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COMPARISON OF SOXHLET AND ULTRASOUND METHODS FOR OIL EXTRACTION FROM SPANISH FLAXSEEDS

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ABSTRACT

Flaxseed oil contains an outstanding amount of the essential fatty acid α-linolenic acid (18:3n-3), as well as other ω-3 fatty acids, which can provide potential benefits in individuals with cardiovascular diseases. This explains the interest of food industries to improve the efficiency of oil extraction from flaxseed. In this sense, the feasibility of two extraction methods (Soxhlet and ultrasounds) has been evaluated through the determination of the amount of oil obtained from 9 flaxseed trademarks (*Linum usitatissimum* L.). The Soxhlet method extracted more oil than the ultrasounds, with mean values being 36.9 and 20.0%, respectively. Aside from the extraction method, flaxseed trademark was also an influencing variable. In this context, the highest extraction yield (53%) was detected when the Soxhlet method in combination with petroleum ether were used with trademark 1 (raw golden flaxseed). However, further studies are needed to evaluate the quality and safety of the extracted oils.

Keywords: *Linum usitatissimum*, oil yield, process optimization, seed oil extraction

INTRODUCTION

Essential unsaturated fatty acids are abundantly present in fish oils, which unfortunately are a limited source from an environmental point of view. Despite this, the relationship of ω-3 consumption with the prevention of cardiovascular disease **(Zhang *et al.*, 2012)** has increased the interest of food and pharmaceutical industries to explore new and more sustainable sources in order to obtain unsaturated fatty acids. A potentially interesting option is flaxseed oil. In this vein, the high amount of the essential fatty acid α-linolenic acid (ALA, 18:3n-3) and other ω-3 fatty acids in flaxseeds (*Linum usitatissimum* L.) is a unique feature among edible plant sources **(Ganorkar *et al.*, 2013; Singh *et al.*, 2011)**. This implies the need for protocols that allow highly performing, efficient, and economical extraction methods. On this basis, it is important to define the best processes that allow manufacturing of flaxseed oils into rich, high quality and functional components.

It is important to highlight the interest that vegetable-oil bioactive compounds are acquiring **(Thongson *et al.*, 2004).** In this context, the multiple beneficial properties on health, both preventive and curative, especially if the product is included in the daily diet, have increased the interest of the consumers for flaxseeds and their based-products, mainly oil **(Pan *et al.*, 2009).** Flaxseed oil has significant nutritional benefits, mainly due to the abundance of ω-3 fatty acids, has a considerable content of lignans and contains polyphenolic compounds, which are mostly responsible for its high antioxidant capacity **(Turatti, 2000; Best, 2004).** The consumption of these components is associated with beneficial effects on hormonal regulation, as well as in the prevention of diseases, such as cancer, diabetes and cardiovascular problems **(Morris, 2001).** Altogether, this explains why worldwide researchers are looking for plant oil extraction methods that are efficient, economic and environment-friendly.

Since the extraction method has a significant impact on the quality of the commercial oil and its functionality, we have decided to explore this aspect in the present study by using different flaxseed trademarks. The decision to carry out trials with different flaxseed brands was to analyze if there are differences among them when using the same oil extraction method, i.e., if the efficiency of the oil extraction may differ among the trademarks analyzed or, for a particular sample, obtaining higher performance is associated with a given method of extraction. This is due to the fact that each sample has particular biological and physical-chemical characteristics, which could produce a better or worse response to certain solvents or methods. To our knowledge, there are no comprehensive studies on the effect of different protocols on flaxseed oil extraction. Therefore, it is necessary to evaluate the effect of various parameters influencing the extraction of oils from commercial brands of flaxseed, including solvent, extraction time, temperature, and the amount of sample and the volume of solvent used.

MATERIAL AND METHODS

Materials

Nine samples of gold and brown flaxseeds obtained from drugstores, food and nutrition stores and large farms were used. Flaxseed samples arrived to the laboratory in plastic bags identified with a bar code, in 250 or 500 g formats. Once registered, flaxseed samples were grinded in a domestic Taurus grinder, model Aromatic, 230V-50 Hz, 150W Barcelona (Spain), including samples 7-9. Afterwards, the samples were taken to the CSA (Food Quality and Safety) Research Group facilities for analysis. This preparation of the samples was essential to allow the solvent to penetrate into the seed cells and achieve a complete removal/extraction of the oil.

