LWT - Food Science and Technology 58 (2014) 71–76

Contents lists available at ScienceDirect

LWT - Food Science and Technology

journal homepage: www.elsevier.com/locate/lwt

Changes in quality and composition of sunflower oil during enzymatic degumming process

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ARTICLE INFO

Article history:
Received 22 October 2013
Received in revised form
3 February 2014
Accepted 14 February 2014

Keywords:
Enzymatic degumming
Sunflower oil
Stability
Quality

ABSTRACT

The objective of this study was to examine the effect of the enzymatic degumming process on composition of crude sunflower oil.

To analyze the crude and degummed oils, different parameters were determined. Phosphorous content, metal traces, phospholipids, acid value, peroxide value, anisidine value, moisture, color and stability oxidative, were measured.

The degumming assays were performed in a batch system with continuous stirring using a phospholipase A1 (Lecitase® Ultra) and an acyltransferase (LysoMax® Oil). The assays were carried out at 50 °C, pH 5 and an enzyme dosage of 200 U/kg of oil during 60 min.

The degumming process with both enzymes decreased the phosphorus content in crude sunflower oil below 3 mg/kg.

Phospholipid content showed a drastic decrease with the enzymatic degumming process. The calcium and magnesium content in crude sunflower oil was extremely high, and it was reduced noticeably with the enzymatic treatment. Induction time for oxidative stability of the crude oil was 17.25 h. In the degummed oil samples with phospholipase A1 and acyltransferase, the oxidative stability index was found to be 4.18 h and 4.33 h respectively. These results indicate that the enzymatic degumming process affected several quality and stability characteristics of crude sunflower oil.

1. Introduction

Crude vegetable oils are traditionally refined by physical or chemical processes. Degumming is the first step in the refining process of vegetable oils, and it removes phospholipids and mucilaginous gums that affect quality and storability. Some enzymatic degumming processes have been suggested for laboratory and pilot plant scales (Clausen, 2001; Dayton, Staller, & Berkshire, 2010; J.G. Yang, Wang, Yang, Mainda, & Guo, 2006; Yang, Zhou, Yang, Wang, & Wang, 2008), and have led to the process on an industrial scale (Autino, 2009). This technique improves the quality and the oil yield (Dijkstra, 2010).

The different enzymes that are commercially available for processing vegetable oils vary in the way they act on phospholipids, but all of them increase the yield of the oily phase recovered by the degumming process (Galhardo & Hitchman, 2011). There are several types of enzymes of interest for the treatment of vegetable oils, such as phospholipase A1 (PLA1) and phospholipase A2 (PLA2) that remove the fatty acid from positions 1 and 2 respectively with respect to glycerol (Dijkstra, 2010; Galhardo et al., 2010). The phospholipase B (PLB) eliminates both fatty acids from the glycerol group (Galhardo et al., 2010). The phospholipase C (PLC) catalyzes the hydrolysis of the phosphate-glycerol bond in phosphatidylcholine and phosphatidylethanolamine (Dijkstra, 2010). There are another group of microbial enzymes, the acyltransferases, which transfer a fatty acid to a sterol present in the oil, in order to convert it into a fatty acid ester (Dijkstra, 2011). All enzymes cause less oil to be retained by the gums by decreasing the amount of gums and their oil retention, which also contributes to an improved oil yield (Dijkstra, 2010).

The oil refining objective is to remove impurities with the least possible effect on desirable components present in the crude vegetable oils in order to obtain an odorless, bland and oxidatively stable refined vegetable oil (Medina Juarez, Gamez, Ortega, Noriega, & Angulo, 2000). Quality and stability are the main factors in the production acceptance and marketing of the vegetable oil product (Brevedan, Carelli, & Crapiste, 2000). These properties depend on seed quality, seed treatment, extraction method and

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http://dx.doi.org/10.1016/j.lwt.2014.02.024
0023-6438/

processing conditions. They are influenced by the presence of minor components such as free fatty acids, phospholipids, trace metals and waxes, which have pro-oxidant or antioxidant properties (Autino, 2009).

Metallic particles have a catalytic effect on the oxidation reaction of oil, as they promote the formation and decomposition of hydroperoxides. These compounds are decomposed into aldehydes, ketones, organic acids, alcohols, and volatile compounds, which are called secondary oxidation products (Villa Salinas, 2009). The presence of trace metals is an important factor as far as the quality of edible oil is concerned (Pehlivan, Arsian, Gode, Altun, & Ozcan, 2008).

