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Flax and hemp
Lin et chanvre

Effect of ultrasonic treatment on extraction and fatty acid profile of flaxseed oil

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Abstract – The aim of this study was to optimize extracion of flaxseed oil process by ultrasonic treatment and determination of its effect on ω-3 fatty acid. The extraction of flaxseed oil was optimized by using different solvents like methanol, acetone, petroleum ether, ethanol, hexane and dichloromethane. The ultrasonication treatment was optimized with respect to amplitude (20–80 kHz), temperature (25–40 °C), sonication time (20–80 min) and solid to solvent ratio (1:5, 1:10 and 1:15) for extraction of flaxseed oil. The extracted oil was subjected to GC analysis to determine ω-3 fatty acid. The recovery of flaxseed oil was higher with hexane followed by dichloromethane. The ultrasonic treatment at frequency of 40 kHz, temperature of 30 °C, extraction time of 40 min and solid to solvent ratio: 1:10 gave best results for extraction. The ultrasonic assisted extraction improves the extraction yield by 11.5% with similar amount of solvent. The chromatograph showed that there were no significant effects on α-Linolenic acid (ω-3) by the ultrasonic assisted extraction. This will beneficial to the oil extractor to recover higher amount of oil from same amount of the raw materials.

Keywords: Extraction / ultrasound / flaxseed oil / polyunsaturated fatty acid / linolenic acid (ω-3)

Résumé – Effet du traitement ultrasonore sur le rendement de l'extraction et sur les acides gras oméga-3 de l'huile de lin. L'objectif de cette étude est l'optimisation du processus d'extraction de l'huile de lin par traitement aux ultrasons et de déterminer son effet sur les acides gras ω-3. L'extraction de l'huile de lin a été optimisée en utilisant différents solvants tels que le méthanol, l'acétone, l'éther de pétrole, l'éthanol, l'hexane et du dichlorométhane. Le traitement ultrasonore a été optimisé en termes de fréquence (20–80 kHz), de température (25–40 °C), de temps de sonication (20 à 80 min) et du ratio solide/solvant (1:5, 1:10 et 1:15) pour l'extraction de l'huile de lin. L'huile extraite a été soumise à une analyse par chromatographie gazeuse (GC) pour déterminer la teneur en acides gras omega-3. Le meilleur résultat d'extraction de l'huile de lin a été obtenu avec de l'hexane comme solvant suivi par le dichlorométhane. Le traitement ultrasons à la fréquence de 40 kHz, à la température de 30 °C, avec une durée d'extraction de 40 min et un ratio solide/solvant de 1/10 a donné le meilleur résultat pour l'extraction. L'extraction assistée par ultrasons améliore le rendement d'extraction de 11,5 % avec la même quantité de solvant. L'analyse des acides gras a montré qu'il n'y avait pas d'effet significatif de l'extraction assistée par ultrasons sur l'acide α-linolénique (ω-3). Le traitement ultrasonore permet ainsi de récupérer une plus grande quantité d'huile à partir de la même quantité de matières premières.

Mots clés : Extraction / ultrasons / acides gras polyinsaturés / acide linolénique (ω-3)

1 Introduction

Flax *(Linum usitatissimum* L.) is an annual plant belonging to the genus *Linum* and the family Linaceae (Sultan, 1992). Different cultivars of Linum have been developed for production of fibre and oilseed. Cultivars of *Linum* used for fibre use are called flax, whereas the oilseed cultivars called linseed, oilseed flax or just flax. Flaxseed is also known with different local names such as Alsi, Jawas, and Aksebija. The sensory attributes of flaxseed showed crisp and chewy texture and pleasant nutty taste (Carter, 1996). The important flaxseed growing countries include Canada, China, Russia, India, United States, and Ethiopia. Canada is the world's largest producer of flaxseed (368.300 MT), whereas India rank

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forth (214.38 MT) (FAOSTAT, 2013). In India, flaxseed is mainly cultivated in Madhya Pradesh, Uttar Pradesh, Bihar, Chattisgarh and Maharashtra (AGROPEDIA, 2014).

