Connor and Sadras, 1992). On the other hand, in oilseed industries it is very common to handle mixtures of different hybrids.

The amount of carbohydrates present in sunflower meals differs depending on the amount of shell present. Düsterhöft et al. (1992) characterized the major non-starch polysaccharides from sunflower meal, which mainly consist of cellulose, pectic polysaccharides, and the rest by glucomannans, glucoronoxylans and fucoxyloglucans.

The aim of this work was to study the oil extraction from two different sunflower genotypes (black-hull oilseed hybrid and striped-hull oilseed hybrid) using hexane assisted by pectinase. We also analyzed the kinetics of tocopherol extraction in both hybrids, and compared them with respect to the conventional process. This data would help to adapt the conventional process to the enzyme-assisted extraction process.

2. Materials and methods

2.1. Materials and chemicals

Two sunflower hybrids were used for testing: ACA 884 (Asociación de Cooperativas Argentinas, Pergamino, Argentina, striped hull) and Paraíso 20 (NideraS.A., Junín, Argentina, black-hull). The hybrids were obtained through the courtesy of the Instituto Nacional de Tecnologia Agropecuaria Balcarce Experimental Station (INTA, Argentina). The sunflower seeds were grown in Balcarce (37°45′S, 58°18′W), Buenos Aires province, Argentina.

Pectinase enzyme (0.048 PEU mg^{-1}; Pectinol, Röhm, Germany) and analytical grade *n*-hexane (solvent) were used in the tests.

2.2. Preparation and characterization of the samples

The whole seeds were ground in a coffee grinder (Moulinex, Argentina) and screened to a particle size of 0.420–1.000 mm for the different tests, corresponding to the optimal milling values suggested in the consulted literature (Patricelli et al., 1979; Chien et al., 1990; Myint et al., 1996). Particles smaller than 0.420 mm (fine) were discarded, while those larger than 1.000 mm (thick) were ground again and re-screened. This operation was repeated several times. The samples with these characteristics are called "meal". Particle size and size distribution were characterized using a Horiba LA-910 laser-scattering particle size analyzer (HORIBA, Japan). The particle size is calculated by the analyzer based on the average value of particle's geometrical lengths measured through different orientations of incidence scattering light.

The initial moisture and oil content of the ground seeds (0.420–1.00 mm) were determined by IUPAC 1.121 and 1.122 (IUPAC, 1992), respectively. Standard AOCS official methods were used to determine crude fiber and protein content ($N \times 5.3$ factor) (AOCS, 2009).

Acid-detergent fiber (ADF), Neutral-detergent fiber (NDF) and lignin were determined by the sequential method, using α-amylase and without sodium sulfite, according to the procedure described by Van Soest et al. (1991) in a bath processor (Ankom Technology Corp., Fairpoint, NY, USA). Cellulose was calculated as the difference between ADF and lignin and hemicellulose, between NDF and ADF.

2.3. Scanning electron microscopy (SEM)

Seeds were sliced into longitudinal sections with a razor blade, after being plunged into liquid nitrogen to ensure the maintenance of their internal structure. They were adhered to a cover slip, coated with a thin gold film in a sputter coater (Pelco 9100) and observed under a scanning electron microscope (model EVO40 VP, JEOL, Tokyo, Japan) at 5 kV accelerating voltage, with magnification ranges between 1000 and 2000. Endosperm cell dimensions were measured using the AnalySIS 3.0 system (Soft Imaging Systems GmbH).

2.4. Oil extraction data

The oil extraction data were obtained in a thermostate-regulated batch system using *n*-hexane as solvent. Preliminary tests and values suggested by the literature consulted (Sineiro et al., 1998; Ramadan and Moersel, 2009) allowed us to determine suitable operating conditions. These conditions were: enzyme:meal ratio 2% (w/w); temperature 50 °C; meal:solvent ratio 1:17 (w/vol); pH, natural seed and stirring speed of 200 rpm ($N_{Re} = 11,355$).

The enzyme was diluted in distilled water at a 2% enzyme/substrate ratio and sprayed onto the solid sample, thus the moisture content in each sample was adjusted to the desired level (12% d.b.).