Twenty grams of flaxseeds were used to perform the oil extraction by the Soxhlet method, while 1 g of flaxseeds was used for the ultrasound method. These quantities were used to ensure that sufficient amount of oil could be extracted. The sample was placed in a paper filter bag. The evaluation of the performance of the oil extraction from flaxseeds was performed based on the total dry mass of the sample under analysis and the extracted oil content. Thus, the performance was determined by the ratio of the mass of oil obtained and the total mass of the sample. Results were expressed as a weight percentage, the according equation:

Fat (%) = (W' – W / W'') x 100

where, W = weight (g) of the filter bag; W' = weight (g) of the filter bag with fat; W'' = weight (g) of the sample.

Oil extraction

The samples of 9 commercial brands of ground flaxseeds were used for oil extraction (Table 1). The total supply of digestible rumen undegraded protein (RUP) was not significantly different between the two seed types (Khan, Booker, & Yu, 2014). Extraction was carried out using 2 methods (Soxhlet and ultrasounds), and with three different types of solvents (petroleum ether, diethyl ether and *n*-hexane). The extract was concentrated on a rotary evaporator for further analysis. The oil obtained from extraction was stored in amber jars and frozen until analysis. After the extraction, the oil presented a pale yellow color.

Table 1 Main characteristics of the samples used in the study

Trademarks	Golden	Brown	Presentation
1	✓		Raw seeds
2	✓		Raw seeds
3	✓		Raw seeds
4	✓		Raw seeds
5	✓		Raw seeds
6	✓	✓	Raw seeds
7	✓	✓	Grinded
8	✓	✓	Milled
9	✓		Milled

Soxhlet extraction method

The Soxhlet method involves a solid-liquid extraction, and its main advantage is that the sample is continuously in contact with the solvent, which helps to shift the balance of the transfer towards the solvent. It is also a simple method that allows extracting greater amounts of sample than most modern methods. Some disadvantages of the Soxhlet extraction compared to other techniques include the time required for the extraction and the use of large amounts of solvent. In addition, samples are taken to the boiling point of the solvent for a long period of time, increasing the possibility of thermal decomposition of certain components of the oil. The Soxhlet apparatus used in this experiment, J.P. Selecta, model 60003286, (Selecta, Barcelona, Spain) does not allow for stirring, which would accelerate the extraction process. Due to the large amount of solvent used, evaporation or concentration after the extraction with the rotary evaporator R-205 was required (Buchi, Flawil, Switzerland) under reduced pressure (100 mbar) and at a temperature of 40 °C (Figure 1).

Extraction of oil by ultrasounds

The application of ultrasounds (sound waves of high frequency, 20-40 kHz) facilitates the release of oil from the cell walls of plant material subjected to the extraction process. Ultrasounds are formed of high frequency energy waves undetectable by the human ear. The resulting vibrations provoke the expansion and compression of the material. This oscillating motion forms bubbles or cavities in the liquid where, by effect of the waves, the temperature is increased causing the breakdown of the surface tension. This favors the solubility by penetrating the solvent into the plant material and dragging with it the active components due to concentration differences (Azuola & Vargas, 2007). Therefore, this extraction technique is based on the mass transfer of solid components into the solvent that is subjected to ultrasound, using as a phenomenon of transport by diffusion through the cell wall and convection in the pores of the solid component. The effect of the ultrasounds resulted in solid and liquid particles in constant vibration and acceleration, causing the rapid passage of the solute located in the solid phase to the solvent (Azuola and Vargas, 2007). Approximately 1 g of flaxseeds samples were used for the application of the ultrasonic waves, using different solvents. The samples were exposed continuously to sound waves in a bath, in cycles of 3 h at 40 kHz of frequency (Hojatti, 2008) (Figure 1).

Statistical analysis

The statistical analysis was carried out using STATGRAPHICS Centurion XV software (StatPoint Technologies, Inc. Warrington, VA, USA); likewise, significance was defined at $p \leq 0.05$. Data were reported as mean, standard deviation and the minimum and maximum values. Inter-group statistical comparisons were performed using the analysis of variance with three factors (method, solvent and trademark), the one-way analysis of variance, the Levene's test for the study of homogeneity among variances, as well as the Kruskal-Wallis hypothesis tests followed by Fisher's least significant difference (LSD) in order to determine which means were significantly different from the others. A rank transformation of the data was performed, although the obtained results were essentially the same. A box-and-whisker plot was used to graphically represent the data.