Flaxseed is the richest plant source of the ω-3 fatty acid *i.e.* α- linolenic acid (ALA) (Gutte *et al.*, 2015). Flaxseed oil is low in saturated fatty acids (9%), moderate in monosaturated fatty acids (18%), and rich in polyunsaturated fatty acid (73%) (Cunnane *et al.*, 1993). ALA can be metabolized in the body into docosahexaenoic acid (DHA) (ω-3) and eicosapentaenoic acid (EPA) (ω-3) due to the action of some kinds of enzyme (Chen, *et al.*, 2002). The health benefits of all ω-3 fatty acids (ALA, EPA and DHA) have been widely reported for several conditions including cardiovascular disease, hypertension, diabetes, cancer, arthritis, osteoporosis, autoimmune and neurological disorders (Gogus and Smith 2010; Simopoulos, 2000). Flaxseed also used in minimization of diseases such as hyperlipidemia (Vijaimohan *et al.*, 2006), colon tumor (Dwivedi *et al.*, 2005), mammary cancer (Thompson *et al.*, 1996; Wang *et al.*, 2005), atherosclerosis (Prasad, 1997; Yamashita, 2005).

Edible oil commonly extracted by conventional mechanical press, mechanical screw presses (oil expellers) and solvent extraction with organic solvents. The conventional mechanical press bears many impurities such as free fatty acids, coloured and others gummy materials which are known to be detrimental to oil flavour and stability (Bera *et al.*, 2004). Mechanical screw presses (oil expellers) are relatively inefficient, leaving about 8–14% of available oil in the de-oiled cake (Singh and Bargale, 2000). Organic solvent extraction is dependent on various factors such as the nature of the solvent, reaction time, size of seeds, process temperature and the solid/solvent ratio. The most severe shortcomings of solvent extraction are the long-time involved and the larger volumes of organic solvents are not desirable can harmful to human and environment released in the atmosphere.

The recent studies have shown that the ultrasound-assisted extraction of can enhance the extraction efficiency through acoustic cavitation and some mechanical effects (Chemat *et al.*, 2012, 2004; Rostagno *et al.*, 2003). Acoustic cavitation can disrupt cell walls facilitating solvent to penetrate into the plant material and allowing the intracellular product release. Another mechanical effect caused by ultrasound may also be the agitation of the solvent used for extraction, thus increasing the contact surface area between the solvent and targeted compounds by permitting greater penetration of solvent into the sample matrix. The ultrasonic-assisted extraction (UAE) also required shorter extraction times and reduced solvent consumption (Li *et al.*, 2004; Mason *et al.*, 2011; Nazir *et al.*, 2009; Zhang *et al.*, 2008). Whereas Hromadkova *et al.* (1999) reported that, ultrasound-assisted extraction can be carried out at a lower temperature which can avoid thermal damage to the extracts and minimize the loss of bioactive compounds.

The aim of this study was to determine the effects of ultrasonic assisted extraction on yield of flaxseed oil and fatty acid profile of flaxseed oil. The results from UAE were compared with that obtained from the conventional solvent extraction and the fatty acid compositions of flaxseed oils extracted by UAE was measured using gas chromatography (GC) to investigate the effect of ultrasound on the quality of flaxseed oil extracted.

2 Materials and methods

2.1 Flaxseed

Flaxseed *(Linum usitatissimum* L.) procured from a farmer market from Gangakhed, District Parbhani, Maharashtra, India. The flaxseed was cleaned to remove the foreign materials such as other seeds, stones and small stalks. These flaxseeds were stored in dry and cool place until further use.

