Photo and thermal stress of linseed oil and stabilization strategies

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Abstract The thermal and light stability of linseed oil has been studied by monitoring the concentrations of fatty acids and lignans, as main nutraceutical components. Linseed oil was subjected to stressing light and temperature conditions, in accordance with the ICH international rules, and monitored by UV–vis spectroscopy and HPLC–DAD. The change of UV spectra along the photodegradation tests, setting the irradiation power at 350 W/m², confirmed a significant overall sensitivity of linseed oil to light. At the same time, the HPLC determination of the major fatty acids showed a marked variation in their concentration up to a residual concentration of 62.3 and 67.2% for α -linolenic and linoleic acid, respectively, after 18 h. In contrast, thermal tests at 60 °C showed some stability, with a concentration of residual fatty acids in the range 82–95% after 48 h. The examined lignans showed significant stability when exposed to both light and heat. Several photoprotection approaches have been also studied to increase the photostability of linseed oil. A significant increase in the stability of fatty acids has been observed using amber glass containers or ascorbic acid or by combining the two protection factors.

Keywords Fatty acids · Lignans · Linseed oil · Photo-thermal degradation · Photoprotection

Introduction

Linseed oil (LO), also known as flax oil, is usually obtained by mechanical extraction for pressure under temperature-controlled from the seeds of the plant *Linum Usitatissimum*, which belongs to the Linaceae family. Nowadays, LO is considered a functional food because it is a good source of ω -3 and ω -6 polyunsaturated fatty acids, lignans, dietary fibers and other minor nutrients. In particular, the ω -6 linoleic acid (LLA) and ω -3 α -linolenic acid (LNA) are important as precursors of other fatty acids of both series. Significant amounts of oleic acid (OLA) are also contained. The unsaturated fatty acids help to prevent cardiovascular diseases, type II diabetes, immune and inflammatory disorders (Rodríguez and Christophe 2005; Endo and Arita 2016; O'Connell et al. 2017). Unfortunately, the major fatty acids contained in LO are less stable than the saturated congeners containing the same number of carbon atoms or even less (Shin et al. 2003). This is the main problem associated with the use of LO as a source of omega-3/6 fatty acids and lignans in food industry. The oxidation of fatty acids not only produces rancid odors and flavors, but can also lead to a lowering of nutritional and beneficial properties. Secondary products potentially toxic to human health could also be formed, generally hydroperoxide products and subsequently aldehydes. (Frankel 1996).

In an interesting review of 2006, the quality properties of LO for food uses are discussed as well as the factors influencing its quality. Information on the negative influence of oxidation on taste, color and smell of oil is reported. Studies about the effects of storage on the composition of LO and methods to promote its quality, including agronomic, chemical, biotechnological and microbiological methods, are detailed (Nykter et al. 2006).

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Another review, published in 2012, summarizes the positive aspects on human health of the polyunsaturated fatty acids, however, highlighting their high sensitivity to oxidation that leads to loss of shelf life, consumer acceptability, functionality, nutritional value and to safety (Arab-Tehrany et al. 2012).

LO also contains polyphenolic compounds known as lignans, considered to be analogues of phytoestrogens, given the marked resemblance of their chemical structure with the natural estrogens (Dixon 2004). For this reason, the lignans are studied for their potential pharmacological use in pathologies related to hormones (Westcott and Muir 2003). The quantity of lignans usually present in LO is between 10 and 26 mg/g (Milder et al. 2005). The main lignan is secoisolariciresinol (SEC), mainly in the form of diglucoside (Attoumbré et al. 2011). Other lignans in discrete quantities are lariciresinol (LAR) and matairesinol (MAT). The properties of lignans make them excellent for the preparation of nutraceutical formulations with beneficial effects on human health. Continuous intake of these compounds can serve as a synergistic aid to endogenous protection systems both qualitatively and quantitatively against cellular damage. Consumption of lignans reduces cardiovascular risk and inhibits the growth of some types of diabetes (Touré and Xueming 2010; Herchi et al. 2014). Determination of SEC in flax seeds has been performed by HPLC–DAD (Bravi et al. 2011).