A sample of 5.00 ± 0.1 g of meal was weighed, and conditioned to the desired moisture (12% d.b.). Both the sample and the solvent were heated to 50 °C before mixing them together. Experiments were carried out from 10 to 1080 min (considered long enough to reach equilibrium state). After the preset time, the miscella (oil + solvent) was separated from the solid material by filtration through a Whatman No. 42 filter paper. The hexane was distilled off under vacuum using a rotary evaporator (Büchi Laboretechnik AG, Flawil, Switzerland) at 45 °C. Residual hexane was removed under a nitrogen stream to constant weight. The amount of oil was measured gravimetrically on a Sartorius balance (Model: PB211D, precision: 0.1 mg, Germany). The oil obtained was stored in brown flasks under refrigeration (4 °C) until further analysis. The control samples were also processed under the same experimental conditions, except for the enzyme addition.

2.5. Fatty acid composition

Fatty acid composition was determined by gas chromatography and expressed as a percentage of total fatty acid content as described by Izquierdo et al. (2002).

2.6. Tocopherol content

Tocopherol content for each oil sample extracted at different preestablished times of the kinetics curves (control and enzymatic) was measured by HPLC using AOCS method Ce 8-89 (AOCS, 2009). A HPLC Hewlett Packard system (HP 1050 Series, Waldbronn, Germany) equipped with a flourescence detector, excitation wave-

length set at 292 nm, emission wavelength 330 nm, and a LiChrosorb Si-60 column (250 × 4 mm i.d., 5 μm particle size, HiCHROM, Theale, Berkshire, UK) was used. The column was eluted with n-hexane:isopropanol (99.5:0.5 v/v, HPLC solvent, J.T. Baker, Phillipsburg, USA) at a flow rate of 1.5 mL min^{-1}. Areas were converted to concentrations using a standard curve of α-tocoferol (Sigma T#3251, 95%) in *n*-hexane ($R^2 = 0.9982$) in the concentration range relevant for the sample concentrations. Tocopherol content was expressed as μg g^{-1} of meal dry weight.

2.7. Statistical analysis

Values represent the means and standard deviations of two replicates. Statistical analysis was carried out by Analysis of Variance using the Infostat software (Di Rienzo et al., 2011). Tukey method was used to compare the means of pairs of treatment with a significance level $p \leqslant 0.05$.

3. Results and discussion

The particle size distribution of the meal samples resulted in an average diameter of 672 ± 18 and 642 ± 42 μm for ACA 884 and Paraíso 20 (P20), respectively (Fig. 1). Statistically, no significant differences were observed in particle size between both samples ($p = 0.446$), therefore it was considered that this parameter could not effect the efficiency of the extraction process.

The approximate composition of moisture-free ACA 884 and P20 ground sunflower seeds used in the experiments is shown in Table 1. Both samples had a similar moisture content ($p = 0.813$). Oil content was statistically different ($p \leqslant 0.01$) between the hybrids, although the values obtained were within the range reported for these hybrids in Argentina (Aguirrezábal and Pereyra, 1998). Reports indicated that black-hull hybrids (P20) usually produce seeds with a higher oil content than striped-hull hybrids (ACA 884) (Izquierdo and Aguirrezábal, 2008).

The samples differed significantly in their content of protein ($p = 0.014$), cellulose ($p = 0,001$), hemicellulose ($p = 0.041$) and lignin ($p = 0.044$). The hybrid ACA 884 had the lowest protein and lignin content, and higher cellulose content. The differences between the samples in oil, protein and fiber content could be attributed to the type of hybrid or the degree of fruit ripening. At the same time, the fiber content is directly associated with the percentage of hulls of the sunflower seeds. The hybrid P20 presented a lower hull/kernel ratio than ACA 884; 21.26% and 34.38% d.b., respectively (de Figueiredo et al., 2011).

Table 1
Chemical composition of two sunflower meal samples.

Parameter (% d.b.)	Hybrid ACA 884	Hybrid Paraiso 20
Moisture	6.66 ± 1.02[a]	6.44 ± 0.61[a]
Oil	43.72 ± 0.15[a]	49.53 ± 1.00[b]
Protein	15.04 ± 0.21[a]	19.74 ± 0.78[b]
Cellulose	31.72 ± 0.39[b]	20.26 ± 0.12[a]
Hemicellulose	3.97 ± 0.63[b]	0.94 ± 0.07[a]
Lignine	4.17 ± 0.01[a]	4.95 ± 0.17[b]

d.b.: dry basis.
Different letters in the same row indicate significant differences according to Tukey's Test ($p \leqslant 0.05$).

