PERFORMANCE OF GREEN SOLVENTS IN THE EXTRACTION OF SUNFLOWER OIL FROM ENZYME-TREATED COLLETS

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Abstract

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The main goal of this work was to evaluate the extraction of sunflower oil from enzyme-treated collets using ethanol and isopropanol as solvents. The sunflower collets were pretreated with the multienzyme complex Viscozyme® L prior to solvent extraction by the Soxhlet method. The influence of the moisture content of the collets, pretreatment, processing time, and solvent type on the amount of total extracted material and the oil extraction efficiency was studied. Some quality parameters such as phospholipid content of the oil and chlorogenic acid content of the residual meal were also analyzed. At low moisture content (7 %) the solvents exhibited similar oil extraction ability (98-99 %), but with increasing moisture the extraction efficiency of ethanol decreased to about 85 %, while no significant differences were observed for isopropanol. The enzymatic treatment increased the extraction efficiency for all times, specially for ethanol. It was observed that isopropanol was more efficient in the extractioncompared to ethanol, and the amount of nonlipid material was reduced by approximately 70 %. In addition, the oil extracted with isopropanol had a lower phospholipid content and the residual meal presented a higher chlorogenic acid content.

Practical Applications: This work would contribute towards the use of green solvents in the extraction of sunflower oil from collets. Ethanol and Isopropanol, used as solvents, present attractive advantages, including low toxicity, good operational security, as well as being obtained from a renewable source. The obtained data provide up-to-date information on the use of these alcohols in the extraction of sunflower oil from collets and the influence of operating conditions, such as moisture content and enzymatic pretreatment of the collets and the extraction time. Information about oil and meal quality is also reported.

Keywords: Sunflower oil, Collets, Ethanol, Isopropanol, Viscozyme® L.

1. Introduction

Hexane has been used for edible oilseed extraction since the 1930's. It has become the solvent of choice due to some of its attributes, such as simple recovery, non-polar nature, low latent heat of vaporization and high selectivity.[1,2] Although alternative solvents such as ethanol, isopropanol, and water, among others, have been examined since the 1950's, they have been unable to displace hexane. Nowadays, with the growing awareness of environmental protection and increasing restrictions on emissions of volatile organic compounds (VOCs), there are many attempts to find alternative solvents to hexane given its harmful effects on health and environment.[3] These alternative solvents, framed within green chemistry, must not only have certain typical characteristics, such as low toxicity, being easily recyclable, inert and non-polluting, but they should also be obtained from renewable and ecological resources.[2,3]

In the ongoing search for alternatives, the use of ethanol and isopropanol should be considered based on their high availability, bio-renewability and low toxicity.[4] There is data in the literature on the alcoholic extraction of oil from cottonseed [5,6], sunflower seeds and collets [7,8,9] and soybean.[10,11] The use of alcohols allows obtaining better quality oils and meals, since they reduce the levels of free fatty acids in the oils and remove anti-nutritional compounds such as gossypol, aflatoxins and chlorogenic acids, improving the nutritional quality of the meals obtained after extraction.[4] In addition, some of these compounds, as well as chlorogenic acid, can be recovered and used as natural antioxidants, which could be beneficial both from a technological and biological point of view.[12] However, one of the disadvantages of

these alcohols is their low selectivity towards triglycerides, extracting together with the oil a greater amount of sugars, phospholipids, pigments, waxes and other compounds. The lipid solubility is also affected by the water content of the solvent and the extraction temperature, reducing the extraction ability.[13]

Water is considered the greenest of solvents overall, but it is known to be a poor solvent for non-polar or some semi-polar compounds, and its use is restricted by the low oil yield.[14,15] Enzyme-assisted extraction processes have become a promising technological approach to increase oil yield and/or extraction rates. Enzymes provide high selectivity, mild treatment conditions, allow for the recovery of high-quality products from the extraction by-products, and the simultaneous recovery of oil and protein.[15,16] However, these treatments have only been applied to aqueous extractions or as pre-treatments for hexane extraction.[14,17] The enzymatic efficiency will depend on the type of oilseed hybrid and the presentation of the seed (whole or meal). Therefore, it is essential to have knowledge of the structure of the oilseeds and the appropriate operating conditions.[14,17] Several studies on the optimization of the extraction process using response surface methodology, algorithm and other statistical methods have been conducted for different oilseed matrices to determine the operational variables that maximize the process.[18]

While there are studies on the enzyme-assisted aqueous extraction applied as pretreatment to sunflower seeds, and scarce information on the use of alcohols in the oil extraction from sunflower collets, no reports on the combination of both processes could be found in the literature. Therefore, the aim of this work was to study the oil extraction performance from enzyme-treated sunflower collets using ethanol and isopropanol as solvents, and their effect on the quality of the oils and meals obtained.

2. Materials and methods

2.1. Characterization of raw material, oil and meal

Sunflower collets (porous cylinders obtained from pressed sunflower cake by expanding) were kindly donated by a local company. They were stored in polyethylene containers with screw caps in the dark and refrigerated at 4 ºC until submitted to the extraction process.

IUPAC methods 1.121 and 1.122 [19] were used to determine initial moisture and oil content, respectively. Protein content (N x 6.5 factor) was measured according to standard AOCS official methods.[20] Acid-detergent fiber (ADF), neutral-detergent fiber (NDF) and lignin were determined by the sequential method, using alpha-amylase and without sodium sulphite, in an Ankom analyzer (Fairpoint, NY, USA).[21] Minor components in the oils such as tocopherols and phospholipids were determined. The tocopherol content was measured using a Waters 600 HPLC system with a fluorescence detector (Waters Associates, Milford, MA, USA) and α-tocopherol (Sigma Chemical Co, St. Louis, MO, USA) as external standard, according to AOCS Ce8-89 method.[20] The phospholipids were concentrated using diol solid-phase extraction cartridges (J.T. Baker Inc, Phillipsburg, NJ, USA). They were quantified by the external standard method in HPLC with a UV detector (Waters Associates, Milford, MA, USA), according to the methodology described by A. A. Carelli, M. V. Brevedan, G. H. Crapiste [22], using reference standards of L-α phosphatidylethanolamine (PE), L-α phosphatidylinositol (PI), L-α phosphatidylcholine (PC) and phosphatidic acid (PA) (Sigma Chemical Co, St. Louis, MO, USA).