2.2 Screening of different solvents for extraction oil from flaxseed

Different polar and non-polar solvents namely methanol, acetone, petroleum ether, *n*-hexane and dichloromethane were screened to check the extractability of flaxseed. The samples of grinded flaxseed (10 g) were placed in 250 ml beaker. In extraction solid to solvent ratio (1:10 w/v.) was maintained. This mixture was agitated with magnetic stirrer at 100 rpm for 10 min and the samples were hold for overnight. The obtained micelle was filtered using a vacuum filtration to remove suspended solids. Subsequently, the solvent was evaporated from the oil using rotary vacuum evaporator and extracted oil was collected in the receiving flask.

2.3 Ultrasound-assisted extraction of oil from flaxseed

The UAE experiments, a 250 W, 20 to 100 kHz ultrasonic emulsifier with a 2.00 cm flat tip probe were used. The ultrasonic output power could be set to a desired level ranging from 0 to 100% of the nominal power by the amplitude controller. Ultrasonic output powers were determined calorimetrically and ranged from 10 to 100 W according to the method described by Li *et al.* (2004). The ultrasound-assisted extraction used in this study was similar to that described by Zhao *et al.* (2007) with slight modifications. The flaxseed powder was mixed in 100 ml *n*-hexane contained in a 250 ml plastic beaker. The ultrasonic probe was inserted into the mixture directly. The samples were extracted under continuous ultrasonic waves at 20 kHz to 100 kHz at different levels of power output. During extraction, the temperature was controlled at a desired level within ±1 °C. Further the extracts were carried out similarly as the procedure described in conventional method.

2.4 Yield determination

The yield of extracted oil from flaxseed was calculated as follows

$$\text{Yiled } (\%) = \frac{We}{Wt} \times 100$$

where,

We = weight of extracted oil and Wt = weight of sample taken for extraction.

Table 1. Fatty acid composition (% of total fatty acids) of flaxseed oil extracted by different methods.

Fatty acid	Solvent extraction	Ultrasonic assisted extraction
	Saturated fat	
C 16:0	4.94	6.69
C 18:0	4.83	5.50
C 20:0	0.21	0.20
C 22:0	0.28	0.30
C 24:0	0.17	0.19
Total saturated fasts	10.44	12.87
	Monounsaturated fats	
C 18:1n9c	21.26	20.34
C 20:1	0.10	0.10
C 24:1	0.09	0.12
Total monounsaturated fats	21.44	20.56
	Polyunsaturated fats	
C 18:2n6c	12.74	14.19
C 18:3n6	1.81	0.00
C 18:3n3	53.57	52.37
Total polyunsaturated fats	68.12	66.56

2.5 Gas chromatographic analysis

Fatty acid compositions of the oils extracted by conventional method and ultrasound-assisted extraction (UAE) were determined by Gas Chromatography (GC). The extracts were converted to fatty acid methyl esters (FAME) for subsequent chromatographic analysis. Obtaining fatty acid methyl esters (FAME) initially, the oil samples obtained were esterified according to the AOAC (2000) method and injected into the chromatograph. FAME separation and identification were carried out on the gas chromatograph. Chromatography was performed with Unicam 610 Series gas chromatograph equipped with a flame-ionization detector and a 60 m × 0.25 mm i.d. column coated with a 0.25 µm film of HP-23. Split injection (split ratio 1:50) was performed, with hydrogen as carrier gas at a flow rate of 43 m s^{-1}. The column temperature was maintained at 160 °C for 1 min after injection then programmed at 2.75° min^{-1} to 215 °C, which was held for 2 min, and then at 40° min^{-1} to 230 °C, which was held for 2 min. The injection port and detector temperatures were 270 °C.

