

# Enzymatic hydrolysis and pressing conditions effect on borage oil extraction by cold pressing

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## Abstract

Borage seed oil extraction using cold pressing produces a good oil quality, but it has a low-yield. In a previous study on a borage oil extraction process by cold pressing using commercial enzymes, the oil yield was enhanced in comparison to the control without enzymes. The aim of this work was to further evaluate the effect of temperature, moisture and time of enzymatic hydrolysis; and the effect of this treatment under selected conditions on the pressing stage and on product qualities. The best treatment condition with Olivex–Celluclast was 45 °C, 20% moisture over 9 h of treatment. When the extraction of the pre-treated borage meal was carried out by double pressing (20 min each) on preheated matter, 95% of the oil was recovered. The enzymatic treatment did not affect the oil quality and the residual meal was more valuable due to its lower fibre content.

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## 1. Introduction

Gamma-linolenic acid (GLA) is metabolized *in vivo* to dihomo-gamma-linolenic acid (DGHLA) which is an important precursor of prostaglandins (series 1) and Leucotrienes (series 3) molecules with anti-thrombotic and anti-inflammatory properties (Barre, 2001; Senanayake & Shahidi, 2000). In nature, DGHLA does not exist in high-quantities and GLA synthesis from linolenic acid (LA) is low (5–10%) (Sprecher, Luthria, Baykousheva, & Mohammed, 1996). Due to these facts, GLA is recommended for treatment of several diseases such as arthritis and dermatitis (Barre, 2001).

Among more than 40 vegetables species that contain GLA, borage seed (*Borago officinalis* L.) has the highest content of GLA. It has 30% of oil with about 60% polyunsaturated fatty acids (PUFA) of which 23% is GLA (Berti, Wilckens, Fischer, & Araos, 2002; Chen & Ju, 2001; Guil-

Guerrero, García-Maroto, & Jiménez, 2001; Padley, Gunstone, & Hardwood, 1994).

Borage seeds oil recovery should be carried out under mild process conditions, reducing the oil oxidation. Cold pressing oil extraction is a good alternative in comparison to the traditional high-temperature solvent extraction, but it affords low-yields. Enzyme incorporation could improve the oil extraction yield as described for rosehip seeds (Concha, Soto, Chamy, & Zúñiga, 2004), grape seeds (Tobar, Moure, Soto, Chamy, & Zúñiga, 2005), canola (Sosulski, Sosulski, & Coxworth, 1988; Sosulski & Sosulski, 1993; Zúñiga, Chamy, & Lema, 2001), chilean hazelnut (Zúñiga, Soto, Mora, Chamy, & Lema, 2003) and borage seeds (Soto, Chamy, & Zúñiga, 2004). In the aforementioned paper (Soto et al., 2004), it is reported that the use of an Olivex–Celluclast (1:1) enzyme mixture in a 0.25% E/S ratio an 84% borage oil recovery was obtained in comparison with 77.7% achieved for control samples.

However, results obtained differ according to the type (source) of seed, extraction technique, and enzymatic treatment conditions. The hydrolysis temperature is a critical

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parameter for enzymatic processes (Sharma, Khare, & Gupta, 2002). When temperature increases, enzymes could be reactivated or inactivated (Illanes, 1999), but its effect was not as important in other oil extraction processes evaluated (Zuñiga et al., 2001). Regarding the hydrolysis, lower water systems increase the thermal stability of enzymes due to enzyme immobilization by water adsorption on the solid substrate (Tuena de Gómez-Puyou & Gómez-Puyou, 1998; Zuñiga, 1998); on the other hand, an enzymatic treatment with high-water content (moisture) can be more effective due to high-enzymatic activity. Also, a high-moisture operation is adverse due to economic reasons, because of the subsequent dryer stage required before pressing, and because the drying operation could be harmful for the seed structure. Pressing conditions also influence the oil yield (Singh & Bargale, 2000).