Total phenolic content in the residual meals was also determined. The phenolic components were extracted by successive washings with ethanol:water (80:20) and mechanical stirring for 30 min. The supernatants were combined and the ethanol was evaporated. The volume of the aqueous phase was adjusted with water and the quantification was carried out by spectrophotometric method (λ= 760 nm).[23] The quantification assay was based on the Folin-Ciocalteu using chlorogenic acid as standard, and expressed as mg of chlorogenic acid by g of meal.[24,25]

2.2. Moisture conditioning prior to alcoholic extraction

The sunflower collets were conditioned at 7, 12, 25, 40 and 65 % (d.b.) moisture contents by spraying them with precalculated quantities of distilled water. Each sample was mixed and then stored in closed containers in the refrigerator at 4 °C for at least 48 h to allow for a homogeneous moisture distribution. The moisture content was determined with an infrared OHAUS analytical balance (model MB 45) using a temperature of 105 °C. Moisture analysis was performed in triplicate to estimate the inherent variability of the measurement. Each sample was taken out of the refrigerator and allowed to stand at room temperature for approximately 2 h before extraction.

2.3. Enzymatic pretreatment

A multienzyme complex produced from a selected strain of *Aspergillus Aculeatus* (VISCOZYME® L, Novozymes) containing a wide range of carbohydrases was used. The unit of enzyme activity was 112 FBGU g^{-1} (beta-glucanase units). The optimal

temperature and pH ranges recommended by the supplier are 45-55 ºC and 3.3-5.8, respectively.

The enzymatic pretreatment was performed in jacketed agitated vessels using an impeller with a centrally-located stirrer shaft. The collets were suspended in citrate buffer (0.1 M), pH= 5, with a 10:1 (mL/g) buffer-to-collet ratio. Each experiment was carried out at 249 rpm, 1.72 % enzyme to collets ratio, at 42 °C for 52 min. These conditions were obtained from an enzymatic aqueous extraction study conducted at laboratory scale. Response surface methodology (RSM) based on a central composite design was used to obtain the optimal oil yield and also a protein-rich meal after extraction.[18]

After the pretreatment, the suspension was separated by vacuum filtration using a Whatman N^{o} 4 filter paper to separate the solid and liquid phases. The solid phase was collected and refrigerated at 4 °C until further use. This solid material will be called hereafter pretreated collets.

2.4. Extraction procedure

2.4.1. Alcoholic extraction

Extraction experiments were carried out with untreated and pretreated collets. The extractions were performed in a Soxhlet equipment using ethanol (96 % m/v azeotropic composition) and isopropanol (98 %, analytical grade) at the boiling temperature of the solvent for 1, 2, 3, 4 and 6 h. After the preset time, the solvents were distilled off under vacuum using a rotary evaporator (Büchi Laboretechnik AG, Flawil, Switzerland) at 50 °C, until reducing the volume by 90%.

2.4.2. Phase separation

The concentrated miscella (solvent-extracted material) was transferred to a Falcon tube and *n*-hexane was added. Then it was stirred and centrifuged for 15 min at 1600*xg* (3000 rpm). The phase boundary was observed with the naked eye since the two phases were clearly separated: The upper phase was the hexane-soluble fraction, while the lower phase was rich in nonlipid material. The upper phase was removed with a Pasteur pipette. The extraction stages and separation phases are shown in Fig. 1. This procedure was repeated several times and the aliquots were collected. Then the solvents in both phases were removed with a stream of nitrogen to constant weight.

The hexane-soluble fraction consisted of the lipid material, and the hexane-insoluble fraction consisted of the nonlipid material (remnant) including polyphenols, pigments, soluble sugar, soluble proteins, etc. Thus, from the amount of hexane-soluble (m_o) and alcohol-soluble components (m_r), the amount of total extracted material (M_T) was calculated as:

$$M_T = m_o + m_r \quad (1)$$

The oil extraction efficiency (E_o), defined as the amount of hexane-soluble components (hereafter called oil) obtained by alcoholic extraction (m_o) with respect to the initial oil content of untreated and treated sunflower collets (m_i), according to the case, can be expressed as:

$$E_o = \frac{m_o}{m_i} * 100 \quad (2)$$

Thus, the nonlipid fraction W_r with respect to the amount of total extracted material can be expressed as follows:

$$W_r = \frac{mass\ of\ nonlipids\ in\ the\ total\ extracted\ material}{mass\ of\ total\ extracted\ material} * 100 \quad (3)$$

2.5. Statistical analysis

The statistical analysis was carried out by ANOVA using the Infostat software.[26] Tukey's test was used to compare the treatments with a significance level of $p \leq 0.10$.

3. Results and discussion

3.1. Characterization of the raw material and pretreated collets

The chemical composition of the collets, expressed as percentages on dry basis (d.b.), were: moisture 7.03 ± 0.10 %, oil 22.95 ± 0.08 %, crude protein (N x 6.25) 40.92 ± 0.37 %, neutral detergent fiber (NDF) 38.03 ± 0.66 %, acid detergent fiber (ADF) 26.39 ± 0.21 %, and lignin 6.02 ± 0.16 %. The obtained values were within the range reported for these raw materials in Argentina.[8,27,28]

The oil and protein content of the pretreated collets was also quantified, with values of 19.74 ± 0.19 % (d.b.) and 38.70 ± 0.60 % (d.b.), respectively. Approximately 14 % of the oil and 5 % of the soluble proteins was extracted during the enzymatic pretreatment.

3.2. Effect of moisture content

The amount of total extracted material (M_T) and the oil extraction efficiency (E_o) at different moisture contents using *n*-hexane, ethanol and isopropanol (IPA) as solvents after 6 h of extraction are shown in Fig. 2 and 3, respectively.