3 Results and discussion

3.1 Effect of different solvents on extraction of oil from flaxseed

Various polar and non-polar solvents were screened for their efficiency to extract oil from flaxseed on the basis of yield obtained were recorded and presented in Table 1. The extraction yield with hexane was highest (14.53%) followed by dichloromethane (13.37%), petroleum ether (13.09%), ethanol (12.78%), acetone (11.00%) and methanol (9.68%), respectively (Fig. 1). This could be attributed to a general understanding that more percentage of non-polar lipids in comparison to polar lipids (glycol and phospo) in plant seeds (Dhoot *et al.*, 2011; Matthus and Bruhl, 2001), since seeds contain both polar and non-polar lipids combination of polar and non-polar

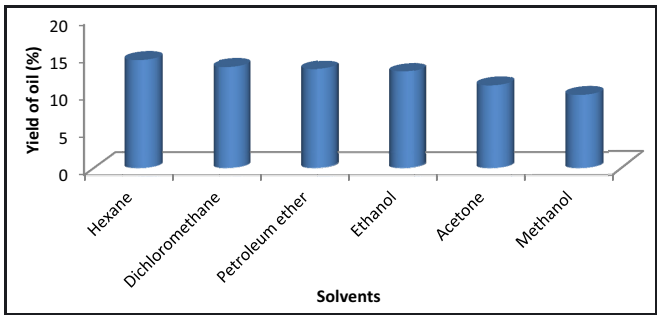

Fig. 1. Effect of different solvents on extraction of oil from flaxseeds.

solvents were studied. Ethanol has a worthy candidate to investigate as an alternative solvent, since it has low cost and it may be produced from a large variety of biological materials using simple technology (Suzana *et al.*, 2003). Other studies shown that drying methods have significant effects on oil extraction efficiency, physical properties and chemical compositions of aromatic plants (Hsu *et al.*, 2003; Omidbaigi *et al.*, 2004). Several studies have been carried out, both at laboratory and pilot scales, aimed to replace hexane with other hydrocarbons (Conkerton *et al.*, 1995; Wan *et al.*, 1995), or alcohols (Sineiro *et al.*, 1998), as solvents for oil extraction. Among hydrocarbon solvents, heptane and iso-hexane were recommended as potential substitutes for hexane to extract oil from cotton seeds (Conkerton *et al.*, 1995; Wan *et al.*, 1995). With respect to the use of alcohol, iso-propanol and ethanol were the most promising solvents for the oil extraction from cotton seeds (Hron *et al.*, 1995).

3.2 Optimization of ultrasound assisted extraction of flaxseed oil

3.2.1 Effect of ultrasonic frequencies

The flaxseeds were treated with different ultrasonic frequencies and obtained results are depicted in Figure 2. It

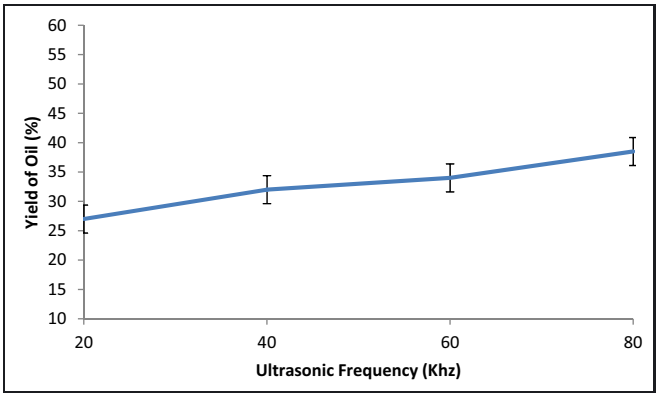

Fig. 2. Effect of ultrasonic frequency on yield of flaxseed oil.

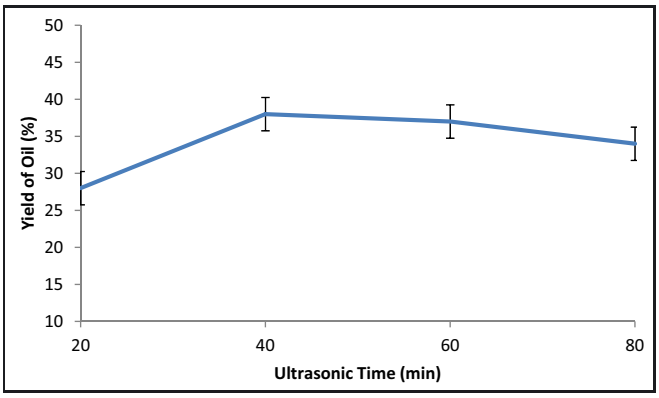

Fig. 3. Effect of ultrasonic extraction time on yield of flaxseed oil.