Fig. 2 shows the comparative oil yields versus the extraction time for the control samples and the enzymatically treated samples of hybrids ACA 884 and P20. Statistical analysis of variance (ANOVA) of the control data showed significant differences between the samples ($p < 0.0001$) and among times ($p = 0.0001$), but no significant interaction between variables ($p = 0.1143$). ANOVA of the hybrids showed that they had a different behavior from each other. Hybrid P20 presented a significant increase in oil yield over oil extraction time ($p < 0.001$). Tukey's test showed, with an error of less than 5%, that after 60 min there were no significant differences in average oil yield over time (up to 1080 min). Time was not a significant variable ($p = 0.6531$) for hybrid ACA 884.

From the results of these sunflower seed samples, it was observed that oil was extracted at different rates in each hybrid. The analysis of the performance data versus time shows that in the case of ACA 884 the oil was released more easily and remained constant with time, whereas for P20 the extraction rate was slower. This could account for the differences observed in the structure of the chemical composition of both hybrids (Table 1).

SEM image of the endosperm surface of the unextracted hybrids is shown in Figs. 3 and 4. Broken vegetable structures (cells) that contained free oil phase are evident. Typical hexagonal cells of the endosperm of both hybrids, which exhibited variation in size and the number and size of the lipid bodies, were observed. The mean cell diameters estimated were of 41 and 22 μm for ACA 884 and P20, respectively.

The relationship between the content of lipids and proteins provides an approximate idea of the size of the lipid bodies (Tzen et al., 1993). In this particular case, P20 would contain smaller lipid bodies since its lipid:protein ratio was 1.67, whereas for ACA 884 it was 2.02, as it was confirmed in the microphotographs.

In the literature, oil extraction is explained by two mechanisms of mass transfer:the first one is the transfer of the oil from broken

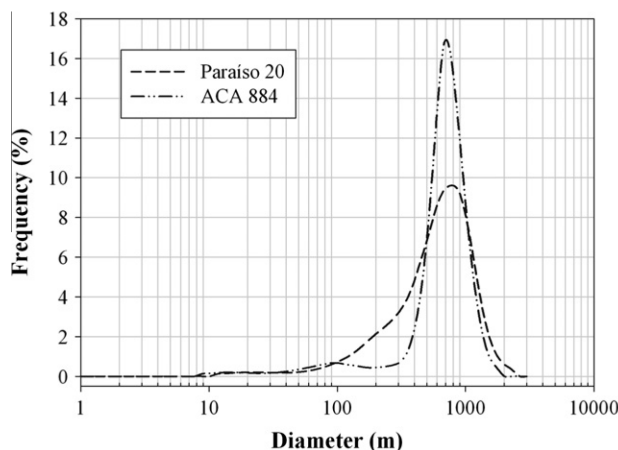

Fig. 1. Particle size distribution of extraction meal of hybrids ACA 884 (– –) and Paraíso 20 (- - -).

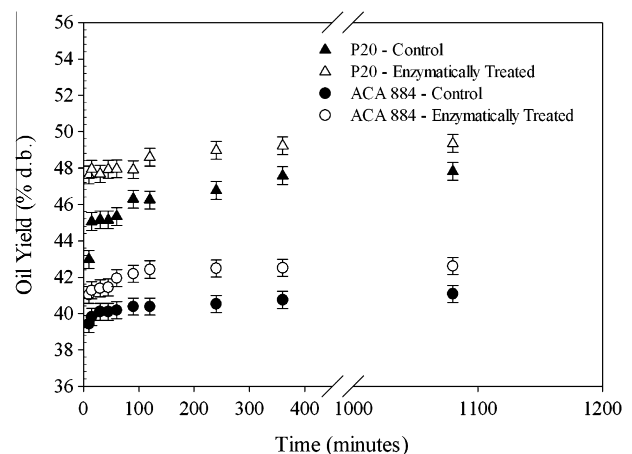

Fig. 2. Oil yield extraction of hybrids ACA 884 and Paraiso 20 (P20).