Nowadays, the interest in functional foods is remarkable and closely related to the potential of their bioactive components in providing health benefits. The growing recognition of these benefits has been particularly recognized for omega-3 long-chain polyunsaturated fatty acids (Wijesundera et al. 2011).

However, recurrent episodes of failures in maintaining the quality of functional foods have been reported (de Goede et al. 2010; Destailats 2011). Due to the known sensitivity of fatty acids contained in LO, proper storage of oil is essential to ensuring its good properties. For this reason, stabilization studies with chemical or physical approaches have multiplied in recent years.

Multilayer emulsions have been proposed to encapsulate and protect LO, using tween-20 and lecithin as surfactants to stabilize the emulsion. The oxidation stability has been improved by the simultaneous encapsulation in the emulsion of chitosan and α -lipoic acid (Huang et al. 2018). In another work, the oxidative stability of the omega-3-containing emulsions is investigated, by testing a series of both natural and synthetic surfactants. Among these, Quillaja saponin has consistently produced the most stable emulsions to oxidation, probably due to its high capacity for scavenging free radicals (Uluata et al. 2015).

The different types of formulation and packaging can drastically affect the chemistry of the fatty acids and their

durability. Some papers are full of information on the requirements for proper storage of foods containing polyunsaturated fatty acids and on the right packaging standards. Lehnert et al. report as necessary prerequisites for the success of products enriched with omega-3 the optimization of extrinsic factors such as light exposure, high temperature and oxygen. It is also emphasized that it is very difficult to predict the oxidative stability of the various additives and that the optimal composition and transformation conditions should be evaluated for each product (Nielsen 2015).

Other studies have proposed to reduce the oxidation of the fatty acids through the addition of antioxidants. The antioxidant property of tocopherols and ascorbic acid in inhibiting the decomposition of hydroperoxides has been shown to be fundamental in preserving the quality of food by reducing the formation of aldehydes (Frankel 1996). Another study uses phenolic compounds such as myricetin, (+)–catechins, genistein and caffeic acid as antioxidants. Their protective effect was assessed by monitoring the form of hydroperoxide and the residual concentration of antioxidants. Among these, myricetin showed a significant reduction in the oxidation of linoleic acid (Michotte et al. 2011). The influence of various natural antioxidants on the preservation quality of oleic, linoleic, linolenic fatty acids in linseed oil, also mixed with other oils, has also been studied. The use of these antioxidants in a quantity of 5% has been shown to increase the oxidation resistance of the oil mixtures by 1.2–1.7 times (Lehnert et al. 2018).

The adoption of appropriate analytical techniques is fundamental for the correct monitoring of the degree of oxidation of LO and its main components. UV and IR spectroscopy techniques are widely used in the analysis and characterization of food matrices (Kapoulas and Andrikopoulos 1987; Ragno et al. 2006a; Gonçalves et al. 2014; Hirri et al. 2015). FTIR has proved to be particularly effective in classifying oil or oil seeds of different geographic origin or different processing methods (Terrouzi et al. 2011; De Luca et al. 2016). However, it has been reported as inappropriate in the photostability studies of vegetable oils (Spatari et al. 2017).

For the fatty acids determination, in particular ω -3 and ω -6, the most used technique is HPLC. They have been determined by HPLC after specific extraction (Guarrasi et al. 2010; Zeb and Murkovic 2013) and the photodegradation tests performed in accordance with international standards (ICH 1996; Ragno et al. 2006b; Dinç et al. 2012; De Luca et al. 2013).

This paper aims to investigate any variations in the composition of the main components of LO when exposed to normal or stressing light and temperature. The study will focus on monitoring the main polyunsaturated fatty acids and lignans, given their importance for their nutraceutical

properties. The study also intends to define some strategies to minimize the sensitivity of LO to light or temperature. Physical protection approaches will be studied through tests on containers variously light-permeable whereas chemical protection will be measured after addition of antioxidants. The synergy between both the protection methods will also be studied.

Materials and methods

Chemicals

LO was purchased commercially and stored in the dark at 4 °C. The oils purchased were of good quality, meeting the requirements in terms of internationally recognized fatty acids (Symoniuk et al. 2017). n-hexane for UV–vis analysis, sodium hydroxide, hydrochloric acid, petroleum ether, formic acid 98% were lab grade; acetonitrile and methanol were of chromatographic grade; LLA, LNA, OLA, LAR, SEC, MAT and ascorbyl palmitate (AP) (standard grade), were purchased from Sigma-Aldrich (Milan, Italy).