It has been reported that enzymatic pre-treatment improves the products' quality (oil and residual meal), reducing the fibre content (Dominguez, Núñez, & Lema, 1994; Sosulski & Sosulski, 1993; Zuñiga et al., 2003), preserving the protein properties in defatted meal (Moure, Dominguez, Zuñiga, Soto, & Chamy, 2002); and producing oils with low-peroxide and free fatty acid values (Che Man, Suhardiyono, Azudin, & Wei, 1996; Cintra, López-Munigua, & Vernon, 1986; Zuñiga et al., 2001). Zuñiga et al. (2001) reported that a good quality canola oil was obtained through enzymatically-assisted process, which had 50% free fatty acids (FFA) as compared to the control samples and the same peroxide value. However, Sosulski and Sosulski (1993) in a similar enzymatic-aided oil extraction process, recovered canola oil with a higher FFA than a conventional extraction process.

Enzyme hydrolysis in oil extraction processes may be different depending on the oilseed. The goal of this work was to evaluate the effect of hydrolysis conditions on borage seed oil extraction yield when an Olivex–Celluclast (1:1) mixture was employed, and to study how this treatment affects the pressing process and the product qualities.

## 2. Materials and methods

### 2.1. Seeds

Borage seeds were provided by LONCOPAN S.A. (Santiago, Chile). Its proximate composition was reported previously (Soto et al., 2004).

### 2.2. Enzymes

A mixture of two commercial enzymes (50% w/w of each) was previously selected (Soto et al., 2004). Olivex (mainly pectinase, cellulase, and hemicellulase activities) and Celluclast (mainly cellulase and hemicellulase activities) enzymes were obtained from Novozymes A/S (Madrid, Spain).

### 2.3. Experimental procedure

Borage seeds were cleaned, crushed in a manual screw mill (Corona) to obtain a particle size equal to or smaller than 2.0 mm and thermally conditioned at 100 °C for 20 min, and then water and enzymes were added for the enzymatic treatment, which was carried out under different conditions. After the enzymic hydrolysis, samples were dried under a vacuum at 40 °C, and then pressed in a hydraulic laboratory press (Carver Press, New York) at 49 MPa, 20 min and at  $11.0 \pm 0.5\%$  moisture in the sample. When the effect of the enzymatic treatment under selected conditions on the subsequent pressing operation was evaluated by applying double pressing, each one of them was done for the same period of time.

The oil extraction yield is the relationship between the oil extracted and the total oil present in the seed. Extracted oil is the difference between the original total oil and the residual oil in the cake, which is determined by soxhlet extraction.

Control trials were carried out using water instead of enzyme solution. Some control samples were compared to those with no treatment (only crushed and thermally conditioned) before pressing, and they did not show significant differences, obtaining for both procedures a borage oil extraction yield of  $77.7 \pm 1.17$  ( $P < 0.01$ ).

### 2.4. Hydrolysis conditions

The enzyme-crushed seed (E/S) ratio was 0.25%. The temperature effect was analyzed between 35 and 55 °C. The effect of moisture (20–50%) during the treatment was also evaluated. The desired moisture levels were obtained by pre-dissolving the enzyme in an appropriate amount of water and adding it to the meal sample.

### 2.5. Analytical methods

Oil and meal characteristics were mainly determined according to the AOAC (1990) methods.

## 3. Results

The temperature of the enzymatic treatment did not affect the yield of oil extraction for the control samples ( $77.7 \pm 1.7\%$ ) in the range of 35–55 °C. As observed in Fig. 1 for 35 °C, the oil yield is modified when the enzymatic hydrolysis is carried out for 3 h from  $76.7 \pm 2.3\%$  up to  $81.9 \pm 0.6\%$  for 12 h of treatment. However, the latter result is lower than that obtained at 45 °C for 3 h. The oil extraction yield for enzymatically-treated samples improves when the temperature increased from 35 to 45 °C, but at higher temperatures the recovered oil decreased. This behavior is similar to that presented by Concha et al. (2004) for rosehip oil and by Zuñiga et al. (2003) for Chilean hazelnut oil extraction.