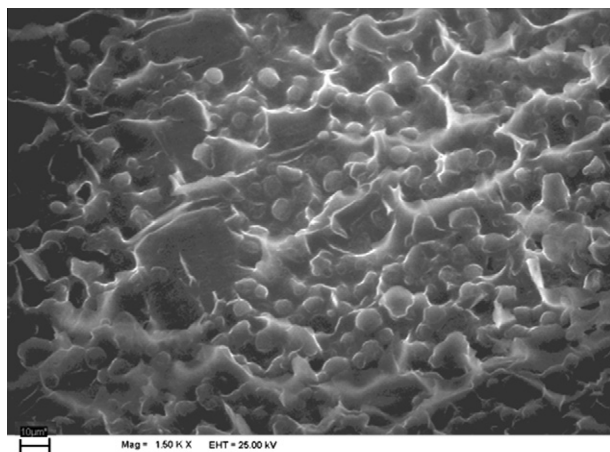

Fig. 3. SEM image of the endosperm section of unextracted striped-hull oilseed hybrid (ACA 884).

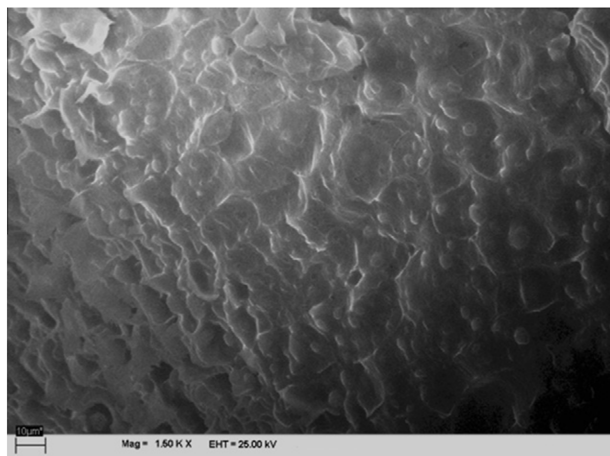

Fig. 4. SEM image of the endosperm section unextracted of black-hull oilseed hybrid (Paraiso 20).

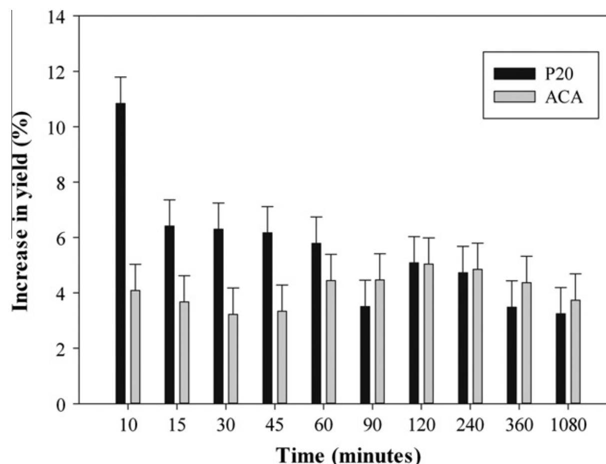

Fig. 5. Percentage increase in oil yield in the extraction time of hybrids ACA 884 and Paraiso 20 (P20).

cells, where the solvent phase controls the extraction process (washing stage); in the second one, the solid phase controls the mass transfer by a diffusion mechanism (Perez et al., 2011; Fernández et al., 2012). In the hybrid ACA 884 it can be observed that the washing step was very fast because the first linear portion of the extraction curve was scarcely present. The grinding pretreatment before extraction on this hybrid, which had larger lipid bodies, could have produced a large number of broken cells on the surface of the particle, being easily removed.

Similarly to the control samples, an increase in oil yield with increasing extraction time was detected in all the tests of the extraction process with enzymatic treatment (Fig. 2). A significant difference in oil yield between samples ($p < 0.0001$) and among extraction times ($p = 0.0176$) was observed, but no significant interaction was found between both variables ($p = 0.9619$). Although the statistical analysis of ACA 884 and P20 showed significant differences in oil yields,there were no differences in yields over extraction time. This proves the effect of the enzyme on the fiber structure, allowing a higher oil release and a quick extraction.

Efficiency of the treatment was measured in terms of the percentage increase in oil yield compared with the control sample (Fig. 5). It was observed that the mean increases were not significantly different over time ($p = 0.9295$) in the case of hybrid ACA 884 (4.12% d.b.). Hybrid P20 showed a significant increase ($p = 0.0001$) at 10 min (10.85% d.b.), but then remained constant over time (4.89% d.b.).