There are studies on the changes in chemical composition that affect the quality and stability of vegetable oils obtained by solvent extraction. These characteristics are primarily dependent on extraction solvents, extraction temperature and pretreatment of seeds (Jung, Yoon, & Min, 1989; Yoon & Min, 1986). Tasan, Gecgel, and Demirci (2011) reported the influence of industrial oilseed extraction methods (pre-pressed, solvent-extracted, full-pressed and mixed oil) on the quality and stability of crude sunflower oil. The effect of pressing conditions on nonrefining sunflower oil has been presented by Turkulov, Dimic, Karlovic, and Vuksa (1998). The influence of water degummed oil in the phosphatide content was discussed by Crapiste, Brevedan, and Carelli (1998). The changes in composition and quality of sunflower oils during extraction and water degumming were described by Brevedan et al. (2000).

Furthermore, there are several studies about the effect of enzymatic degumming on the phosphorus content (Chakrabarti et al., 2009; Jahani, Alizadeh, Pirozifard, & Qudsevali, 2008; Jiang et al., 2011; Soe & Brown, 2011; B. Yang, Wang, & Yang, 2006; J.G. Yang et al., 2006), the quantitative and qualitative analysis of phospholipids (Clausen, 2001; Galhardo et al., 2010) and the acid value achieved (B. Yang et al., 2006). However, there are just few studies reported in the literature concerning changes in physical and chemical characteristics of sunflower oil during the enzymatic degumming process.

The aim of this work was to investigate and analyze the effect of the enzymatic degumming process on composition, quality and oxidative stability of sunflower oil.

2. Materials and methods

2.1. Materials

Crude sunflower oil extracted by hexane, provided by a local Company was used. The oil was stored under refrigeration and protected from light until it was used.

Lecitase® Ultra, an acidic phospholipase A1 (EC 3.1.1.32) from *Thermomyces lanuginosus* expressed in *Aspergillus oryzae* was acquired from Novozymes (Bagsvaerd, Denmark). This enzyme exhibits phospholipase A1 activity at pH values from 4.5 to 6 at 50 °C according to indicated by the manufacturer.

LysoMax® Oil, a microbial lipid acyltransferase (EC 2.3.1.43) with phospholipase A2 activity, was provided by Danisco & Genencor (Arroyito, Córdoba, Argentina). This enzyme exhibits activity at pH values from 5 to 10 at temperature range from 45 to 80 °C according to indicated by the manufacturer.

All reagents were of analytical grade. Citrate buffer pH 4.94 was prepared by mixing sodium citrate solution (0.1 mol/L) and sodium hydroxide solution (0.1 mol/L). Both reagents were made with twice-distilled water, in the amount in which the stock solutions required, in order to yield a desired pH value in each case.

2.2. Oil degumming assay system

The assay system consisted of a jacketed reactor fitted with lid, a propeller and a thermometer. The reactor was connected to a water bath with water pump and flexible tube (Fig. 1).

The assay was performed in a batch system with continuous stirring, at pH 5. One Liter of sunflower oil was loaded in the reactor which was kept at about 50 °C. Followed by the addition of 2 mL/100 mL buffer/substrate ratio and 200 U/kg of oil enzyme dosage solution, the mixture was stirred with a mechanical mixer to provide a safe large surface area through emulsification. Aliquots of the reaction mixture were taken at 10, 20, 30, 40, and 60 min and heated for 30 min at 100 °C to stop the enzymatic reaction. Then, they were centrifuged (10 min at 2400 × *g*) to recover oil and water phases. The processed samples were stored until analysis at 5 °C, protected from light.

2.3. Analytical methodology

To analyze the crude and degummed oils, different parameters were determined by standard official methods.

2.3.1. Phosphorus content analysis

The phosphorous content was determined by ashing the sample in the presence of zinc oxide followed by the spectrometric measurement of phosphorous as a blue phosphomolybdic acid complex (Ca-12 55,AOCS, 2009).The absorbance was measured at 650 nm by using a Shimadzu 160 UV-VIS spectrophotometer (Shimadzu 160 Japan, equipped with a computer assisted system for data acquisition).

Fig. 1. Reactor used for the enzymatic degumming process of crude sunflower oil samples.

2.3.2. Acid value analysis

Acidity was measured by titration with a standardized ethanolic solution of potassium hydroxide and phenolphthalein as indicator. The analysis was determined according to IUPAC Standard Method 2.201 (IUPAC, 1992).

2.3.3. Moisture and volatile matter determination

Moisture and volatile matter was determined using AOCS Official Method Ja 2a-46.