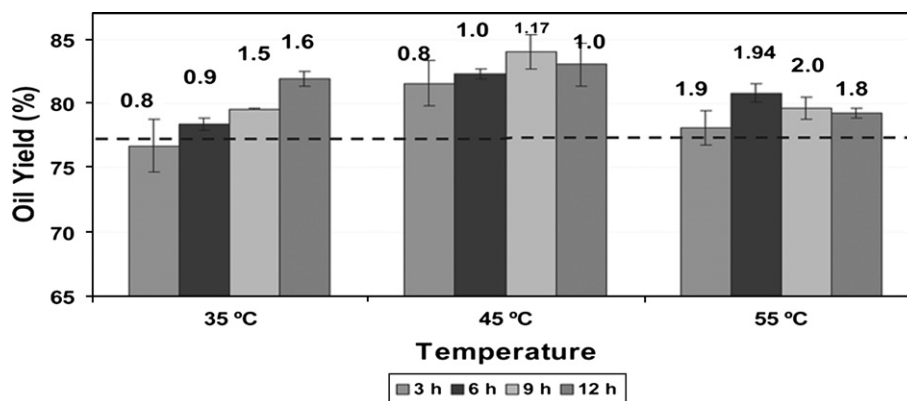


Fig. 1. Effect of hydrolysis temperature on borage oil extraction yield using 0.25% w.b./d.b. E/S of an Olivex–Celluclast (1:1) mixture and 30% moisture. Soluble sugar concentration (numbers).

The thermal inactivation of enzymes explains this behavior. Enzymes are intrinsically labile, but, temperature could produce opposite effects on its stability and reactivity becomes an important variable in any process that includes biocatalysts (Illanes, 1999). At 55 °C the commercial enzymes used (Olivex and Celluclast) were reported as being stable (Novozyme Products Sheets, 1997, 1998) and as observed in Fig. 1, at this temperature for 6, 9 and 12 h of treatment, the differences were not significant ( $P > 0.05$ ), possibly due to the low-water hydrolysis condition which could provide a high-thermal stability, as reported by Tuena de Gómez-Puyou and Gómez-Puyou (1998).

Another explanation could be the decreasing soluble sugar production for higher enzyme hydrolysis at higher temperatures. These sugars could be caramelized in the subsequent drying stage prior pressing, limiting the oil release from the cells (Zuñiga et al., 2001). Fig. 1 shows an increase of reducing sugars ( $1.94 \pm 0.11\%$ ) at 55 °C in comparison to that achieved at 45 °C ( $1.02 \pm 0.11\%$ ). This result suggests that the higher hydrolysis achieved at 55 °C in comparison to that at 45 °C is not expressed as an

increase in the oil extraction yield because it could be decreased by sugar caramelization or/and by inhibition by hydrolysis products (Mansfield, Mooney, & Saddler, 1999).

The temperature of 45 °C was selected to evaluate the effect of moisture during the enzymatic treatment. Results displayed in Fig. 2 show minor differences in the extracted oil when the moisture of the hydrolysis is modified in the range studied. For treatments at 40% moisture, the oil yield was  $82.46 \pm 0.29\%$  ( $P > 0.05$ ), independently from the period of hydrolysis between 3 and 12 h. Better oil extraction yield of 85.5% was achieved after 9 h of treatment at 20% moisture, which is consistent with the higher enzyme hydrolysis.