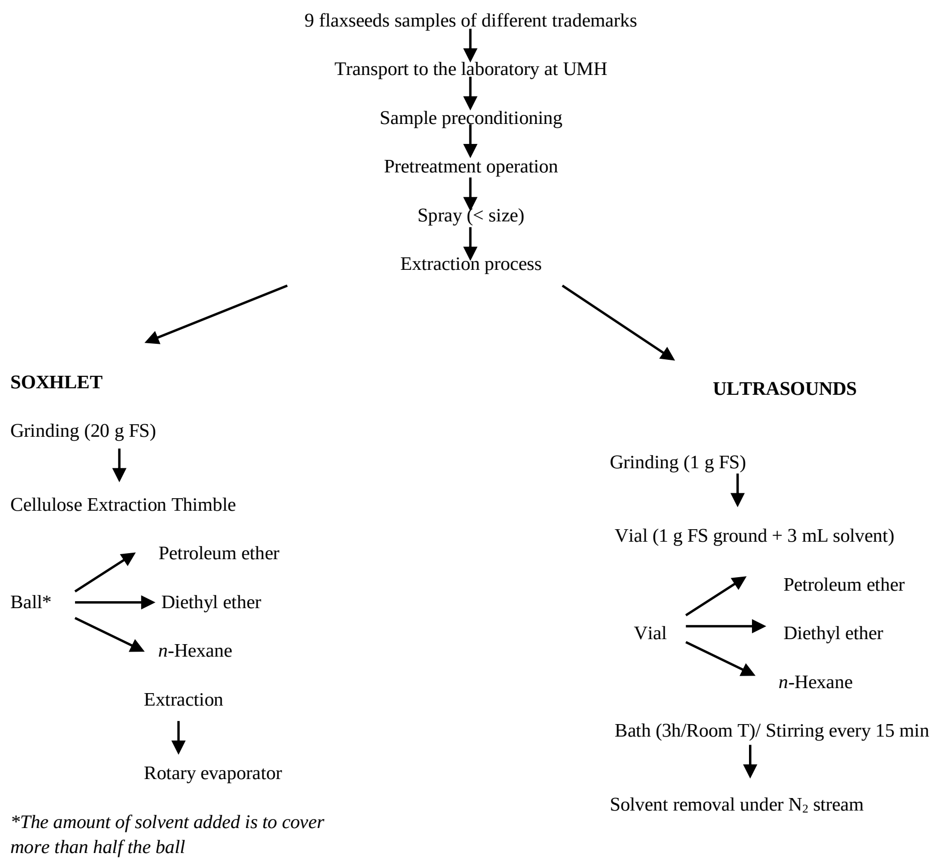

Figure 1 Flow diagram showing the two protocols used for the extraction of the oil from the flaxseed samples. Abbreviations used: RT, room temperature; UMH, University Miguel Hernandez.

RESULTS AND DISCUSSION

The oil extraction from flaxseed samples is basically a mass transfer procedure, and depends on key parameters such as the choice of the most adequate extraction method, the type of solvent used, as well as the nature of the sample to be extracted. The selection of solvents used is mainly due to their polarity and their affinity for lipid components. One of the disadvantages of the use of solvents in these extraction methods is that there are legal restrictions on the maximum residues that may be left in the oils, especially when the oils are the raw material for the production of foods or nutraceutical products. These limits are based on current legislation and may vary in the future, but always towards lower contents to guarantee the safety of consumers. Furthermore, it is believed that prolonged exposure of the flaxseed samples to the solvents can cause oxidation of fatty acids **(Singh *et al*., 2011).**

Tables 2-7 show the results of the oil percentage obtained from samples of different flaxseed brands/trademarks. Each table summarizes the results obtained for each trial and its replicate, indicating the method applied and the flaxseed sample used. Also, the charts provide relevant statistical parameters such as the average value, its standard deviation, as well as the upper and lower values obtained in each test.