Table 2 shows the content of palmitic ($C_{16:0}$), stearic ($C_{18:0}$), oleic ($C_{18:1}$) and linoleic acids ($C_{18:2}$) in both hybrids. Control samples of ACA 884 and P20 differ significantly in the content of stearic ($p = 0.0062$), oleic ($p = 0.0001$) and linoleic acid ($p = 0.0004$), but not of palmitic acid ($p = 0.2115$). Hybrid P20 presented the highest content of oleic acid. Pectinase did not produce significant differences in the percentage of the four analyzed fatty acids in hybrid ACA 884 ($C_{16:0}$, $p = 0.0829$; $C_{18:0}$, $p = 0.1253$; $C_{18:1}$, $p = 0.8570$; $C_{18:2}$, $p = 0.9999$), whereas its effect on the concentration of stearic and oleic acids in hybrid P20 was significant ($p = 0.002$, $p = 0.0019$, respectively). Sitohy et al. (1993) reported differences in fatty acid composition during the extraction of sunflower oil using different enzymes in aqueous media. They also observed that the oil extracted with pectinase contained relatively high contents of oleic acid.

The ratio of total content of saturated to unsaturated fatty acids increased slightly in oils extracted by enzymes compared to the untreated samples in both hybrids. The same tendency was observed in the fatty acid profile of goldenberry pomace oils obtained using cellulase and pectinase in the enzyme-assisted aqueous extraction (enzyme-assisted aqueous) and the enzyme-solvent extraction (Ramadan et al., 2008; Ramadan and Moersel, 2009).

Total tocopherol content in the oils extracted by Soxhlet was 434 ± 8 ppm (190 μg α-tocopherol g dried meal^{-1}), 557 ± 15 ppm (276 μg α-tocopherol g dried meal^{-1}) for hybrid ACA 884 and P20, respectively. In all samples only the presence of alpha- and beta-tocopherol was identified, and the level of alpha-tocopherol was higher than 97%. Although these concentrations were within the range reported by other authors for crude oil obtained from whole sunflower seeds, they were near the lower values of the range (Nolasco et al., 2004; Izquierdo et al. 2007).

Table 3 shows total tocopherol content for the control and enzyme-treated samples at different extraction times, expressed as μg of total tocopherol extracted at a given time with respect to the dry mass of the sample. The mean values of the tocopherol content in hybrid P20 were found to be much higher than those of the Soxhlet extraction method for all times. Some authors have reported on the sensitivity of the fat-soluble vitamins to the extraction method used, such as tocopherols (vitamin E), due to changes in oxygen, light and pH (Turner et al., 2001; Sabliov et al., 2009). Matthäus and Brühl (2001) observed that temperature increases above 75 °C cause a decrease in the recovery of tocopherols. In the present case, the difference between the batch system and Soxhlet extraction was approximately 20 °C. On the other hand,

Table 2
Average fatty acid composition of oil extracted from the hybrids ACA 884 and Paraíso 20 at different extraction times.

Fatty acid		$C_{16:0}$		$C_{18:0}$		$C_{18:1}$		$C_{18:2}$		S/u ratio* (%)	
Sunflower Hybrid	Time (min)	CS	ETS	CS	ETS	CS	ETS	CS	ETS	CS	ETS
ACA 884	10	6.4^{a}	6.6^{a}	2.9^{a}	2.8^{a}	19.2^{a}	18.9^{a}	71.4^{a}	71.7^{a}	10.3	10.4
	45	6.2^{a}	6.7^{a}	2.6^{a}	2.9^{a}	19.0^{a}	18.6^{a}	72.1^{a}	71.9^{a}	9.6	10.6
	120	6.8^{a}	6.7^{a}	2.8^{a}	2.8^{a}	18.8^{a}	18.6^{a}	71.6^{a}	71.9^{a}	10.6	10.5
	1080	6.2^{a}	6.7^{a}	2.6^{a}	2.8^{a}	19.0^{a}	19.6^{a}	72.2^{a}	71.8^{a}	9.6	10.5
Paraíso 20	10	6.2^{a}	6.2^{a}	2.9^{a}	3.5^{bc}	27.5^{a}	24.1^{b}	63.4^{a}	66.2^{a}	10.0	10.7
	45	6.1^{a}	6.0^{a}	3.0^{ab}	3.5^{bc}	27.5^{a}	23.9^{b}	63.5^{a}	66.6^{a}	10.0	10.5
	120	6.3^{a}	6.4^{a}	2.9^{a}	3.0^{ab}	27.5^{a}	27.7^{a}	63.4^{a}	62.9^{a}	10.1	10.4
	1080	6.3^{a}	6.1^{a}	3.0^{ab}	3.6^{c}	27.5^{a}	24.2^{b}	63.2^{a}	66.2^{a}	10.3	10.7

CS: Control Sample, ETS: Enzymatically-Treated Sample.
Different letters indicate significant differences between treatments for each fatty acid and each hybrid according to Tukey's test ($p \leqslant 0.05$).
* Ratio of saturated fatty acids to unsaturated fatty acids.