2.3.4. Peroxide value analysis

AOCS Official Method Cd 8–53 (AOCS, 2009) for peroxide value (acetic acid–chloroform method) was employed to measure peroxides and other similar compounds that oxidize potassium iodide as primary oxidation products.

2.3.5. Anisidine value analysis

The anisidine value was determined by AOCS Official Method Cd 18–90 (AOCS, 2009), which measures the amount of aldehydes (principally 2-alkenals and 2,4-dienals) as secondary oxidation products.

2.3.6. Determination of phospholipids by HPLC (High-Performance Liquid Chromatography)

Quantitative determination of phospholipids was carried out by enrichment using diol solid phase extraction cartridges (J.T. Baker Inc., Phillipsburg, NJ) and subsequent analysis by high-performance liquid chromatography (Ja7b-91, AOCS, 2009). Waters HPLC e2695 Alliance systems with a Waters 2998 Photodiode Array Detector (PDA) set at 206 nm and an EC 250/4.6 Nucleosil 50-5 de Macherey-Nagel column were used. The mobile phase used was n-hexane/2-propanol/acetate buffer pH 4.2 (8:8:1, v/v/v). The flow rate was set at 2 ml/min.

2.3.7. Metals

The determination of metals was performed by atomic emission spectrophotometry flame. The oil sample was treated with open flame and then it was carried to ashes in a muffle furnace at 550 °C. The ashes were taken up with concentrated hydrochloric acid and hot distilled water. Determinations were performed using an Atomic Emission Spectrometer Inductively Coupled Plasma (ICP-AES), Shimadzu 9000 Simultaneous High Resolution by EPA Standard 2007 (EPA 6010-C 2007, US EPA, 2007). The measurements were performed in all cases by external calibration using Chem-Lab certified standards patterns, Zedelgem B-8210, Belgium.

2.3.8. Rancimat test

The Oxidative Stability Index (OSI), expressed as induction time, was determined by the Rancimat method (12b-92, AOCS 2009). The experiments were carried out with a Metrohm 679 Rancimat apparatus (Metrohm AG, Switzerland) at 110 °C. Air flow rates were set at 20 L/h for all determinations.

2.3.9. Color measurement

To perform a color experiment of the oil samples, the HunterLab UltraScan XE tristimulus colorimeter (Hunter Associates Laboratory, Inc., Reston, VA) was used. An aliquot of each sample was taken to fill a glass cuboid cell (10 mm thickness), and the total color transmitted through the sample was measured at 10° observer angle with D65 illuminant. Results were expressed as the Hunter Lab scale parameters "L" (lightness: 0 = black, 100 = white), "a" (greenness (−), redness (+)) and "b" (blueness (−), yellowness (+)). These values indicate how much a standard and sample differ from one another and they are often used for quality control.

2.4. Statistical analysis

All experiments were performed in quadruplicate. The results were expressed as mean value ± standard deviation.

The differences in mean values between samples were assessed with Student's *t*-test. The effects were considered significant at $P < 0.05$.

3. Results and discussion

3.1. The efficiency of the degumming process

The efficiency of the enzymatic degumming process over time was assessed by phosphorus content determination. The monitoring of the process was conducted in order to meet the demands of industrial refining. Physical refining requires phosphorus content below 10 mg/kg in order to be applied (Jahani et al., 2008). Chakrabarti et al. (2009) stated that enzymatic degumming is probably the best process available for reducing the phosphorus content of vegetable oils below 10 mg/kg.

As showed in Table 1, the phosphorus content of crude sunflower oil was 474.7 ± 14.7 mg/kg. This content was strongly affected by the process using phospholipase A1, reaching a value of 2.61 ± 0.22 mg/kg after 60 min of reaction. At the same time of reaction, total phosphorous content was of just 2.54 ± 0.20 mg/kg in the degummed oils obtained with acyltransferase.

As showed in Fig. 2, the phosphorus content decreased by increasing reaction time. The least of the reaction time that oils were degummed to less than 10 mg/kg of phosphorus was 60 min. These results are consistent with the values detected in previous studies using the same enzymes (Lamas, Ceci, Constenla, & Crapiste, 2010; Lamas, Ceci, Crapiste, & Constenla, 2011, 2012). B. Yang et al. (2006) informed that values of 3 mg/kg of phosphorous content are low enough for physical refining process.