Instruments

Forced photodegradation tests were conducted by using the Suntest CPS + camera (Heraeus, Italy), equipped with a Xenon lamp, whose radiant spectrum is largely overlapping with the spectrum of the solar radiation. The instrument allows to select specific spectral regions by interposing specific filters; the internal temperature can be adjusted by means of a refrigerant unit to avoid sample heating due to the lamp.

UV analysis was performed by Agilent 8453 UV Spectrophotometer equipped with a diode array detector (Agilent Technologies, CA, USA). The spectra were recorded at the following conditions: 10 mm quartz cell; wavelength range 200–350 nm; spectral band 1 nm.

HPLC analysis was performed using an HP1100 pump equipped with a G1315B (Agilent Technologies) diode detector and a Rheodyne 7725 manual injector. A LC-C18 Gemini column (Phenomenex) 250 × 4.6 mm × 5 µm, at room temperature, was used to separate both the fatty acids and lignans.

Standard solutions

500 mg of LO were diluted in 10 mL of hexane and the solution was further diluted 1:10 with the same solvent. These samples (n = 10) were used to record the UV spectra of LO before degradation tests.

The standard solutions of LLA, LNA and OLA, stored at 4 °C before the use, were prepared by solubilization of

1.60, 3.02 and 5.76 mg of the products, respectively, in 1 mL of methanol and further filtration through a 0.22 µm filter. Analogously, the standard solutions of MAT, LAR and SEC were prepared by weighing 1.02, 1.08 and 1.03 mg, respectively, and diluting in methanol up to obtain concentration ranges between 0.06 and 0.50 mg/mL. All the samples were analyzed by HPLC to carry out the regression equations correlating the signal areas with the concentration of the analytes. These equations were then used along the degradation experiments to calculate the analyte concentrations of the samples tested.

Analysis by UV and HPLC

The UV spectra of LO along the degradation tests were performed after appropriate dilution, in agreement with the procedure above described. Determination of the fatty acids was performed after appropriate extraction as follows: 5 g of LO were hydrolyzed with 50 mL of 0.5 M NaOH at 25 °C for 3 h under stirring. 50 mL of petroleum ether were added twice to the dispersion, in order to remove the unsaponifiable fraction. The aqueous phase was acidified with 1 M HCl to pH 2.9 and then extracted twice with 50 mL of petroleum ether. The organic solvent was removed at 40 °C under pressure. Finally, the analytes were recovered with 4 mL of methanol and filtered through 0.22 µm filters before the HPLC injection. The chromatographic separation was performed on C18 column in isocratic mode by means of a mobile phase acetonitrile/methanol (90:10 v/v) adjusted with formic acid to pH 5.5. Flow rate was 1 mL/min; injection volume 20 µL.

Determination of the lignans was made by extracting 45 g of LO with methanol (3 × 20 mL). The methanol phase was then concentrated under pressure and diluted in acetonitrile 4 mL. This solution was treated with hexane (3 × 10 mL), concentrated under pressure, diluted in methanol 1 mL, filtered through a 0.45 µm PTFE filter and then analyzed by HPLC (Montedoro et al. 1992). Separation of the lignans was achieved on the same column above reported at room temperature in isocratic mode. In this case, a mixture methanol/water (70:30 v/v) adjusted with formic acid to pH 3.4 was used as a mobile phase. Flow rate was set at 0.65 mL/min; injection volume 20 µL.

All of the experiments were repeated three times and the significance of concentration differences were calculated using analysis of variance (ANOVA, 95% confidence level) by SPSS statistic 23 (IBM, Chicago IL, USA).