M_T values obtained with n-hexane were lower than those obtained with alcohols over the studied moisture range (Fig. 2). The variations in the total extracted material indicate that more components were extracted with the alcohols due to their low selectivity to oil. Alcohols tend to extract other compounds together with the triglycerides, such as phospholipids, polyphenols, pigments and soluble sugars.[6,7,29] The nonlipid fraction did not change significantly with moisture content in the case of isopropanol, but it increased with moisture for ethanol.

As for oil extraction efficiency, it varied with moisture content and the solvent (Fig. 3). At the initial moisture content of the collets (7 %), n-hexane, ethanol and isopropanol exhibited similar oil extraction ability (99.99 ± 0.01 %, 98.55 ± 0.64 % and 99.52 ± 0.07 %, respectively). Oil extraction with hexane decreased with higher moisture values (> 25%); extraction with ethanol decreased to about 85 % at 25-40 % moisture and then remained approximately constant, while no significant differences with moisture were observed for IPA ($p = 0.1150$).

These results can be explained in terms of the effect of moisture on oil solubility and the solvent-solid structure interaction. The water sorption isotherm of sunflower meals and collets can be divided into three regions.[28,30] At low moisture, in the monolayer region, water is strongly retained by hydrophilic bonds on the polar sites in the solid matrix (mainly carbohydrates and proteins), so it practically does not interact with the solvent. Monolayer values have been reported in the 4-5.7 % d.b range for sunflower meals and collets.[28,30] In the intermediate region, water is retained mainly by adsorption in multilayers with weaker bonds in the microcapillaries and the fibrous structure of the solid. Adsorbed water produces the expansion and disruption of the structure, enabling the adsorption and diffusion of the solvent. At high moisture, most of the condensed water is mechanically retained

in the empty spaces and macrocapillaries of the solid and has a similar behavior to free water. The free water molecules could interfere with the penetration of the solvent when it is immiscible as hexane, reducing the extraction rates and the oil yield. Some authors studied the effect of the moisture content of the collets on oil quality and oil yield with hexane extraction, obtaining at moisture contents of 12.40 and 18.92 % d.b. similar yields to those found in the present work, and slightly higher at 5.65% d.b.[28] Fig. 3 shows that this effect increases significantly at higher moisture contents (>25%).

In the case of water-miscible solvents such as alcohols, with high content of free water, there is a transfer of moisture from the solid to the solvent that can change the composition, and consequently, the properties of the solvent. The solubility of oil in ethanol is strongly affected by temperature and water content. When the alcohol concentration is reduced, the solubility of the oil decreases sharply because the polarity of the solvent increases, and the extraction of other components soluble in polar solvents also increases.[4,13] E. R. Baümler, M. E. Carrín, A. A. Carelli[8] reported that at low moisture content, the ethanolic extraction of sunflower oil has a higher final performance but a lower rate compared to hexane. Other authors obtained an efficiency of 98 %, 90 % and 86 % for pure solvent and ethanol:water ratios of 90:10 and 80:20, respectively, in the Soxhlet extraction of soybean oil for 6 h.[32] The oil yield was similar (98-99.5 %) after 10 h of extraction, suggesting that moisture affected mostly the extraction rate. At temperatures above 70 ºC, soybean oil is miscible in all proportions with near absolute ethanol, and soybean flakes are dried to 3-5 % moisture to prevent the consequential loss of oil solubility.[33] Isopropanol is much more tolerant to the moisture content of the flakes than ethanol; flakes with 7 % moisture were in equilibrium with isopropanol azeotrope.[33] The

solubility of soybean oil in IPA is significantly higher than in ethanol, being miscible at temperatures higher than 50 °C.[32] Also the increase in solvent hydration from absolute alcohols to the azeotropic mixtures negatively affects the oil extraction yield for both ethanol and IPA at different temperatures.[34]

The enzymatic treatment changes the solid matrix of the collects by disrupting the cell structures, favoring the diffusion of the solvent and the miscella, the accessibility to the lipid bodies and the release of other cell components. In addition, the material obtained after the enzymatic treatment presented a moisture content of 48-65 % d.b., which would require the incorporation of an intermediate drying stage to condition the moisture of the solid. The obtained results show that there was no significant difference in oil extraction efficiency at relatively high moisture between both solvents (Fig. 3). For this reason, the enzymatically-treated collets were not dried before the alcoholic extraction.

3.3. Alcoholic extraction

The extractive capacity of alcohols at different times for both the untreated (control) and enzyme-treated collets was determined in order to study the simultaneous effect of the enzymatic treatment and solvents. The average values of total extracted material (M_T) obtained during alcoholic extraction are given in Fig. 4. ANOVA presented significant differences between the control and the treated samples at each extraction time ($p < 0.008$) for both solvents using Tukey's test, with the control samples presenting a significant increase in M_T.

Table 1 shows the oil extraction efficiency by alcoholic extraction for the control and treated collets after the separation of the fractions. The statistical analysis showed significant differences in extraction efficiency between the samples and between

extraction times for both alcohols ($p < 0.10$). From the results, it follows that the extraction efficiency of isopropanol was greater than that of ethanol in the unpretreated samples. Some authors have reported that ethanol exhibits a lower performance than solvents of intermediate polarity such as isopropanol. This can be explained by the dielectric constant (a measure of molecular polarity), since ethanol and isopropanol present a dielectric constant value of 22.29 and 17.30, respectively.[34] The performance obtained with isopropanol may also be due to its effect of opening the cell walls to allow more thorough solvent extraction of the cell contents, as well as some specific interaction involving hydrogen bonding with the ester groups of the triglycerides.[35]

Due to the significant time-solvent ($p < 0.0001$) and treatment-solvent ($p = 0.0002$) interactions found, the effect of each treatment on both solvents was analyzed separately at different times using Tukey's test. Oil extraction increased with time independently of the solvent and the treatment. The ethanolic extraction efficiency for the control and the treated samples varied in the first 3 h ($p < 0.001$), and then achieved asymptotically the maximum extraction efficiency in the 80-84% range. The treated samples presented a significantly higher (>20%) E_o than the control samples for all times, with a maximum of 98.7 %. These results could be explained in terms of the enzymatic action, since enzymes can break the cell structure, allowing a higher oil release and a quick extraction.[17,33,36] The extraction with IPA presented a similar behavior to that with ethanol, but the effects were less important because of the higher extraction rates and yield. The statistical analysis showed significant differences ($p < 0.001$) in extraction efficiency for the control samples at all times, achieving a maximum of 98.6 % at 6 h. On the other hand, significant differences in E_o were observed for the treated collets at between 1 and 3 h of extraction ($p >$

0.093), but no significant differences ($p > 0.13$) were observed at longer times, obtaining a maximum of 99.2 %. Treated samples showed a slightly higher extraction efficiency than control, but the differences were not statistically significant at most times. The improvement achieved with the enzymatic treatment in the case of IPA was relatively low, in the order of 5%, because the untreated sample also showed high extraction efficiency.