Jahani et al. (2008) reported that in rice bran oil the residual phosphorus content decreased to less than 5 mg/kg, when the reaction time was increased to 6.5 h, by treatment with Lecitase® Ultra. Using the same enzyme in the soybean oil, the phosphorous content was 6 mg/kg after 5 h of reaction (Yang et al., 2008). In the

Table 1
Quality and stability characteristics obtained for crude and enzymatic degummed oils.

Analytical determination	Samples		
	Crude sunflower oil	Degummed phospholipase A1 oil	Degummed acyltransferase oil
Phosphorous content (mg/kg)	474.7 ± 14.7[b]	2.61 ± 0.22[a]	2.54 ± 0.20[a]
Acidity (mg KOH/g)	2.22 ± 0.04[b]	3.95 ± 0.07[c]	1.65 ± 0.05[a]
Moisture (g/100 g)	0.0945 ± 0.0170[a]	0.1895 ± 0.0107[c]	0.1700 ± 0.0126[b]
Peroxide value, PV (meq/kg)	4.63 ± 0.18[a]	5.38 ± 0.12[c]	4.88 ± 0.20[b]
Anisidine value, AV	3.67 ± 0.18[a]	3.50 ± 0.05[a]	3.66 ± 0.10[a]
TOTOX = 2 PV + AV	12.93	14.26	13.42
Phospholipids (g/100 g)	0.631 ± 0.065[b]	0.014 ± 0.004[a]	0.013 ± 0.003[a]
Metal content (mg/kg)			
Fe	8.37 ± 0.66[c]	1.02 ± 0.10[a]	3.05 ± 0.23[b]
Cu	3.41 ± 0.55[c]	0.62 ± 0.06[a]	1.32 ± 0.11[b]
Ca	95.34 ± 3.67[b]	17.72 ± 2.08[a]	18.14 ± 1.54[a]
Mg	105.04 ± 3.24[c]	4.28 ± 0.68[a]	10.75 ± 1.47[b]
OSI at 110 °C (h)	17.25 ± 1.45[b]	4.18 ± 0.21[a]	4.33 ± 0.40[a]

Results are mean values ± standard deviation ($n = 4$).
Different letters in the same line indicate significant differences ($P < 0.05$).
("a" means the lowest value, "c" means the highest value).

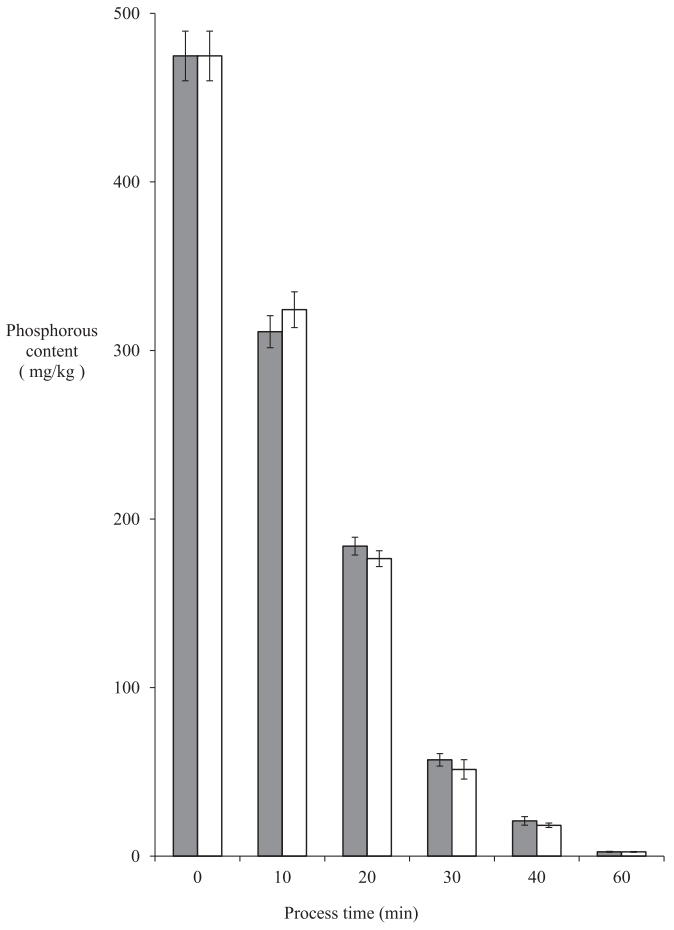

Fig. 2. Change of phosphorous content depending on the duration of enzymatic degumming and enzyme applied. Enzymes applied: (■) phospholipase A1, Lecitase® Ultra, (□) acyltransferase, LysoMax® Oil. Results are means values ($n = 4$). Error bars represent the standard deviations of mean values.

case of rapeseed oil, the phosphorous decreased to less than 5 mg/kg, using a similar phospholipase A1 (Clausen, 2001). However, in rice bran oil, the phosphorus content was just reduced to 18 mg/kg with this enzyme. Only after bleaching the enzyme-degummed oil the phosphorus content decreased to less than 5 mg/kg (Roy, Rao, & Prasad, 2002). The phosphorous content in soybean oil degummed by phospholipase B was 4.9 mg/kg (Jiang et al., 2011).