Photodegradation experiments

LO samples were subjected to light irradiation without any pretreatment. Photodegradation experiments were conducted under light conditions miming the effect of the

sunlight outside by cutting the radiations below 290 nm. The irradiation power was set at 350 W/m², corresponding to 21 kJ/m² min, and the wavelength range was cut under 290 nm by interposing a UV-filter glass. The heating due to the lamp was neutralized keeping the internal temperature of the irradiation chamber at 25 °C by a cooling unit. UV and HPLC analyses were recorded at 6 h intervals up to 48 h, after specific treatment of the samples, above described. In order to minimize any interference by the extraneous light, all laboratory procedures were executed in a dark room equipped with a red lamp of 60 W at a distance of at least 2 m.

Thermal degradation

Thermal degradation of LO was performed by placing the samples in a water bath maintained at 60 °C in the dark for 48 h and monitoring the samples by UV and HPLC, after specific treatment above reported, at 6 h intervals.

Chemical and physical protection

LO photostabilization experiments were carried out under the same forced irradiation conditions reported above. The LO samples were placed in quartz and transparent glass containers and the results were compared with those obtained by the adoption of amber glass. A second series of photoprotection experiments was performed by adding to pure LO samples aliquots of the antioxidant AP with concentrations in the range 0.01–0.20% w/v.

Results and discussion

UV and HPLC analysis of linseed oil, fatty acids and lignans

LO was analyzed via UV spectrophotometry after appropriate dilution in hexane up to a final oil amount of 5 mg/mL, in such a way as to detail the characteristic bands around 230 nm and 280 nm, due to double and triple conjugated bonds, respectively. The high absorbance around 210–220 nm is due to $n \rightarrow \sigma^*$ transitions of carbonyl chromophores and to $\pi \rightarrow \pi^*$ transitions of single ethylene chromophores.

HPLC analysis of the main fatty acids, LLA, LNA and OLA, was defined on a mixture of the standard solutions of the three components. The chromatographic conditions were optimized by testing different solvent mixtures to respect the lipophilicity of the analytes and optimize their resolution. The best result was reached by using a mixture 90:10 methanol:water pH 5.5 (formic acid). The retention

times (RT) were 4.7, 5.4 and 6.5 min for LNA, LLA and OLA, respectively, as shown in Fig. 1a.

The quantification of the fatty acids in the LO samples, before or during the degradation tests, was carried out through the respective calibration curves, built on the data from HPLC analysis of the standard samples in the range 0.015–0.909 mg/mL for LLA, 0.028–1.152 mg/mL for LNA and 0.016–0.800 mg/mL for OLA. Likewise, the separation of the lignans in HPLC was optimized using a mixture of methanol:water acidified at pH 3.5 with acetic acid. The RT values were 4.6, 6.0 and 7.2 min for SEC, LAR and MAT, respectively, as shown in Fig. 1b. The regression parameters, used to quantify fatty acids and lignans, are listed in Table 1 and the curves are shown in Fig. 2.

Photodegradation testing

As a preliminary experiment, three samples of LO were subjected to forced photodegradation in quartz flasks to study their behavior in the absence of any interference due to the container.

The UV spectra, shown in Fig. 3, were recorded before and during the photodegradation experiments at time intervals above detailed. A clear change was observed, consisting of an increasing trend of both characteristic bands around 230 and 280 nm. In contrast, the absorption band in the range 210–220 nm has increased due to the formation of carbonyl chromophores. Many authors have actually reported that the oils undergo a radical-type degradation that initially forms hydroperoxides which in turn are transformed into alcohols, acids, aldehydes, ketones and lactones (Guillén and Cabo 2002; Chen et al. 2011).

The amount of the fatty acids along the stressing irradiation test was monitored by HPLC analysis of the samples at time steps of 6 h up to 48 h. A clear decrease of all analytes was demonstrated. Figure 4 shows the chromatograms recorded at time 0 and after 18 h of the stressing photodegradation test.

Monitoring of LNA and LLA in linseed oil exposed to light in quartz flasks showed rapid degradation with a reduction of the initial amount to 70% after only 6 h. Subsequently, the transformation proceeded more slowly until a residual amount of about 35% at the end of the experiment (48 h of total exposure). OLA showed a higher stability, with a residual percentage of 84% after 6 h and 43% after 48 h of irradiation. These results were calculated on the data collected from three samples tested. The photodegradation profiles of the individual fatty acids are plotted in Fig. 5.