Table 2 Oil yield (5) obtained after extraction by the Soxhlet method using petroleum ether

SOXHLET EXTRACTION METHOD			
Solvent: petroleum ether			
Trademarks (20 g)	**Oil (%)**	**Replicates**	**Oil (%)**
1	36.50	1	**53.00**
2	31.20	2	30.60
3	41.45	3	38.70
4	46.00	4	37.45
5	40.90	5	36.00
6	40.00	6	38.85
7	35.40	7	36.55
8	**11.35**	8	**11.80**
9	**48.05**	9	45.05
Mean	36.76		36.44
SD	10.85		11.19
Upper range	48.05		53.00
Lower range	11.35		11.80

Table 3 Oil yield (%) obtained after extraction by the Soxhlet method using diethyl ether

SOXHLET EXTRACTION METHOD			
Solvent: diethyl ether			
Trademark (20 g)	**Oil (%)**	**Replicates**	**Oil (%)**
1	38.50	1	41.50
2	42.25	2	33.75
3	44.10	3	43.75
4	41.45	4	44.65
5	41.20	5	43.05
6	**45.75**	6	45.55
7	42.80	7	40.15
8	**15.80**	8	**15.45**
9	44.00	9	**49.00**
Mean	39.54		39.65
SD	9.14		10.00
Upper range	45.75		49.00
Lower range	15.80		15.45

Table 4 Oil yield (%) obtained after extraction by the Soxhlet method using n-hexane

SOXHLET EXTRACTION METHOD			
Solvent: *n*-hexane			
Trademarks (20 g)	**Oil (%)**	**Replicas**	**Oil (%)**
1	25.70	1-1	35.75
2	29.00	2-1	30.55
3	37.75	3-1	38.10
4	24.50	4-1	15.70
5	40.35	5-1	45.30
6	**50.60**	6-1	42.35
7	36.80	7-1	41.90
8	**14.75**	8-1	**14.10**
9	50.20	9-1	**49.60**
Mean	34.41		33.98
SD	12.01		12.55
Upper range	50.60		49.60
Lower range	14.75		14.10

Table 5 Oil yield (%) obtained after extraction by the ultrasounds method using petroleum ether.

ULTRASOUNDS EXTRACTION METHOD			
Solvent: petroleum ether			
Trademarks (20 g)	**Oil (%)**	**Replica**	**Oil (%)**
1	16.90	1-1	26.60
2	17.70	2-1	**32.20**
3	19.60	3-1	23.80
4	**23.40**	4-1	30.60
5	20.30	5-1	27.80
6	20.60	6-1	27.50
7	19.90	7-1	22.30
8	**7.70**	8-1	**10.40**
9	16.90	9-1	24.70
Mean	18.11		25.10
SD	4.41		6.34
Upper range	23.40		32.20
Lower range	7.70		10.40

Table 6 Oil yield (%) obtained after extraction by the ultrasounds method using diethyl ether.

ULTRASOUNDS EXTRACTION METHOD			
Solvent: diethyl ether			
Trademarks (20 g)	**Oil (%)**	**Replica**	**Oil (%)**
1	21.30	1-1	18.30
2	20.40	2-1	14.80
3	15.40	3-1	18.10
4	24.20	4-1	21.40
5	24.10	5-1	20.60
6	21.80	6-1	19.50
7	25.70	7-1	18.80
8	**7.80**	8-1	**6.10**
9	**26.50**	9-1	**23.30**
Mean	20.80		17.88
SD	5.90		5.01
Upper range	26.50		23.30
Lower range	7.80		6.10

Table 7 Oil yield (%) obtained after extraction by the ultrasounds method using n-hexane.

ULTRASOUNDS EXTRACTION METHOD			
Solvent: *n*-hexane			
Trademarks (20 g)	**Oil (%)**	**Replicas**	**Oil (%)**
1	23.20	1-1	16.70
2	25.20	2-1	14.10
3	24.70	3-1	13.00
4	24.50	4-1	17.40
5	21.00	5-1	16.90
6	24.10	6-1	15.60
7	25.50	7-1	13.90
8	**10.70**	8-1	**5.70**
9	**28.10**	9-1	**21.10**
Mean	23.00		14.93
SD	4.98		4.22
Upper range	28.10		21.10
Lower range	10.70		5.70

The extraction efficiency depends on several factors, such as the extraction method used (including the solvent) and the oil content in the seeds. Regarding the extraction method, data on Tables 2-4 show that high percentages of oil were obtained by the Soxhlet method when using the 3 assayed solvents (36.6% petroleum ether, 39.6% diethyl ether and 34.2% *n*-hexane). Data on Tables 5-7 indicate that lower percentages of oil were extracted by the ultrasounds method using the 3 assayed solvents (21.6% petroleum ether, 19.3% diethyl ether and 19.0% *n*-hexane), as compared to those above shown for the Soxhlet method.