The results obtained in the present study, indicated the high efficiency of the enzymes to achieve the phosphorous content required for physical refining. Moreover, these results validated the subsequent analysis of the quality and stability characteristics of crude and degummed oils. The analytical determinations to evaluate these properties were performed on samples obtained after 60 min of reaction.

The quality and stability characteristics of the crude sunflower oil and enzymatic degumming oils are summarized in Table 1.

3.2. Quality indexes

The acid value of crude sunflower oil was 2.22 ± 0.04 mg KOH/g of oil. The average acidity was in the range from 1.95 to 3.34 mg KOH/g of oil detected in a previous study for such oils (Brevedan et al., 2000).

The acid value of crude sunflower oil was increased from 2.22 ± 0.04 to 3.95 ± 0.07 mg KOH/g of oil in the degummed oil by Lecitase® Ultra. This increase was due to the fatty acids released during enzymatic hydrolysis of the phospholipids. It should be noted that obtained-acid values does not represent drawback for its reduction during refining process. B. Yang et al. (2006) reported that in the cases of rapeseed and soybean oils the enzymatic degumming process using phospholipase A1 caused a slight increase of acid value. Similarly, in the case of rice bran oil was reported that Lecitase® Ultra increased the free fatty acids content (Jahani et al., 2008).

As observed in Table 1, for enzymatic degumming testing using LysoMax® Oil, there was a slight decrease of acid value compared to crude oil (1.65 ± 0.05 mg KOH/g of oil), due to the behavior of the acyltransferase enzyme. Dijkstra (2011) informed that this enzyme can extract a fatty acid fraction of a phosphatide and transfer it to a present sterol in the oil making fatty acid ester.

The moisture content of the enzyme degummed oils was greater than twice that of crude oil. The source of this variability could be due to the addition of the buffer aqueous solution necessary for the enzymatic reaction.

The peroxide value of the crude sunflower oil was 4.63 ± 0.18 meq/kg. Similarly, Crapiste, Brevedan, and Carelli (1999) informed that the peroxide value was 3.92 meq/kg in industrial crude sunflower oil obtained by hexane extraction.

For degummed oils using Lecitase® Ultra and LysoMax® Oil, the peroxide value was found to be 5.38 ± 0.12 meq/kg and 4.88 ± 0.20 meq/kg respectively. The higher peroxide values of degummed oils might be due to oxidation caused by moisture achieved with the enzymatic process. Similarly, Jung et al. (1989) observed the correlation between peroxide value and moisture, in soybean oil using traditional degumming process.

It is well known that hydroperoxides are the primary products formed during oxidation, but they are labile intermediate compounds that decompose into several secondary oxidation products. Thus, even though peroxide value is a common indicator of lipid oxidation, its use is limited to the earlier stage of oxidation (Crapiste et al., 1999).

The anisidine value, which measure secondary oxidation compounds, was lower than 4 in all samples. No significant differences were observed for this index in the oil samples analyzed. Peroxide value increased slightly from crude oil to degummed oils, while anisidine value remained approximately constant with enzymatic degumming. That behavior was certainly owed to the generation and decomposition of hydroperoxides without removal of secondary decomposition products during the enzymatic degumming.

The total oxidation (TOTOX) index was calculated as a combination of peroxide value and anisidine value parameters, as indicated in Table 1. This index is a good indicator of oil oxidation history (Autino, 2009).

The total oxidation values obtained were lower than 15 in all cases. The TOTOX analysis results were consistent with the indexes taken to its calculation suggesting only a possible primary oxidation.

Phospholipids are natural components of oilseeds that pass to oil during extraction.

The phospholipids content in the crude oil was 0.631 ± 0.065 g/100 g. This content was strongly affected by the enzymatic degumming process. The phospholipids composition was reduced up to 2.2 g/100 g by enzymatic degumming with Lecitase® Ultra. Carelli, Ceci, and Crapiste (2002) found that total phospholipid content of the crude sunflower oil decreased up to 7 g/100 g, by the enzymatic degumming treatment with phospholipase A1. Nearly 98 g/100 g of the total phospholipids were removed from crude oil by enzymatic degumming with LysoMax® Oil.