was reviewed from the figure that the extraction of oil was gradually increasing with increase in the ultrasonic frequency. As the larger amplitude ultrasonic wave travelled through a liquid medium, more bubbles were created and collapsed (Hemwimol *et al*., 2006). Since the temperature and pressure were very high inside the bubbles and the collapse of bubbles occurred over very short time, the violent shock wave and high-speed jet were generated which could enhance the penetration of the solvent into the cell tissues and accelerate the intracellular product release into the solvent by disrupting the cell walls. Moreover, the violent shock wave and high-speed jet might have caused the molecules to mix better enhancing the mass transfer rate. Due to the presence of the hard cell walls which are not so permeable, the large increase in ultrasonic power resulted in a moderate rise in yield. Similar results were obtained by Li *et al.* (2004) and Sivakumar *et al*. (2007), in the ultrasound-assisted extraction of oil from soybean and tannin from myrobalan nut.

3.2.2 Effect of time

The extraction time is an important parameter for oil extraction. Extractions of oil from flaxseed at different times were carried out and obtained results are depicted in Figure 3. As the extraction time increases the yield of oil was increased up to 40 min and slightly lowered after 40 min. This process indicates that the effect of ultrasound is more effective in the

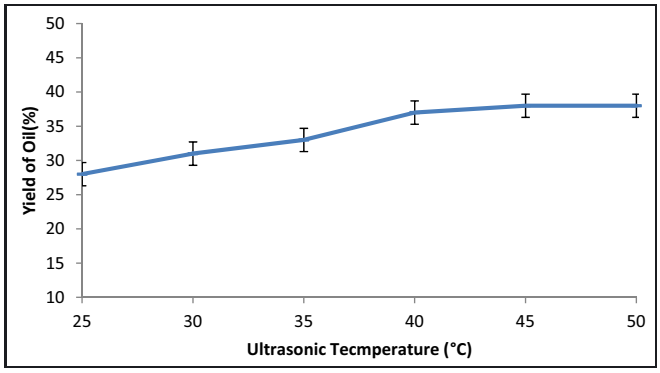

Fig. 4. Effect of ultrasonic temperature on yield of flaxseed oil.

first 40 min. It may be that ultrasonic wave could disrupt the cell walls, so larger contact area between solvent and material was created and more oil was appeared on the surface in ultrasonic assisted extraction than that in maceration extraction. However, this effect would be increasingly weak on the inner cell walls as the distance is increased. It should be noted that both processes occur very rapidly at the beginning of the extractions. Ultrasonic assisted extraction has the greatest rise in yield. Perhaps the ultrasonic waves affect the mass transfer rate mainly in the solvent penetration stage. It should of course also enhance the dissolved oil to transfer out of the solid structure. Similar findings were reported by Zhao *et al.* (2007), Hemwimol *et al*. (2006) and Balachandran *et al*. (2006). In the research of Balachandran *et al*. (2006), it had been confirmed that the ultrasound continued to be effective even during the later stage of extraction through an enhancement to the internal diffusivity, although the effects were smaller. In any case, based on the results so far, 30 min was found to be an optimum operating time for ultrasonic assisted extraction.

3.2.3 Effect of temperature

The flaxseed samples was pre-treated with ultrasonic treatment at various temperatures i.e. 25 °C, 30 °C, 35 °C, 40 °C, 45 °C and 50 °C. The yield of oil is gradually increasing with increase in the temperature up to 40 °C, whereas beyond 40 °C there was no significant effect on extraction (Fig. 4). An increase of temperature reduces the oil kinematic viscosity with an increasing mobility of biopolymers in cellular walls. Based on these findings reflux temperature (around 80 °C) was taken as optimum temperature for better yield (Panchal *et al*., 2014). This is surprising as well, one reason for this may be that the vapour pressure of solvent increased with the increase of temperature and the vapour pressure had a great influence on the occurrence and the intensity of acoustic cavitation. At lower temperature, the vapor pressure is lower. Ultrasound produces a few cavitational bubbles as a result of high acoustic cavitation threshold. However, the bubbles explode with relatively greater force, which enhanced cell tissues disruption during extraction. At higher temperature, the vapor pressure was higher and more bubbles were created, but they collapsed with less intensity due to a smaller pressure difference between inside and outside of bubbles (Hromadkova *et al.*, 1999).