The data in tables 2 and 5 indicate that the extraction method leading to the highest mean oil content, when using petroleum ether as solvent, was Soxhlet (36.6%) as compared to ultrasounds (21.6%). This result was also observed in tables 3 and 6, where diethyl ether was used as solvent (39.6% with Soxhlet, 19.3% with ultrasounds); as well as in tables 4 and 7, where *n*-hexane was used (34.2% with Soxhlet, 19.0% with ultrasounds). Therefore, with respect to the methodology used for extraction, the results support that Soxhlet was the higher performing method. This is partly due to the steam being not directly in contact with the solvent in the liquid state and therefore less amount of the oil was degraded when the temperature was increased **(AOAC, 1998)**. In this context, the considerable oil yield obtained with Soxhlet is a result of the mass transfer between samples and solvents at high temperature due to increased vapor pressure, which decreases viscosity.

Regarding the solvent used, diethyl ether yielded the best results in the Soxhlet extraction method, confirming previous reports indicating that this solvent is more efficient in extracting acyclic hydrocarbons **(Tornabene *et al.*, 1982).** On the other hand, the best results for the ultrasound extraction method were obtained when petroleum ether was the selected solvent. These are positive results because the two solvents have low toxicity for both humans and the environment and it is relatively simple to treat and dispose of **(Metherel *et al.*, 2009).** In any case, the solvents used in this study are recognized as having a low risk for human health **(European Medicines Agency. 2009).**

Regarding the brand, sample 9 (milled golden flaxseed) presented the highest performance values when using the Soxhlet method, reaching up to 49.0% (Tales 3 and 4). In this context, the oil content in the flaxseeds depended on the variety and climate conditions of the location where they were cultivated **(Gallegos, 2008; InfoAgro, 2014).** The difficulty that can arise in the oil extraction is that flaxseeds oil is strongly trapped in the intracellular oil-containing granules. Additionally, seeds have a woody structure. For this reason, the material must be previously grinded in order to allow a greater contact area between the solid material and the solvent. The small particles resulting from the grinding facilitate the solvent extraction, by decreasing the distances that the oil and solvent must travel, inside and outside the seeds **(Morris, 2001; Best, 2004; Turatti, 2000).** This explains why trademark 9 gave the higher extraction yield, because milling allowed smaller particle sizes.

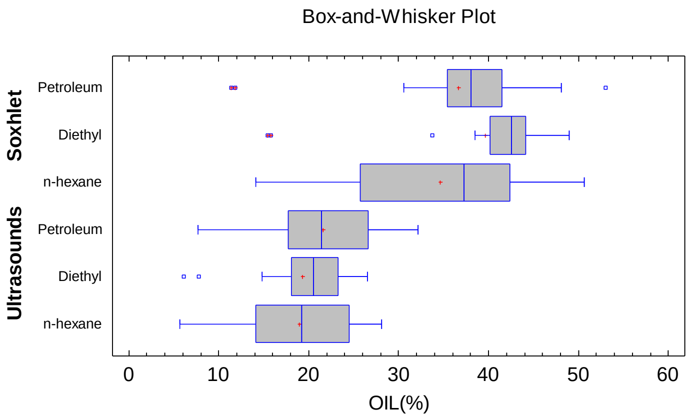

Figure 2 Percentage of the fat content as affected by the extraction method (Soxhlet or ultrasounds) and the solvent (petroleum ether, diethyl ether and n-hexane)

Several studies have shown that the oil composition may vary depending on the extraction method used **(Thongson *et al.*, 2004).** Therefore, it is important to identify all the parameters participating during the extraction. These will affect the oil properties and composition as well as their effects after consumption. For this reason, not only are performance and trademark important factors; other parameters must be considered, such as the extraction time, and the temperature of the process. The established time, which is different for the Soxhlet and ultrasounds methods, are 360 and 180 min, respectively. Regarding the extraction temperature, the Soxhlet method is performed at 40ºC, while the ultrasound extraction is generally performed at room temperature (22-24ºC).