The calcium and magnesium content in crude sunflower oil was extremely high, and it was reduced noticeably with the enzymatic process. This may indicate that the higher percentage of ions was

complexed with phospholipids. Dijkstra (2010) stated that the calcium and magnesium phospholipids salts present in the oil as non-hydratable phospholipids leave the oily phase after the enzymes action.

Copper and iron metals are among the most common contaminants. These metals, particularly copper ions, act accelerating the deterioration of the oil (Autino, 2009; Brevedan et al., 2000).

When phospholipase A1 was added to the oil and mixed at 50 °C for 60 min, the iron content of the crude oil decreased from 8.37 ± 0.66 to 1.02 ± 0.10 mg/kg. The iron content of crude sunflower oil was reduced to 3.05 ± 0.23 mg/kg after enzymatic degumming using acyltransferase, at the same reaction time. The copper content in the crude and enzymatic degummed oils was the lowest. It has been reported previously that water degumming reduced significantly the iron and copper concentration (Brevedan et al., 2000).

Crude vegetable oils contain various natural color substances. The color of crude oil can be directly affected by oilseed/crude oil storage conditions, as time, availability oxygen and temperature (Tasan et al., 2011). Oxidation has an important effect on the color of fats and oils (Carrín & Carelli, 2010).

As shown in Table 2, the "L" value obtained for the crude sunflower oil was lower compared to enzymatic degummed oils, which indicates that crude oil is darker, closer to black. The parameter "a" was positive, indicating an approach to the reds in the crude sample. In the degummed oil samples with phospholipase A1 and acyltransferase, "a" value was found to be −2.02 ± 0.21 and −1.52 ± 0.08, respectively. The negative values for this parameter refer to an approach to the green. The "b" value was the highest for the enzymatic degummed samples, but all the samples tested positive for this parameter, indicating a tendency to yellow tones.

Darkening of the crude sunflower oils could be due to the oxidation of colorless compounds (Tasan et al., 2011). The bleaching is a process whereby the clay absorbent is mixed with the oil, in order to remove unwanted color bodies (Carrín & Carelli, 2010). Color pigments were retained in the gums during the enzymatic treatment. This could mean a benefit for subsequent bleaching.

3.3. Oxidative stability

Oxidative stability is influenced by factors such as temperature, oxygen availability and the concentration of minor compounds with antioxidant or prooxidant characteristics (Autino, 2009; Brevedan et al., 2000; Crapiste et al., 1999).

Induction time for oxidative stability of the crude oil, measured by the Rancimat method, was 17.25 h. In the degummed oil samples with phospholipase A1 and acyltransferase, the oxidative stability index was found to be 4.18 h and 4.33 h respectively. The differences in oxidative stability could be attributed to the concentration of phospholipids in degummed oils, which have strong antioxidant effects. Brevedan et al. (2000) and Alpaslan, Tepe, and Simsek (2001), support these results.

Table 2
Values obtained of the Hunter L, a, b color scale for crude and enzyme degummed oils samples.

Sample	L	a	b
Crude sunflower oil	57.62 ± 2.10[a]	5.43 ± 0.53[c]	35.60 ± 1.65[a]
Degummed phospholipase A1 oil	82.03 ± 2.57[b]	−2.02 ± 0.21[b]	41.73 ± 1.73[b]
Degummed acyltransferase oil	77.65 ± 2.18[b]	−1.52 ± 0.08[a]	42.37 ± 1.38[b]

Results are mean values ± standard deviation ($n = 4$).
Different letters in the same column indicate significant differences ($P < 0.05$). ("a" means the lowest value, "c" means the highest value).

4. Conclusions

The quality and oxidative stability of crude sunflower oil were strongly affected by the enzymatic degumming process.

Acidity was significantly increased by degumming process with Lecitase® Ultra. The transferase activity of LysoMax® Oil caused a slight decrease of acid value. So, it is suggested implement this enzyme for degumming of sterol-rich oil.

Iron and cooper were better extracted by phospholipase A1 than by acyltransferase. Therefore, it is recommended the use of Lecitase® Ultra enzyme when the oil to be treated has high metal content.

The phosphorus content achieved during the degumming process with both enzymes was lower than 3 mg/kg. Hence, the enzyme-degummed oil is fitted for physical refining.