Previous studies have also explored the effects of alcohol type on the oil extraction yield from other oilseeds. A study on the solvent extraction from sesame seeds (for 12 h of extraction) reported a similar efficiency for isopropanol and hexane, while the efficiency of ethanol was lower than the results to that reported in this work.[35] Other authors evaluated the extraction of jojoba oil with isopropanol, obtaining an efficiency of 86 % (for 18 h of extraction).[37] The use of IPA and ethanol as solvents in the extraction of rapeseed oil has also been compared (6 h of extraction), obtaining an efficiency of 83.1 % and 22.8 %, respectively.[38] The alcoholic extraction of corn germ-bran oil, rice bran oil and sesame seed oil, also were studied by several authors, obtaining extraction yields that increased with temperature in the 80-97 % range depending on the material, with the highest values being observed for IPA.[4,34,39] These values are lower than those found in the present work for both alcohols, but the authors used a different extraction method (batch extraction in a single stage). On the other hand, high efficiencies (99 % with ethanol and 99.5 % with IPA) for the soybean oil extraction with Soxhlet for 10 h, using absolute alcohols and their azeotropic mixtures with water were obtained in other works.[32]

As mentioned above, due to the lower selectivity of isopropanol and ethanol towards lipids, other compounds such as phosphatides, polyphenols, pigments, and soluble sugars are also obtained during extraction. Table 2 shows the nonlipid material

obtained after the separation of the fractions. No significant solvent-time (p = 0.9907) and time-treatment (p = 0.8588) interactions were observed according to ANOVA. Ethanol extracted a higher amount of nonlipid material than IPA, with a significant difference between control and the treated collets for both alcohols at all times. The nonlipid fraction extracted from the treated collets was considerably lower compared to the control (up to 50 % with ethanol and up to 20-35 % with IPA). This can be due to the enzymatic treatment, since some nonlipid water-soluble components such as proteins and sugars are separated during the process.[18] Other authors have also observed low selectivity in the extraction with ethanol, performing a separation of the total extracted material. E. R. Baümler, M. E. Carrín, A. A. Carelli[8] studied the ethanolic extraction of oil from sunflower collets, reporting that the material extracted with ethanol contained 31 % of hexane-insoluble compounds or nonlipid fraction, in agreement with the data presented in this work for the control samples. R. J. Sánchez, M. B. Fernández, S. M. Nolasco[40] examined the separation of lipid and nonlipid material in the ethanolic extraction from canola seeds, reporting no significant effect of the microwave pretreatment on the nonlipid fraction (about 10%) and oil yields.

3.4. Minor Components

The content of minor components in the oils extracted by different methods is shown in Table 3. The tocopherol and phospholipid contents in the oil extracted from the untreated collets was 510 ± 18 ppm and 4.62 ± 0.20 g/kg, respectively.

The enzymatic treatment reduced the total tocopherol content by 19 %, but the profile (predominantly α-tocopherol and smaller percentages of β-tocopherol and β-tocotrienol) remained relatively stable.[18] E. E. Pérez, M. B. Fernández, S. M.

Nolasco, G. H. Crapiste[17] observed a similar trend for total tocopherols of enzymatically-treated sunflower samples, presenting some degradation that can be attributed to oxygen, light, pH and temperature effects. In the extraction with IPA, total tocopherol content decreased by 24 % (Table 3), whereas in the oil extracted with ethanol total tocopherol was 6 % higher than in the enzymatically treated samples. This result could be due to the polarity of the solvent, as some authors consider that polar solvents extract more tocopherols than non-polar solvents.[35] However, E. R. Baümler, M. E. Carrín, A. A. Carelli[8] found no significant differences in tocopherol content between ethanolic and n-hexane extractions.

The enzymatic treatment reduces the total phospholipid content and changes its profile, as some hydratable phosphatides can be removed.[18] A significant reduction (55 %) in the total phospholipid content was observed. The phospholipid distribution also changed, mainly the percentage of PI and to a lesser extent of PA (Table 3). This could be attributed to the fact that PI exists as a complex with potassium or magnesium, and due to its hydrophilic inositol group it can be fully hydratable.[41] On the other hand, PA is non-hydratable, but it exists as a partially dissociated acid that could combine with a monovalent metal ion forming a complex that is hydratable; therefore part of the PA could be complexed and extracted in the aqueous phase during the enzymatic treatment.

The oils obtained by alcoholic extraction presented a higher total phospholipid content than the oils from the untreated and treated sunflower collets. Ethanol removes more polar compounds, such as phospholipids, than IPA. While the ethanolic extraction increased the concentration of the four phospholipids in the oil, the extraction with IPA increased mainly the amount of PC (Table 3). These results show the same trend as those reported in bibliography, for samples of rice bran oil

extracted with ethanol and isopropanol and for rapeseed oil.[34,42] They could be explained by the solvent's polarity, as polar lipids are bound by electrostatic forces and hydrogen bridges and require polar solvents for breaking such bonds and releasing them.[43] Ethanol presents a higher dielectric constant than IPA [34,35] and removes more polar lipids, including those released from the cell structure due to the enzymatic treatment.