In canola and rosehip oil extraction, a higher oil volume was obtained by a similar process when the enzymatic hydrolysis was carried out at 30% moisture (Concha et al., 2004; Sosulski & Sosulski, 1993), and at 40% moisture when chilean hazelnut oil was extracted (Zuñiga et al., 2003). The difference observed for borage oil extraction is possibly due to better water absorption by crushed borage seeds than canola, rosehip seeds or chilean hazelnut,

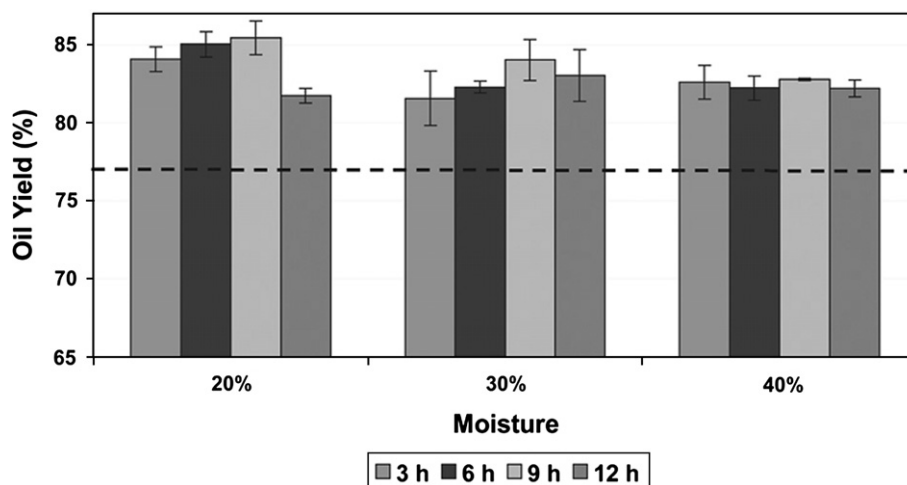


Fig. 2. Effect of moisture during enzymatic treatment on borage oil extraction yield using 0.25% E/S of Olivex–Celluclast (1:1) mixture and 45 °C.

increasing its water activity and then enhancing the enzyme activity which depends on binding water more than the total water of the systems; excessive solvation promotes the loss of the proteins' native structure as suggested by Tuena de Gómez-Puyou and Gómez-Puyou (1998).

The effect of the enzymatic treatment under selected conditions (0.25% E/S ratio; 45 °C; 20% moisture, 9 h) on the subsequent pressing operation was evaluated. Fig. 3 shows that oil extraction kinetics was improved when the samples were enzymatically pre-treated in comparison to the control samples. The pressing operation for more than 20 min did not show any significant differences ( $P > 0.05$ ) in the oil yield recovered. Long extraction periods could produce cellular channels occlusion due to excessive compression that obstructs the free flow of oil. Another explanation for the oil extraction hindrance could be the oil

absorption into the fibre (Ward, 1976). A double pressing and pre-heating of the matter would decrease these problems, opening the channels, reducing the oil viscosity and enhancing the oil fluidity through the cell wall that has not been degraded.

The positive effect of double pressing on pre-heated matter is shown in Fig. 4, improving the oil extraction for both enzymatically pre-treated and control samples for two pressings of 20 min each, reaching up to 94.4% and 89%, respectively (significance levels are  $P < 0.001$  and  $P < 0.05$ , respectively).

The effect of enzymatically-aided oil extraction process on defatted meal composition is presented in Fig. 5. The oil in residual meal obtained by enzyme assisted process was 3.6% compared to 9.5% (d.b.) for the control process; this could have a positive effect on residual meal stability.