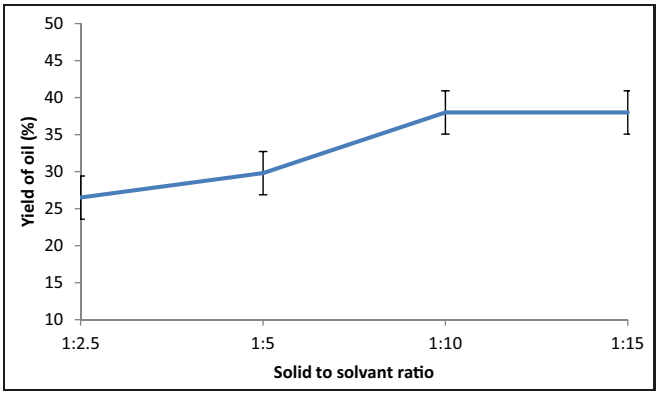

Fig. 5. Effect of solid to solvent ratio on extraction of flaxseed oil.

Another reason may be due to the surface tension which decreased with the increase of temperature affecting the bubble formation and collapse. The bubbles may be so easily collapsed at higher temperature thus reducing the intensity of the mass transfer enhancement.

3.2.4 Effect of solid to solvent ratio

The ratio of solid to solvent is important parameter for oil extraction process. Different solid to solvent ratio *i.e.* 1:5, 1:10, and 1:15 w/v was used for experiment and obtained results are presented in Figure 5. The recovery of oil was increased with increase in solid to solvent ratio. The highest oil recovery found at observed when the solid to solvent ratio 1:10 w/v. The larger liquid (solvent) to solid ratio means a larger concentration difference which facilitates mass transfer. When the liquid to solid ratio is small, this effect is more obvious. Too much liquid would not change much of the driving force any more as the limitation to mass transfer is more confined to the solid interior. Again, ultrasonic assisted extraction outperformed macerated extraction at all liquid-solid ratios tested here. Similar result has also been shown by Zhao *et al.* (2007).

3.2.5 Effect of ultrasonic assisted extraction on fatty acid profile of flaxseed oil

The fatty acid compositions of flaxseed oil extracted by ultrasonic assisted extraction and solvent extraction are given in Table 1. The fatty acid recorded in solvent extraction method and ultrasonic assisted extraction methods were saturated fatty acids (12.87% and 10.44%), monounsaturated fatty acids (20.56% and 21.44%) and polyunsaturated fatty acids (66.56% and 68.12%), respectively. The results showed that ultrasonic assisted extraction improved extraction of all fatty acids and significant observation was recorded in polyunsaturated fatty acids. The major fatty acids observed includes saturated fatty acid such as palmitic acid (16:0), stearic acid (18:0), arachidic acid (20:0), behenic acid (22:0) and lignoceric acid (24:0); monounsaturated fatty acids such as oleic acid (18:1 n-9c), gondoic acid (20:1) and nervonic acid (24:1) and polyunsaturated fatty acids such as linoleic acid (18:2 n-6c), γ-linolenic acid (18:3 n-6) and α-linolenic acid (18:3 n-3) (Metherel *et al.*, 2009).