3.5. Total phenolic content

The residual meals were characterized according to the content of chlorogenic acid. Statistical analysis showed significant differences between the samples ($p<0.0002$). The control presented a chlorogenic acid content of 25.64 ± 0.89 mg/g meal, similar to that reported by other authors.[12,25] The aqueous-enzymatic treatment reduced the phenolic content by 60 % (enzyme-treated, 9.66 ± 0.07 mg/g meal). H. Dominguez, M. Nunez, J. Lema[44] reported a similar behavior for sunflower seeds, with a reduction of chlorogenic acid of 88 % through an aqueous process. The ethanolic extraction from treated collets reduced the chlorogenic acid content to 1.61 ± 0.34 %, representing a 94 % decrease. Taking into account that a fraction of the meal is lost during the extraction process (nonlipid fraction), the chlorogenic acid content does not change significantly in the extraction with isopropanol (14.10 ± 2.26 mg/g meal), which may be due to the polarity of the solvent and its hydration. N. K. Scharlack, K. K. Aracava C. E. C. Rodrigues[45] reported a decrease in chlorogenic acid after the alcoholic extraction from sunflower seed press cake, with a reduction of 75 % and 69 % with ethanol and isopropanol (azeotropes), respectively. Although other authors have also studied the removal of chlorogenic acid from organic solvents [46,47], in those cases the extraction process was carried out from defatted meals but without pre-treatments.

4. Conclusions

In this work the extraction of sunflower oil from enzyme-treated collets using ethanol and isopropanol as solvents was evaluated. The influence of the moisture content of the collets, time, and solvent type was analyzed. Based on the results, both alcohols could be used to extract oil from treated collets, even with high aqueous content (48-65% d.b). IPA exhibited a higher oil extraction efficiency than ethanol for different extraction times, reaching maximum extraction (96%) at 3 hours, and it was not significantly affected by the moisture of the collets. Even though both alcohols presented low selectivity by extracting other polar compounds together with the oil, the nonlipid fraction was higher in the case of ethanol. The treated samples presented less nonlipid compounds than the control, with a decrease of 70 % in the case of IPA. In addition, extraction with IPA showed some better quality characteristics: the oil had a lower phospholipid content (3.53 ± 0.09 g/kg oil) and the residual meal had a lower chlorogenic acid content (14.10 ± 2.26 mg/g meal). Thus, the alcoholic extraction, particularly with IPA, with an enzymatic pretreatment could be an alternative process for the sunflower oil production, as restrictions on the use of hexane may be implemented.

Abbreviations

VOCs, volatile organic compounds; ADF, acid-detergent fiber; NDF, neutral-detergent fiber; PE, phosphatidylethanolamine; PI, L-α phosphatidylinositol; PC, L-α phosphatidylcholine; PA, phosphatidic acid; m_o, amount of hexane-soluble components; m_r amount of alcohol-soluble components; M_T, amount of total material

extracted; E_o, oil extraction efficiency; m_i, initial oil content of untreated and treated sunflower collets; W_r, nonlipid fraction.

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Conflict of Interest statement

The authors have declared no conflict of interest.

Data Availability Statement

Data derived from public domain resources. The data that support the findings of this study are available in Repositorio Institucional CONICET Digital at https://ri.conicet.gov.ar/handle/11336/83401.

References

[1] S. J. Kumar, S. R. Prasad, R. Banerjee, D. K. Agarwal, K. S. Kulkarni, K. V. Ramesh, *Chem. Cent. J*. **2017**, *11*, 9.

[2] M. M. Cascant, C. Breil, S. Garrigues, M. de la Guardia, A. S. Fabiano-Tixier, F. Chemat, *Anal. Bioanal. Chem*. **2017,** *409,* 3527.

[3] S. Ferreira-Dias, D. G. Valente, J. M. Abreu, *Grasas y Aceites.* **2003,** *54*, 378.

[4] S. L. Navarro, M. C. Capellini, K. K. Aracava, C. E. Rodrigues, *Food Bioprod. Process.* **2016,** 100, 185.

[5] G. Abraham, R Hron, S Koltun, *J. Am. Oil Chem. Soc.* **1988**, *65*, 129.

[6] R, Hron, M. Kuk, G. Abraham, P. Wan, *J. Am. Oil Chem. Soc.* **1994**, 71, 417.

[7] J. Sineiro, H. Domínguez, M. Núñez, J. Lema, *J. Am. Oil Chem. Soc.* **1996**, 73, 1121.

[8] E. R. Baümler, M. E. Carrín, A. A. Carelli, *J. Food Eng.* **2016**, *178*, 190.

[9] J. A. Gallegos-Infante, N. E. Rocha-Guzmán, R. F. González-Laredo, D. Gomez-Sánchez, F. Zuno-Floriano, S. A: Vidaña-Martínez, *Journal of Food Quality, **2007**, 30*, 413.

[10] E. Baker, D. Sullivan, *J. Am. Oil Chem. Soc.* **1983,** *60*, 1271.

[11] H. Rittner, *Oleagineux (France).* **1992**, *29.*

[12] G. M. Weisz, D. R. Kammerer, R. Carle, *Food Chem.* **2009**, *115,* 758.

[13] T. A. Toda, M. M. Sawada, C. E. Rodrigues. *Food Bioprod. Process.* **2016** *98*, 1.

[14] M. F. Ramadan, J. T. Moersel, *Int. J. Food. Sci. Technol*. **2009,** *44,* 435.

[15] A. Rosenthal, D. L. Pyle, K. Niranjan, *Enzyme Microb. Technol.* **1996**, *19*, 402.

[16] E. Danso-Boateng, *World Acad. Sci. Eng. Technol*. **2011,** *5*.

[17] E. E. Pérez, M. B. Fernández, S. M. Nolasco, G. H. Crapiste, *J. Food Eng.* **2013**, 117, 393.

[18] L. M. Rodriguez, Doctor's Thesis, *Department of Chemical Engineering. National University of the South*. **2019**.

[19] Paquot, A. Hautfenne, *Standard Methods for the Analysis of Oils, Fat and Derivates*. Blackwell, Oxford Pergamon Press, Oxford, UK **1992**.