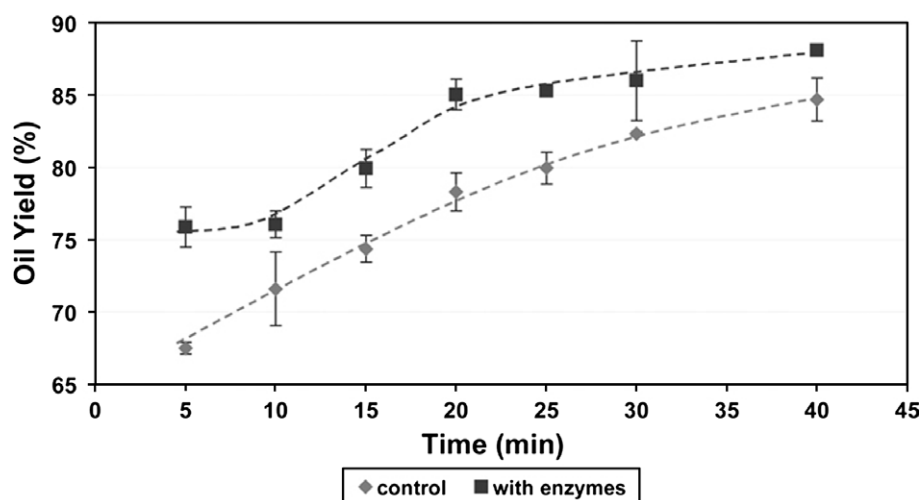


Fig. 3. Oil yield extraction obtained by single pressing for control and enzymatically-treated samples. Hydrolysis conditions: 0.25% E/S of Olivex–Celluclast (1:1) mixture, 45 °C, 20% moisture, 9 h. Pressing conditions: single pressing, 39.2 MPa.

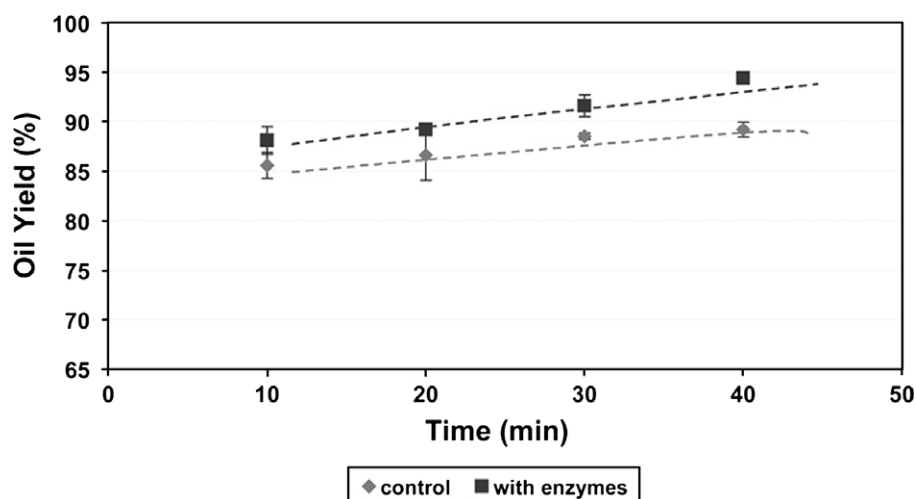


Fig. 4. Oil yield extraction obtained by double pressing with a pre-heating of matter, for control and enzymatically-treated samples. Hydrolysis conditions: 0.25% E/S of Olivex–Celluclast (1:1) mixture, 45 °C, 20% moisture, 9 h. Pressing conditions: double pressing, 39.2 MPa, pre-heating of matter (5 min, 70 °C).

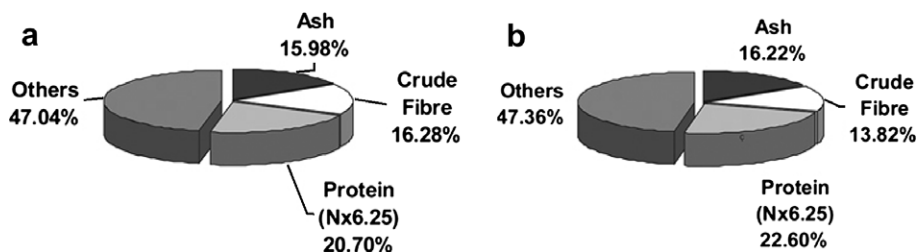


Fig. 5. Proximal composition of residual defatted meal obtained from borage oil extraction process by cold pressing (single and without pre-heating) for (a) control and treated samples (b).