In contrast, the concentration of the lignans did not show a significant variation after the photodegradation tests. The

Fig. 1 HPLC chromatogram of standard mixture samples: **a** fatty acids ω -3 α -linolenic acid (LNA, 0.23 mg/mL), ω -6 linoleic acid (LLA, 0.37 mg/mL) and oleic acid (OLA, 0.60 mg/mL); **b** lignans

secoisolariciresinol (SEC, 0.1 mg/mL), matairesinol (MAT, 0.1 mg/mL) and lariciresinol (LAR 0.25 mg/mL)

residual concentration of all these analytes was between 95 and 105%, values that cannot be attributed to a sure degradation but rather to a statistical variation of the results.

These results demonstrate the lability of LO and in particular of the polyunsaturated fatty acids when exposed to light. This instability requires the adoption of appropriate precautions during the production and storage of this product. In fact, in the past years, light has not been considered important as responsible for degradation in the food and pharmaceutical fields and only in the last 20 years this

capacity been recognized (Ioele et al. 2010, 2014). Even the papers concerning the stability characteristics of LO for food use have always focused the attention above all on temperature and oxygen as main responsible for oxidation processes. Nielsen, in the 2015 review, included among the extrinsic factors responsible for LO degradation the exposure to light as well as high temperature and oxygen. However, the authors emphasize the need to evaluate the most appropriate criteria for each product, given the difficulty in predicting the antioxidant capacity (Nielsen 2015).

Table 1 Parameters of the regression curves for the fatty acids and lignans

Analyte	Slope	Intercept	Correlation coefficient	Validation range (µg/mL)
LLA	37.541	− 2664.8	0.9902	0.016–0.800
LNA	53.025	− 395.2	0.9788	0.015–0.909
OLA	10.325	− 104.61	0.9933	0.028–1.152
LAR	54.846	8498.3	0.9823	0.060–0.500
SEC	20.929	1569	0.9989	0.060–0.500
MAT	21.767	520.18	0.9956	0.060–0.500

LLA ω -6 linoleic acid, LNA ω -3 α -linolenic acid, OLA oleic acid, LAR lariciresinol, SEC secoisolariciresinol, MAT matairesinol

Fig. 2 Regression curves obtained from HPLC data of the standard solutions of fatty acids (a) and lignans (b)

Thermodegradation testing

The thermal degradation of LO was performed by placing the samples in a water bath set at 60 °C, maintained in the dark for up to 48 h. Aliquots of the samples were analysed by UV and HPLC at 6 h intervals. A good resistance of the fatty acids was relieved for all of them with a residual concentration between 94–97% after 12 h and 87–95% after 48 h. In reality, the good stability of the lignans at temperatures lower than 100 °C has already been reported for different vegetable oils. Only at high temperatures, up to 250 °C, the lignans quickly degraded in different oils. However, the lignans contained in the linseed are the most

Fig. 3 Sequential UV spectra of LO in quartz recorded at time intervals along forced irradiation

stable at high temperatures, withstanding for a few minutes even at 250 °C (Gerstenmeyer et al. 2013).

The results obtained are in line with those already presented in some previous works, even if most of these studies monitored the transformation of the oil in the culinary field, therefore when subjected to high temperatures. The effect of strong heating in different oil samples has been assessed by chromatographic and mid-infrared analysis, monitoring the influence of heating on the composition. Many edible oils have shown a drastic reduction in nutritional fatty acids when subjected to high temperatures. In contrast, oils containing high amounts of alpha-linolenic and linoleic acids, such as LO, have shown greater resistance to temperature (Rampazzo et al. 2018).

Really, the studies on the thermal degradation of LO are almost all focused on the use in paints and oil paints and, in recent years on the production of fuel and industrial lubricants. Many studies have therefore focused on improving the drying performance of this oil and on reducing the dangerous properties related to its application rather than its use in the food and nutraceutical field.

Photoprotection strategies

In order to improve the photostability of LO, two different approaches, physical and chemical, were investigated.

Fig. 4 Sequential chromatograms of LO in quartz recorded at time 0 and after 18 h of forced irradiation

Physical protection

The photodegradation experiments were repeated on LO placed in transparent and amber glass containers, which, unlike quartz, absorb much of the UV radiation. During the photodegradation tests, the calculated amounts of fatty acids in the clear glass samples were very similar to those calculated using quartz containers. These results suggested that the radiation responsible for degradation is mainly located in the spectral region of the visible, to which both quartz and glass are transparent.