The anisidine value was not significantly affected by the enzymatic treatment. Peroxide value increased slightly from crude oil to degummed oils. These results revealed the presence of hydroperoxides and oxidized compounds.

The crude sunflower oil was darker than degummed oils. During the process, some color pigments were retained in the gums.

The phospholipid content in crude sunflower oil was reduced noticeably by the enzymatic degumming process. The calcium and magnesium content followed the same trend as the phospholipids. Thus, the metal scavenging capacity of the phospholipids was clearly demonstrated by this study.

The degumming process produced a decrease of phospholipids and an increase in the peroxide value, yielding considerably less-stable oils. To minimize the deterioration of edible oil during enzymatic treatment, it is recommended exclude light and oxygen and remove metals and oxidized compounds. On the other hand, it is suggested examine the effect of the enzymatic process on antioxidant components such as tocopheroles and phenolic compounds, in order to maintain them as high as possible.

References

Alpaslan, M., Tepe, S., & Simsek, O. (2001). Effect of refining processes on the total and individual tocopherol content in sunflower oil. *International Journal of Food Science and Technology, 36*, 737–739.

AOCS. (2009). In D. Firestone (Ed.), *Official methods and recommended practices of the American Oil Chemists' Society* (5th ed.). Champaign, USA: Press.

Autino, H. C. (2009). Temas Selectos en Aceites y Grasas Vol. 1 – Procesamiento. In J. M. Block, & D. B. Arellano (Eds.), *Desgomado* (pp. 97–142).

Brevedan, M. I. V., Carelli, A. A., & Crapiste, G. H. (2000). Changes in composition and quality of sunflower oils during extraction and degumming. *Grasas y Aceites, 51*, 417–423.

Carelli, A. A., Ceci, L. N., & Crapiste, G. H. (2002). Phosphorus-to-phospholipid conversion factors for crude and degummed sunflower oils. *Journal of the American Oil Chemists' Society, 79*, 1177–1180.

Carrín, M. E., & Carelli, A. A. (2010). Changes in quality and composition of sunflower oil during processing and storage. In M. C. Tomas (Ed.), *Advances in Fats and Oils Research* (pp. 25–48). Transworld Research Network.

Chakrabarti, P. P., Rao, B. V. S. K., Roy, S. K., Bethala, L. A. P. D., Karna Narayana, P. R., Vemulapalli, V., et al. (2009). Process for the pre-treatment of vegetable oils for physical refining. United States Patent US 7.494.676 B2 (in English).

Clausen, K. (2001). Enzymatic oil-degumming by a novel microbial phospholipase. *European Journal of Lipid Science and Technology, 103*, 333–340.

Crapiste, G. H., Brevedan, M. I. V., & Carelli, A. A. (1998). *Water degumming of sunflower oil*. In S. S. Koseoglu, K. C. Rhee, & R. F. Wilson (Eds.), *World conference on oilseed and edible oils processing* (Vol. 2); (pp. 32–35). Champaign IL: AOCS Press.

Crapiste, G. H., Brevedan, M. I. V., & Carelli, A. A. (1999). Oxidation of sunflower oil during storage. *Journal of the American Oil Chemists' Society, 76*, 1437–1443.

Dayton, C. L. G., Staller, K. P., & Berkshire, T. L. (2010). Process for improving enzymatic degumming of vegetable oils and reducing fouling of downstream process equipment. United States Patent 7, 713–727 (in English).

Dijkstra, A. J. (2010). Enzymatic degumming. *European Journal of Lipid Science and Technology, 112*, 1178–1189.

Dijkstra, A. J. (2011). Un Nuevo análisis del proceso de desgomado de aceites vegetales. *Aceites & Grasas, 82*, 120–123.

Galhardo, F., & Hitchman, T. (2011). Has degumming with enzymes come of age? *International News on Fats, Oils and Related Materials, 22*, 331–334.

Gallardo, F., Dayton, C., Autino, H., Odone, M., Imoda, V., & Lascano, A. (2010). Desgomado enzimático de aceites vegetales. Comparación de diferentes alternativas de procesos. *Aceites & Grasas, 79*, 204–212.

IUPAC. (1992). Standards methods for the analysis of oils, fats and derivatives. In C. Paquot, & A. Hautfenne (Eds.), *International union of pure and applied chemistry* (7th ed.). Oxford: Blackwell Scientific Publications Inc.

Jahani, M., Alizadeh, M., Pirozifard, M., & Qudsevali, A. (2008). Optimization of enzymatic degumming process for rice brain oil using response surface methodology. *LTW – Food Science and Technology, 41*, 1892–1898.