Table 3
Total tocopherol content in oil of sunflower hybrids ACA 884 and Paraiso 20 at different times.

Time (min)	Total Tocopherol (μg g dried meal^{-1})			
	ACA 884		Paraiso 20	
	Control sample	Enzymatically treated sample	Control sample	Enzymatically treated sample
10	249 ± 5^{a}	172 ± 7^{b}	559 ± 17^{a}	738 ± 15^{a}
15	228 ± 5^{ab}	169 ± 7^{b}	532 ± 16^{a}	716 ± 18^{a}
30	211 ± 5^{abc}	167 ± 7^{b}	484 ± 15^{ab}	614 ± 13^{c}
60	196 ± 5^{bcd}	164 ± 7^{b}	488 ± 15^{ab}	662 ± 17^{b}
90	187 ± 5^{cde}	195 ± 8^{ab}	465 ± 14^{ab}	657 ± 17^{b}
120	184 ± 5^{cde}	220 ± 11^{a}	448 ± 13^{ab}	601 ± 21^{c}
240	178 ± 5^{cde}	205 ± 9^{a}	385 ± 12^{b}	564 ± 23^{d}
360	165 ± 3^{de}	193 ± 7^{ab}	372 ± 11^{b}	529 ± 23^{d}
1080	153 ± 3^{e}	156 ± 7^{b}	373 ± 11^{b}	435 ± 20^{f}

Means within a column marked with different letters are significantly different ($p \leqslant 0.05$).

the difference in tocopherol content between both hybrids could be attributed to the size and amount of lipid bodies. Fisk et al. (2006) reported that tocopherols are directly associated with the membrane of lipid bodies, their function being to protect the oil and stabilize the cell membrane. As mentioned above, the P20 lipid bodies are smaller and therefore this hybrid has a higher proportion of cell membrane, hence it follows that this hybrid would need to increase the amount of tocopherols to protect and stabilize the oil membrane.

A significant different in the second-order interaction Hybrid $*$ Treatment $*$ Time ($p < 0.001$) was found, indicating that each hybrid behaved differently depending on the treatment and extraction time. It was decided to analyze the effect of each treatment on both hybrids separately at different times using Tukey's test (Table 3).

The influence of the type of treatment on total tocopherol content was also observed in oils obtained from goldenberry pomace. Tocopherol content was higher in oils obtained by enzyme-assisted solvent extraction than by enzyme-aqueous or solvent extraction. A close relationship was found between tocopherols and the total polar lipid content (Ramadan et al., 2008).

High concentrations of total tocopherol occured in the initial stages of both control samples, then a decline in content was observed. This decrease could be explained by the fact that tocopherols are unstable toward air and UV light, decomposing α-tocopherol to its more-stable oxidized form (α-tocopherolquinone) (Sabliov et al., 2009). Tocopherol extraction rate curves from almond oil, obtained by extraction with supercritical carbon dioxide, showed the same trend (Leo et al., 2005). Furthermore, it was reported that tocopherol extraction from ground canola seeds and sunflower pellets occurred mainly in the washing step together with the oil (Fernández et al., 2012; Baümler et al., 2010).

The ACA 884 sample treated with pectinase showed a maximum displacement toward 120 min. This may be attributed to the fact that the release of tocopherols is closely related to the morphology and chemical nature of the matrix and its solubility in oil. The effect of the enzyme on this sample can allow the release of other components that modify the solubility of the oil (Turner et al., 2001).

4. Conclusion

The kinetics study of oil extraction from two sunflower hybrids allowed to determine that the oil was released at different rates depending on their structural characteristics, and that the tocopherol extraction was also affected. Applying the enzymatic treatment effectively produced an increase in oil and tocopherol yield for the black-hull hybrid. Thus the effect of the enzyme was found to be beneficial on the cell structure used, which can be used to improve the performance of traditional processes or obtain the same percentage of oil in shorter extraction times.

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