[20] D. Firestone, *Official Methods and Recommended Practices of the American Oil Chemists' Society*. AOCS Press, Champaign, Illinois, USA **2009**.

[21] P. J. Van Soest, J. B. Robertson, B. A. Lewis, *J. Dairy Sci.* **1991**, 74, 3583.

[22] A. A. Carelli, M. V. Brevedan, G. H. Crapiste, *J. Am. Oil Chem. Soc.* **1997**, 74, 511.

[23] M. M. Pedrosa, M. Muzquiz, C. García-Vallejo, C. Burbano, C. Cuadrado, G. Ayet, L. M. Robredo, *J. Sci. Food Agric.* **2000,** 80, 459.

[24] V. L. Singleton, J. A. Rossi, *Am. Soc. Enol. Viticulture.* **1965**, 16, 144.

[25] P. Salgado, Doctor's Thesis, *Faculty of Exact Sciences. National University of La Plata,* **2009**.

[26] J. A. Di Rienzo, F. Casanoves, M. G. Balzarini, L. Gonzalez, M. Tablada, C. W. Robledo, InfoStat software versión. *Grupo InfoStat, FCA*, Universidad Nacional de Córdoba, Argentina, **2017**.

[27] E. R. Baümler, G. H. Crapiste, A. A. Carelli, *J. Am. Oil Chem. Soc. **2010**, 87*, 1489.

[28] E. E. Pérez, E. R. Baümler, G. H. Crapiste, A. A. Carelli, *Eur. J. Lipid Sci. Technol.* **2018**, 121, 1.

[29] R. Hron Sr, S. Koltun, A. Graci Jr, *J. Am. Oil Chem. Soc*. **1982**, 59, 674.

[30] D. A. Cardarelli, M. A. Mattea, G. H. Crapiste, *J. Am. Oil Chem. Soc.* **2009**, 77, 587.

[31] L. A. Johnson, E. Lusas, *J. Am. Oil Chem. Soc.* **1983**, 60, 229.

[32] A. Gandhi, K. Joshi, K. Jha, V. Parihar, D. Srivastav, P. Raghunadh, J. Kawalkar, S. Jain, R. Tripathi, *Int. J. Food Sci. Technol.* **2003**. *38,* 369.

[33] L. A. Johnson, *Technology and Solvents for Extracting Oilseeds and Nonpetroleum Oils*. Eds: P.J. Wan, P.J. Wakelyn. AOCS PRESS, Champaign, Illinois. **1997**, Ch. 1.

[34] M.C. Capellini, V. Giacomini, M. S. Cuevas, C. E. C. Rodrigues, *Ind. Crops. Prod.* **2017**, *104*, 133.

[35] R.Tir, P. C. Dutta, A. Y. Badjah-Hadj-Ahmed, *Eur. J. Lipid Sci. Technol.* ***2012,*** 114, 1427.

[36] M. Mat Yusoff, M. H. Gordon, O. Ezeh, K. Niranjan*, Food Chem.* **2016,** 211, 400.

[37] M. Abu-Arabi, M. Allawzi, H. Al-Zoubi, A. Tamimi, *Chem. Eng. J.* **2000**, 76, 61.

[38] Y. Li, F. Fine, A-S. Fabiano-Tixier, M. Abert-Vian, P. Carre, X. Pages, F. Chemat, *C. R. Chim.* **2014**, 17,242.

[39] M.C. Capellini, L. Chiavoloni, V. Giacomini, C. E. C. Rodrigues, *J. Food Eng.* **2019,** *240*, 145.

[40] R. J. Sánchez, M. B. Fernández, S. M. Nolasco, *Eur. J. Lipid Sci. Technol.* **2018**, 120, 1.

[41] D. L. Lamas, G. H. Crapiste, D. T. Constenla, *LWT-Food Sci. Technol.* **2014**, 58, 71.

[42] M. Citeau, S. Albe Slabi, F. Joffre,P. Carré, OCL. **2018**, 25, D207.

[43] A. S. Brum, L. F. D. Arruda, *Quim. Nova*. **2009**, 32, 849.

[44] H. Dominguez, M. Nunez, J. Lema, *Food Chem.* **1994,** *49*, 271.

[45] N. K. Scharlack, K. K. Aracava C. E. C. Rodrigues, *J. Sci. Food Agric.* **2017**, 97, 4612.

[46] M. Rossi, C. Peri, M. Riva, *Riv. Ital. Sostanze Grasse.* **1980**. 57, 509.

[47] S. González-Perez, K. B. Merck, J. M. Vereijken, G. A. Van Koningsveld, H. Gruppen, A. G. J. Voragen, *J. Agric. Food Chem*. **2002**, 50,1713.

Table 1: Extraction efficiency (E_o) using ethanol and isopropanol as solvents at different times.

	Ethanol		Isopropanol	
Time (h)	Untreated	Pretreated	Untreated	Pretreated
1	38.02 ± 0.30$^{\mathbf{aA}}$	46.75 ± 2.48$^{\mathbf{aB}}$	74.76 ± 0.53$^{\mathbf{aA}}$	78.98 ± 4.63$^{\mathbf{aA}}$
2	61.69 ± 2.39$^{\mathbf{bA}}$	77.98 ± 2.69$^{\mathbf{bB}}$	83.46 ± 2.49$^{\mathbf{bA}}$	87.83 ± 2.64$^{\mathbf{bA}}$
3	79.89 ± 1.26$^{\mathbf{cA}}$	95.21 ± 2.54$^{\mathbf{cB}}$	85.80 ± 1.78$^{\mathbf{bA}}$	95.97 ± 0.79$^{\mathbf{bcB}}$
4	83.88 ± 2.66$^{\mathbf{cA}}$	96.07 ± 0.57$^{\mathbf{cB}}$	92.11 ± 1.24$^{\mathbf{cA}}$	96.23 ± 1.66$^{\mathbf{cA}}$
6	80.43 ± 2.81$^{\mathbf{cA}}$	98.67 ± 0.50$^{\mathbf{cB}}$	98.65 ± 0.43$^{\mathbf{dA}}$	99.22 ± 0.44$^{\mathbf{cA}}$

Data are mean values ± standard error.