Jiang, F., Wang, J., Ju, L., Kaleem, I., Dai, D., & Li, C. (2011). Optimization of degumming process for soybean oil by phospholipase B. *Journal of Chemical Technology and Biotechnology, 86*, 1081–1087.

Jung, M. Y., Yoon, S. H., & Min, D. B. (1989). Effects of processing steps on the content of minor compounds and oxidation of soybean oil. *Journal of the American Oil Chemists' Society, 66*, 118–120.

Lamas, D. L., Ceci, L. N., Constenla, D. T., & Crapiste, G. H. (2010). Enzymatic degumming of sunflower oil. In *101st AOCS annual meeting & Expo, Phoenix, Arizona (USA), 16–19 May* (110 pp.).

Lamas, D. L., Ceci, L. N., Crapiste, G. H., & Constenla, D. T. (2011). Análisis y diseño de experimentos aplicados al estudio del desgomado enzimático. In *XIII Congreso Argentino de Ciencia y Tecnología de Alimentos (CYTAL), Buenos Aires, (Argentina), 19e21 October* (Section 3, pp.1–5).

Lamas, D. L., Ceci, L. N., Crapiste, G. H., & Constenla, D. T. (2012). Estudio comparativo de enzimas fosfolipasas y aciltransferasas aplicadas al proceso de desgomado de aceite de girasol. In *Congreso Latinoamericano de Ingeniería y Ciencias Aplicadas (CLICAP), San Rafael, Mendoza, (Argentina), 28e30 March*.

Medina Juarez, L. A., Gamez, M. N., Ortega, G. J., Noriega, R. J. A., & Angulo, G. O. (2000). Trans fatty acid composition and tocopherol content in vegetable oils produced in Mexico. *Journal of the American Oil Chemists' Society, 77*, 721–724.

Pehlivan, E., Arsian, G., Gode, F., Altun, T., & Ozcan, M. M. (2008). Determination of some inorganic metals in edible vegetable oils by inductively coupled plasma atomic emission spectroscopy (ICP-AES). *Grasas y Aceites, 59*, 239–244.

Roy, S. K., Rao, B. V. S. K., & Prasad, R. B. N. (2002). Enzymatic degumming of rice bran oil. *Journal of the American Oil Chemists' Society, 79*, 845–846.

Soe, J. B., & Brown, A. V. (2011). Process United States Patent Application Publication Pu. No.: 0136187 (in English).

Tasan, M., Gecgel, U., & Demirci, M. (2011). Effects of storage and industrial oilseed extraction methods on the quality and stability characteristics of crude sunflower oil (*Helianthus annuus* L.). *Grasas y Aceites, 62*, 389–398.

Turkulov, J., Dimic, E., Karlovic, D., & Vuksa, V. (1998). *Effect of hydrothermal treatment on the quality of nonrefined edible sunflower oil*. In S. S. Koseoglu, K. C. Rhee, & R. F. Wilson (Eds.), *World conference on oilseed and edible oils processing* (Vol. 1); (pp. 185–187). Champaign IL: AOCS Press, 23–25 September.

US EPA. United States Environmental Protection Agency. (February, 2007). In *Inductively coupled plasma. Atomic emission spectrometry*. Revision. 03. Available at www.epa.gov/epaoswer/hazwaste/test/pdfs/6010c.pdf.

Villa Salinas, V. M. (2009). *Temas Selectos en Aceites y Grasas Vol. 1 – Procesamiento*. In J. M. Block, & D. B. Arellano (Eds.), *Clarificación de aceites y grasas* (pp. 161–210).

Yang, B., Wang, Y. H., & Yang, J. G. (2006). Optimization of enzymatic degumming process for rapeseed oil. *Journal of the American Oil Chemists' Society, 83*, 653–658.

Yang, J. G., Wang, Y. H., Yang, B., Mainda, G., & Guo, Y. (2006). Degumming of vegetable oil by a new microbial lipase. *Food Technology and Biotechnology, 44*, 101–104.

Yang, B., Zhou, R., Yang, J. G., Wang, Y. H., & Wang, W. F. (2008). Insight into the enzymatic degumming process of soybean oil. *Journal of the American Oil Chemists' Society, 85*, 421–425.

Yoon, S. H., & Min, D. B. (1986). Effect of thermally oxidized compounds on the flavor stability of soybean oil. *Journal of the American Oil Chemists' Society, 63*, 425–426.