A summary of the oil yields obtained during extraction with the different solvents is shown in Figure 2. The percentages of flaxseed oil extracted ranged from 6.1% (using ultrasounds with diethyl ether as solvent in trademark 8) to 53.0% (using the Soxhlet method with petroleum ether as solvent in trademark 1). It can also be observed that the method, independently of the solvent used, is the most influential factor regarding the extraction efficiency. Therefore, the Soxhlet method provided higher yields than the ultrasounds method for all three solvents. In particular, the mean oil percentage extracted by the Soxhlet method, 36.9%, was almost twice (1.8X) that obtained by the ultrasound method, 20.0%. The ranges of the Soxhlet method were between the highest value (yield= 53%) obtained with trademark 1 using petroleum ether, and the lowest value (yield= 11.35%) with trademark 8 using the same solvent (Table 2).

The mean oil content detected in this study using Soxhlet, 36.9%, can be considered as adequate, but is certainly much lower than that previously reported by **(Backer *et al.*, 1982),** who obtained results between 80% using mechanical extraction and 80-90% using solvent extraction with a temperature of 121°C, far from the room temperature conditions used in the present study. On the other hand, the percentage of extracted oil using different solvents in the current study were found to be higher than those reported by other authors **(Ahsan *et al.*, 2015; Mata, 2009).**

Table 8 summarizes the ANOVA results for the 3 factors under analysis (extraction method, solvent and trademark). This analysis shows that the method and trademark have a statistically significant effect on the percentage of flaxseed oil extracted ($p < 0.001$), while the choice of solvent does not significantly affect the values (see Table 9). Likewise, the most contributing factor, which explained 49.3% of the total variation of the percentage of oil extracted was the method used, followed by the trademark (29.8%). Again, solvent type did not contribute to variations in the percentages of oil extracted.

Table 8 ANOVA analysis oil percentage affected by the extraction method

Source	**Sum of squares**	**Degrees of freedom**	**Mean square**	***F*-Ratio**	***P*-Value**
Between groups	7768.19	1	7768.19	103.22	0.0000
Within groups	7977.79	106	75.26		
Total (Corr.)	15746.0	107			

Table 9 Multi-factorial variance analysis for the three factor model (A/B/C).

Analysis of Variance for oil % - Type III Sums of Squares					
Source	**Sum of Squares**	**Degrees of freedom**	**Mean Square**	***F*-Ratio**†	***P*-Value**
MAIN EFFECTS					
A: trademark	4700.13	8	587.52	18.05	0.00
B: method	7768.19	1	7768.19	238.60	0.00
C: solvent	152.21	2	76.11	2.34	0.10
RESIDUAL	3125.45	96	32.56		
TOTAL (CORRECTED)	15746.0	107			

†*All F-ratios are based on the residual mean square error*

Table 10 ANOVA analysis of oil percentage affected by trademarks

Source	**Sum of squares**	**Degrees of freedom**	**Mean square**	***F*-Ratio**	***P*-Value**
Between groups	4700.13	8	587.52	5.27	0.0000
Within groups	11045.80	99	111.57		
Total (Corr.)	15746.0	107			

The one-way analysis of variance (Table 10) and Kruskal-Wallis test for the factor trademark showed that there were statistically significant differences (both in means and medians) among the trademarks when extracting flaxseed oil (p<0.001 in both cases). As previously mentioned, the mean percentage of flaxseed oil extracted using the Soxhlet method was 36.9%, whereas using the ultrasound method the value obtained was 20.0%. After analyzing the multiple range tests (both for the original data and the transformed data using the rank function) and applying the 95% LSD procedure, it was observed that the 9 trademarks could be ranked in increasing order of the mean percentage of flaxseed oil extracted in the following manner: {8}<{2}<={4,1,3,7}<={5,6}<={9}. Therefore, the trademark is a matter of consideration when analyzing the amount of oil content in a flaxseed.

Finally, other parameters that have not been considered in this study should also be taken into account, such as the quality of the oil obtained, the behavior of the solvent used for extraction, and the final use of the oil. Quality of the final oil can be assessed by determining the fatty acid composition and oxidation levels. Regarding solvent behavior and final use, the solvent type, toxicity and means used for disposal are instrumental factors to consider.

CONCLUSION

Regarding the optimization of the oil extraction from flaxseed products, the conclusion of this study was that the following conditions are recommended, when only considering extraction efficiency: (i) Soxhlet during an extraction time of 360 min, and (ii) using diethyl ether as solvent. The ultrasound method led to smaller amounts of extracted oil. However, this method has several advantages, including the use of shorter extraction times and lower temperatures, thereby minimizing the risk associated to this process. Finally, further research regarding the quality of the oil extracted is currently being performed in our laboratory.

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