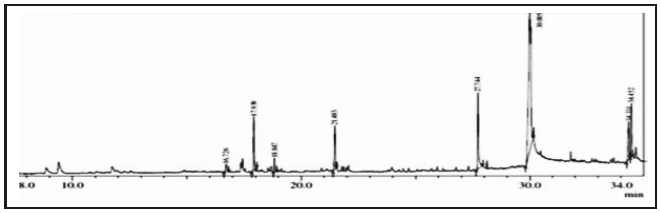

Fig. 6. Chromatograph of oil extracted by solvent extraction method.

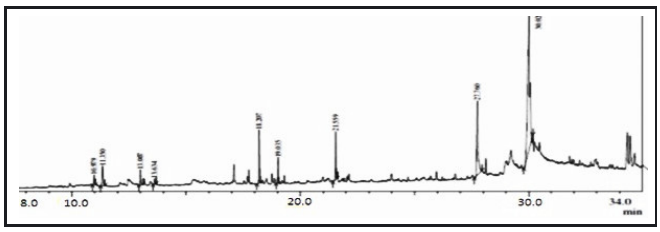

Fig. 7. Chromatograph of oil extracted by ultrasonic method.

Individual fatty acid determinations were most similar to the solvent extraction method with ultrasonic assisted extraction method. Ultrasound-assisted extractions in resulted in a fatty acid profile similar to solvent extraction but palmitic acid (16:0) and stearic acid (18:0) were significantly lower whereas other saturated fatty acid did not showed significant difference. The extraction of mono and poly unsaturated fatty acid such as oleic acid (18:1 n-9c), linoleic acid (18:2 n-6c), γ-linolenic acid (18:3 n-6) and α-linolenic acid (18:3 n-3) were substantially increased by the ultrasonic treatment. The other unsaturated fatty acid did not showed significant variation.

GC analyses combined with NIST 05 database searches confirmed the identification of fatty acid methyl esters across extraction techniques, thereby confirming quantitative measures (Figs. 6 and 7). No differences in the spectra from the standard Folch extraction and the ultrasound-assisted extractions for 20 min at 100% amplitude are seen as demonstrated for alpha-linolenic acid (18:3 n-3).

Some previous researchers have reported that the appearance of off-flavors in some food products containing lipids when subjected to ultrasounds (Pingret *et al.*, 2012, 2013). These off flavour may be due to the oxidation of fatty acid present in the product. Chemat *et al.*, 2004 verified oxidation in oil emulsions after sonication even when in indirect contact with the ultrasound source.

3.2.6 Sustainable approach and scale-up

The low cost of oil extraction is clearly profitable for the proposed UAE method in terms of time, energy, and improved yield. UAE allows a rapid extraction of oil from Flaxseed. Conventional methods such as solvent extraction are often time and/or energy consuming. In this study, higher extraction yield was observed with UAE. Among the others, many advantages can be pointed out when taking into account UAE of oil from flaxseed: (i) the operating procedure is faster, operative and cost reduction (time needed, energy required, equipment size) and (ii) no additional treatment and/or chemicals are required

to realize experiments. An experimental pilot study was carried out in a 30 L extraction tank consisting of a quadriple output of ultrasound at 25 kHz and 4200 Watts by Virot *et al*. (2010) and Pingret *et al*. (2012). From the previous lab study, the selected conditions for the ultrasound extraction pilot study were at the optimum conditions. Although, the yield of oil from the ultrasound extraction pilot scale was equal to laboratory scale investigations and was 11.5% higher than the conventional method. The result showed that the potential use of ultrasound extraction was promising for extraction on an industrial scale. UAE could shorten the extraction time, enhance the final yield, and lower the operating temperature, which can result in considerably lowered operating costs.

4 Conclusions

The highest recovery of flaxseed oil was obtained with hexane followed by dichloromethane. Optimization study of ultrasonic treatment showed that ultrasonic frequency of 40 kHz, temperature of 30 °C, extraction time of 40 min and solid to solvent ratio: 1:10 was best for extraction of oil from flaxseed. The results of extraction showed that the ultrasonic treatment significantly increase recovery and α-Linolenic acid (ω-3) acid. This study will be helpful to improve extraction recovery and α-Linolenic acid (ω-3) acid.

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