Also, the enzymatic treatment reduces meal fibre content, which could enhance its digestibility. Borage meal inclusion resulted in reduced intake and poor nutrient utilization especially for growing pigs (Mustafa, McKinnon, Thacker, & Christensen, 1997). The same authors found that borage meal fibre content and composition was similar to canola meal fibre which suggests that borage meal could gain improved digestibility when its cell wall carbohydrates are enzymatically-hydrolyzed, similar to that reported for canola meal (Slominski & Campbell, 1990) and other meals (Cañeque, Velasco, Sancha, Manzaneres, & Souza, 1998).

As shown in Table 1, enzymatic treatment produces a decrease in hemicellulose and cellulose contents. However, lignin and pectin contents were increased. These results were not expected, but Sosulski and Sosulski (1993) reported that the enzymatic treatment effects on the cell wall structure depended on the type of seed and source of the enzyme. A similar behavior was noted by Zúñiga et al. (2003) who reported lower hemicellulose, lignin and pectin contents and higher cellulose contents when used in an enzymatic oil extraction process for Chilean hazelnut instead of the control process. The protein and pectin contents in borage seeds were remarkable (about 20% and 6%, respectively) suggesting that these compounds could produce interference on NDF and ADF values. NDF determination is based on sodium dodecyl sulfate at neutral pH solubility of some cellular components as starch and proteins between others, and on insolubility of cellulose, hemicellulose and lignin, however, this detergent could produce solubilization of pectin, also a fraction of cellular proteins could be retained in insoluble fraction of NDF. ADF determination is support on cetyl trimethyl ammonium bromide solubility of NDF soluble components plus hemicellulose, maintaining cellulose and lignin in the residue, but pectin could precipitate increasing the ADF value (Jung, Valdez, Hatfield, & Blanchette, 1992; Reeves, 1997; Van Soest, Robertson, & Lewis, 1991).

Table 2 shows that the enzymatic treatment does not affect virgin borage oil properties, nevertheless the peroxide value denotes a high-oil oxidation level maybe caused by samples stored at  $-18\text{ }^{\circ}\text{C}$  (not  $-20\text{ }^{\circ}\text{C}$  as recommended) and without a nitrogen atmosphere.

Khan and Shahidi (2000) showed that borage oil is susceptible to oxidation increasing its peroxide value from 1.65 to 141  $\text{meq}_{\text{O}_2}/\text{kg}_{\text{oil}}$  when the oil was heated for 168 h at  $60\text{ }^{\circ}\text{C}$ . According to these authors, a peroxide value of 18  $\text{meq}_{\text{O}_2}/\text{kg}_{\text{oil}}$  obtained in this work (borage oil obtained by pressing) is similar to what was reported for 24 h of incubation at  $60\text{ }^{\circ}\text{C}$ , showing a possible storage problem.

On the other hand, Molero-Gómez and Martínez de la Ossa (2002) reported that borage oil extracted by a soft supercritical fluid extraction technique achieved a peroxide value of 33.5  $\text{meq}_{\text{O}_2}/\text{kg}_{\text{oil}}$ . The peroxide index does not seem to be related to the enzymatic extraction process. Olive oil showed a lesser peroxide index and better oxidative stability when it was extracted by the enzyme assisted process (Montedoro, Bertuccioli, & Petruccioli, 1975). In corn oil extraction by enzymatic aqueous process a small increase in peroxide value was observed in comparison with the conventional extraction process, but the enzyme extracted oil showed better oxidative stability (Bocevaska, Karlovic, Turkulov, & Pericin, 1993).