More comforting results were obtained when the test was performed on amber glass samples. A significant improvement was observed in the first 12 h of irradiation for all three fatty acids analyzed. The residual amount of LNA increased from a value slightly higher than 50%, for quartz and glass, to a value of 82% in amber glass. The amount of LLA changed from 65 to over 90% while the OLA values increased from 75 to 88%. However, after this test time, the degradation profile for all the analytes practically matched the results obtained with the use of the transparent glass. This behavior suggested that, after some time, radical oxidation proceeded independently of the external light. (Gargouri et al. 2015).

Chemical protection

One of the most common strategies adopted to protect the edible vegetable oils from rancidity is the addition of antioxidant compounds that slow down or prevent the oxidation processes (Tańska et al. 2018). For our study, AP, a well-known vitamin C ester, was selected because often used in the food industry. Its solubility in fat, together with a low toxicity, makes it very versatile as a food additive. The antioxidant activity of ascorbic acid, and in particular of the AP derivative, has often been tested in vegetable oils together with natural tocopherols showing synergistic activity (Cort 1974).

The photodegradation experiments were repeated on the LO samples after addition of AP at concentrations ranging from 0.01 to 0.20%. The experiments were performed in quartz flasks in such a way as to evaluate only the influence of AP on the stability of the fatty acids, avoiding any interference from the container. Preliminary results showed a clear increase in photostability for the whole matrix and for the individual fatty acids. The photoprotection power increased, as expected, with increasing concentration, obtaining however the maximum protection efficacy at AP

Fig. 5 Kinetic study of fatty acids degradation ω -6 linoleic acid (LLA), ω -3 α -linolenic acid (LNA) and oleic acid (OLA) at different experiment conditions: quartz (Q), glass (G), amber glass (AG), ascorbate palmitate (AP) and amber glass + ascorbate palmitate (AG + AP)

concentration of 0.05%. With higher concentrations of AP, the influence on stability remained almost constant.

In Fig. 5, the degradation profiles of fatty acids show a significant increase in stability with a residual percentage between 50 and 60% after 48 h, higher than the values recorded with the adoption of amber containers. However, it should be noted that the protective effect of colored glass in the first 12 h remained even higher for all analytes.

Combined chemical and physical protection

In subsequent experiments, the possible synergistic action of both the studied protective factors was tested. The chromatographic analysis of LO fatty acids in amber glass added of AP 0.05% gave very satisfactory results with concentrations of all fatty acids close to 95% after 12 h of stress irradiation. The residual values at the end of the experiments, after 48 h, never fell below the value of 57%. The combined action of amber glass and antioxidant was

particularly effective, as it was able to slow down the initial oxidation phases of the main components of LO. This protective effect is particularly important because, under normal lighting conditions, it prevents the occurrence of radical oxidation processes, which are the main cause of oil alteration. In order to evaluate the difference between kinetic degradation of the fatty acids at different experimental conditions, one-way ANOVA was carried out. As shown in Fig. 5, all analyses showed significant differences ($p < 0.05$) across the five photodegradation experiments.

Conclusion

The photo and thermal stability of linseed oil has been studied by monitoring the concentrations of the main components, fatty acids and lignans. Analysis by UV spectroscopy and chromatography revealed significant changes in the concentrations of the fatty acids, reduced to 35% after 48 h of forced light exposure. In contrast, the lignans showed a not significant decrease in concentration. Based on these results, the photoprotection of the oil has been investigated by testing the stability efficacy of the amber glass containers and the addition of the antioxidant ascorbyl palmitate. Impressive results were achieved from their combined use by stabilizing the fatty acids at 58–72% after 48 h of stressing irradiation. These results, if compared to those obtained by the experiments without any protection factor, can be considered very satisfactory and could be a huge stimulus to increase the shelf life of linseed oil or other edible oils. The results of this study indicate that the adoption of an appropriate formulation associated with accurate physical protection plays a significant role in maintaining optimal conditions for the linseed oil, which could be exploited in the food and nutraceutical industry.

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