Means within a column marked with different lowercase letters (effect of time) are significantly different ($p<0.10$) according to Tukey's test.

Means within a row marked with different uppercase letters (effect of treatment for each solvent) are significantly different ($p<0.10$) according to Tukey's test.

Table 2: Nonlipid fraction (W_o) extracted using ethanol and isopropanol as solvents at different extraction times.

	Ethanol		Isopropanol	
Time (h)	Untreated	Pretreated	Untreated	Pretreated
1	51.46 ± 3.05[a]	24.64 ± 0.29[b]	19.89 ± 1.30[a]	15.11 ± 0.72[b]
2	41.04 ± 0.94[a]	22.01 ± 0.01[b]	20.41 ± 0.03[a]	13.47 ± 0.56[b]
3	33.56 ± 0.88[a]	15.37 ± 2.46[b]	24.29 ± 0.38[a]	17.47 ± 1.13[b]
4	40.41 ± 1.84[a]	19.58 ± 1.29[b]	19.55 ± 4.97[a]	11.99 ± 2.76[b]
6	39.77 ± 2.02[a]	21.06 ± 1.43[b]	24.81 ± 0.97[a]	20.99 ± 0.75[b]

Data are mean values ± standard error. Means in the same row followed by a different letters (effect of treatment for each solvent) are significantly different ($p>0.10$) by Tukey's test method.

Table 3: Total content of tocopherols and phospholipids in oils obtained under different extraction conditions.

	Untreated + Hexane	Enzyme-treated + Hexane	Enzyme-treated + Ethanol	Enzyme-treated + IPA
Tocopherols (ppm)	510 ± 18^{a}	414 ± 13^{b}	440 ± 9^{c}	313 ± 10^{d}
Phospholipids (g/kg oil)	4.62 ± 0.20^{a}	2.08 ± 0.05^{b}	16.29 ± 0.93^{c}	3.53 ± 0.09^{ab}
PC (%)	55.84 ± 1.31^{a}	61.55 ± 0.72^{b}	45.77 ± 0.05^{c}	78.62 ± 1.69^{d}
PI (%)	30.83 ± 1.19^{a}	24.01 ± 0.69^{b}	42.14 ± 0.18^{c}	17.37 ± 2.22^{d}
PE (%)	6.56 ± 0.10^{a}	6.36 ± 0.04^{a}	7.60 ± 0.08^{b}	3.58 ± 0.32^{c}
PA (%)	6.77 ± 0.23^{a}	8.08 ± 0.02^{b}	4.48 ± 0.05^{c}	4.24 ± 1.15^{c}

Data are mean values ± standard error. Different letters in the same row indicate significant differences according to Tukey's Test ($p<0.05$).

Figure Captions

Fig. 1: Schematic diagram of the alcoholic extraction and phase separation

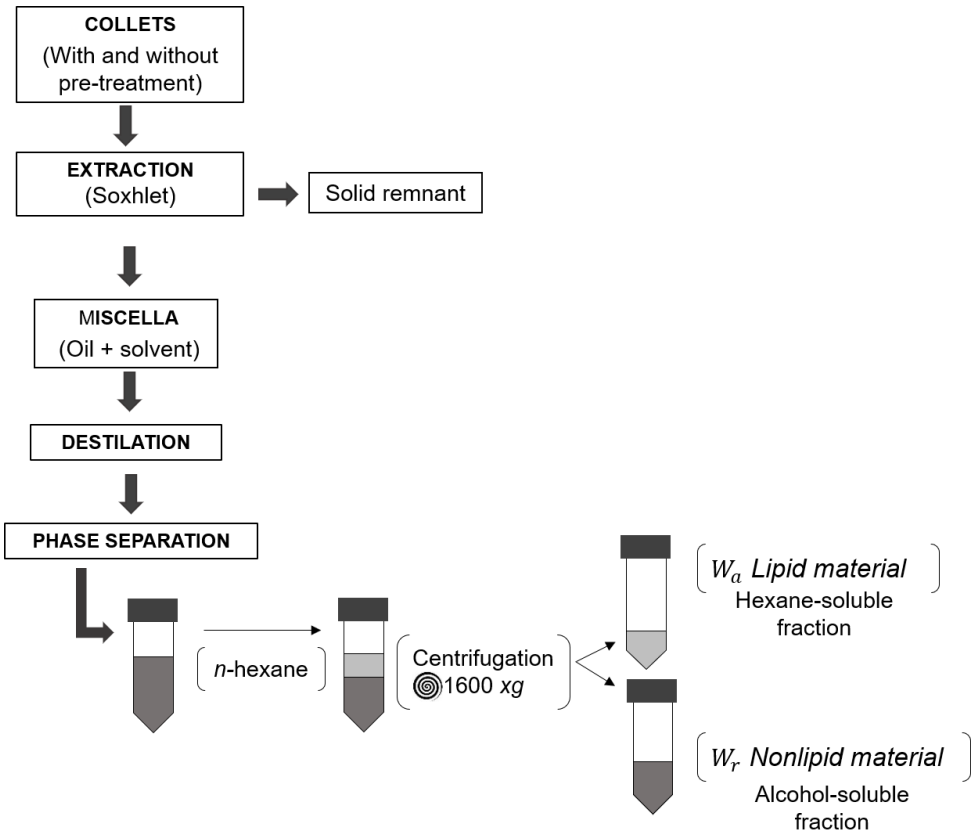
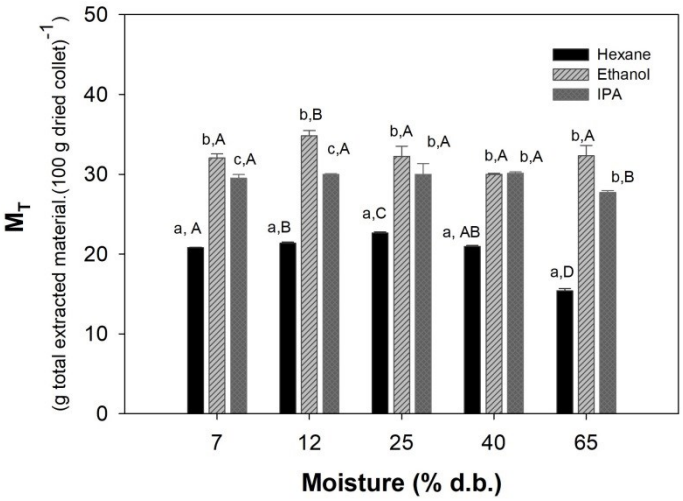


Fig. 2: Total extracted material from sunflower collets according to type of solvent at different moisture contents at 6 hours of extraction.