To determine if the process conditions (cold pressed) and the enzymatic treatment affect the characteristics of the extracted oil, we analyzed two commercial borage oils. The first (Loncopan S.A., Chile) extracted by hexane and refined oil and a second extra virgin cold pressed oil sample (Omega Nutrition, Vancouver, BC, Canada). Both oils showed a similar peroxide value of 48  $\text{meq}_{\text{O}_2}/\text{kg}_{\text{oil}}$ , may be due to the high-PUFA content of borage oil, being

Table 1  
Fibre contents of borage seeds

Component	Control (% w/w)	Enzyme (% w/w)
Hemicellulose		
NDF-ADF	8.0	5.4
Cellulose		
ADF-ALF	32.6	29.0
Lignin		
ALF	13.8	19.2
Pectin	6.4	7.5

NDF, neutral detergent fibre; ADF, acid detergent fibre; ALF, lignin residue from acid detergent fibre.

Table 2  
Borage oil properties extracted by enzymatic-aided and control cold pressing process

Property	Control	With enzymes
Acidity ( $\text{mg}_{\text{NaOH}}/\text{g}_{\text{oil}}$ )	$5.56 \pm 0.04$	$6.55 \pm 0.03$
Saponification value ( $\text{mg}_{\text{KOH}}/\text{g}_{\text{oil}}$ )	$272.49 \pm 14.37$	$289.00 \pm 11.76$
Peroxide value ( $\text{meq}_{\text{O}_2}/\text{kg}_{\text{oil}}$ )	$18.00 \pm 0.15$	$16.62 \pm 0.32$
Iodine value ( $\text{g}/100\text{ g}_{\text{oil}}$ )	$359.28 \pm 8.91$	$371.88 \pm 6.53$



Table 3

Fatty acid profile of borage oil obtained without and with an enzymatic-aided process

Fatty acid	Control (% w/w)	Enzyme (% w/w)
Palmitic acid (16:0)	8.7	9.2
Stearic acid (18:0)	2.6	2.6
Oleic acid (18:1, $\omega - 9$ )	18.9	19.1
Linoleic acid (18:2, $\omega - 6$ )	40.8	40.3
$\gamma$ -Linolenic acid (18:3, $\omega - 6$ )	22.9	22.7
Gadoleic acid (20:1, $\omega - 11$ )	5.3	5.2
Erucic acid (22:1, $\omega - 13$ )	0.85	0.84

highly susceptible to oxidation, proving that the oil obtained by studied extraction process has lesser oxidation than the commercial borage oils tested. Commercial hexane extracted borage oil showed an acidity value of 1.94 mg<sub>NaOH</sub>/g<sub>oil</sub>, which is lower than that obtained for the second oil sample extracted by cold pressing which had a 4.69 mg<sub>NaOH</sub>/g<sub>oil</sub> index which is similar to that reported in Table 2; probably the lower index of the hexane extracted oil is due to its neutralization.

A fatty acid profile of borage oil extracted by pressing shows that the enzyme incorporation does not significantly have an effect on the oil quality maintaining its high-polyunsaturated fatty acid contents (about 70%) with 22% of GLA. A similar behavior was reported by other authors for enzymatically-assisted vegetable oil extraction (Bocevská et al., 1993; Dominguez et al., 1994; Rosenthal, Pyle, & Niranjana, 1996; Sengupta & Bhattacharyya, 1996). The minor differences between the enzyme aided extracted oil and its control displayed in Table 2, are not proportional to the changes observed in fatty acid profile; we suggest this is not significant ( $P > 0.05$ ). Molero-Gómez and Martínez de la Ossa (2002) reported differences on properties as iodine index, saponification value and peroxide value of borage oil extracted by different techniques, but they did not show any changes on fatty acid profile, as also noted in this work (see Table 3).

#### 4. Conclusions

Among the ranges studied, the hydrolysis temperature was a more significant parameter than moisture hydrolysis to improve the borage oil extraction yield by cold pressing. Nevertheless, period and type (double, single, with or without pre-heated matter) of pressing were more significant factors for both processes; the enzymatically-aided and control process. The enzymatic treatment and the subsequent cell wall degradation reduce the cake compression resistance in the pressing stage, allowing the recovery of total oil. The enzyme assisted oil extraction process did not change the oil properties and improved the defatted meal quality in comparison with the control devoid of enzymes.

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