Vertical bars represent mean values ± standard error. Means accompanied by the same lowercase letter for each moisture (solvent effect) are not significantly different at $p > 0.05$. Means accompanied by same uppercase letter (effect of moisture) are not significantly different ($p>0.05$) by Tukey's test method.

Fig. 3: Effect of moisture content on oil extraction efficiency from sunflower collets with *n*- hexane, ethanol and isopropanol.

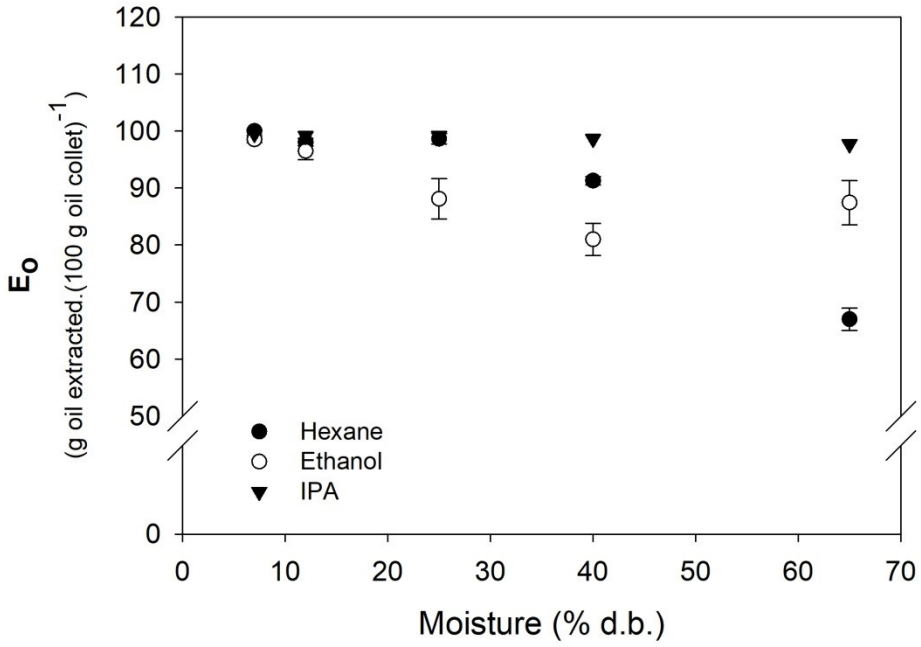
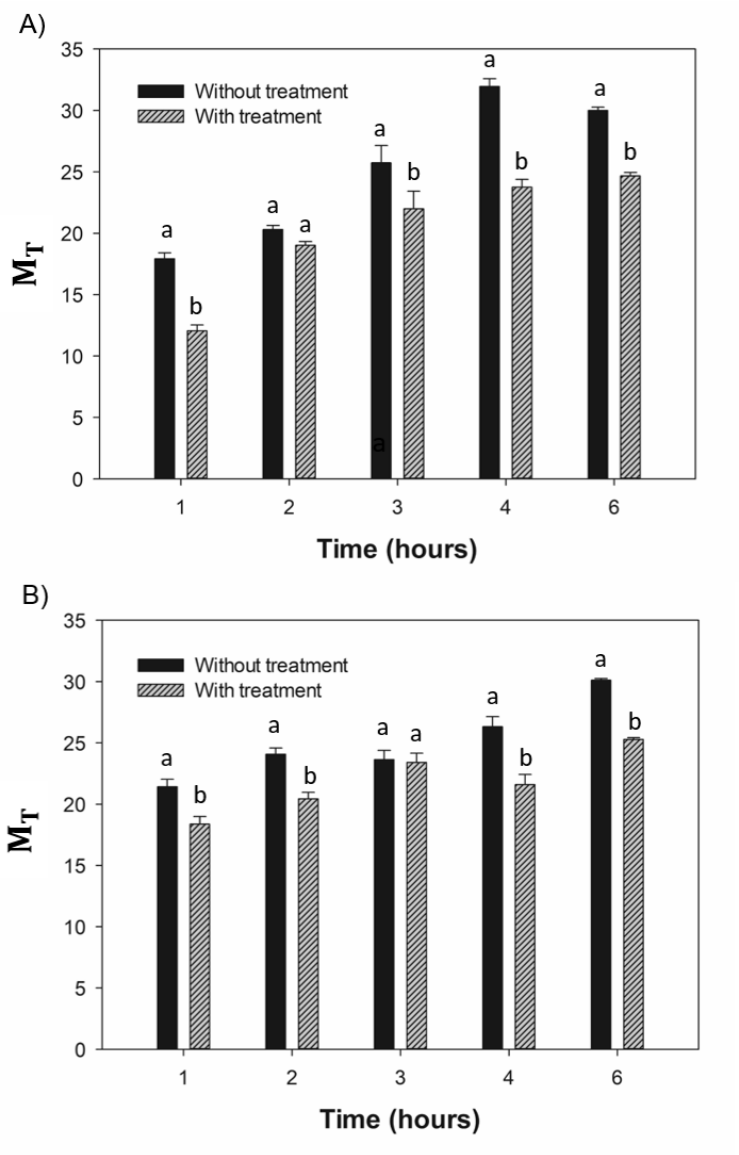


Fig. 4: Percentage of total material extracted (M_T) from untreated and treated sunflower collets with ethanol (A) and isopropanol (B) at different extraction times.



Sunflower oil extraction with green solvents from enzyme-treated collets was obtained and determined the effects of operating conditions on the yield and quality oil.