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Supercritical Carbon dioxide and flaxseed oil like functional food

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Anidride carbonica supercritica e olio di lino come alimento funzionale

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Summary
Literature shows the functional food properties of Flaxseeds (*Linum usitatissimum* L.). Flaxseeds are rich in Alpha-Linolenic Acid (ALA) and are interesting for their dietary fiber and proteins content and for the presence of helpful minor compounds, important for human consumption, like lignans, phytoestrogens that can have hormone-like effects in humans. In fact, diets introducing flaxseed oil show also useful effects on diseases like lowering blood pressure, particularly in middle age dyslipidaemia and reducing the risk of myocardial infarction. Organic solvents are generally used to obtain a high extraction yield of flaxseed oil. An alternative method is expression (mechanical extraction) that offers a remarkable reduction of environmental impact, but with lower extraction yield in comparison with solvents. Another method of extraction could be the use of supercritical fluids. The same authors already studied the Supercritical Carbon dioxide (SC-CO_2) as potential solvent for flaxseed oil extraction. In this study a comparison of the technological and qualitative effects on the flaxseed oils obtained by neat SC-CO_2 extraction and by SC-CO_2 added with ethanol as co-solvent extraction was conducted. The extraction capacity of important polar compounds like polyphenols was determined, too. The results of this study confirm the ability of SC-CO_2 in obtaining a high quality product like flaxseed oil.

Riassunto
La letteratura mostra le proprietà funzionali dell'olio di lino alimentare (*Linum usitatissimum* L.). I semi di lino sono ricchi in Acido Alfa-Linolenico (ALA), e sono interessanti per il loro contenuto in fibra e in proteine e per la presenza di preziosi composti minori, importanti per il consumo umano. Infatti, le diete comprendenti olio di semi di lino dimostrano effetti utili contro disordini come quelli legati alla bassa pressione sanguigna, particolarmente per la dislipidemia e nella riduzione del rischio di infarto del miocardio. Normalmente, per l'ottenimento di olio di semi di lino vengono utilizzati solventi organici, i quali permettono alte rese. Un'alternativa è posta dalla spremitura (estrazione meccanica), che preserva maggiormente il prodotto e l'ambiente, ma con rese inferiori rispetto ai solventi. Un altro metodo potrebbe essere l'utilizzo di Fluidi

Supercritici. Gli stessi autori hanno studiato precedentemente l'impiego di CO_2 supercritica (SC-CO_2) come potenziale solvente per l'estrazione di oli di semi di lino. In questo studio sono stati realizzati dei confronti degli effetti tecnologici e qualitativi su oli di semi di lino estratti mediante l'impiego di SC-CO_2 pura e SC-CO_2 addizionata di etanolo come co-solvente. È stata determinata anche la possibilità di estrazione di importanti composti polari quali i polifenoli. I risultati di questo studio confermano la capacità estrattiva della SC-CO_2 nell'ottenimento di un prodotto alimentare di elevata qualità quale l'olio di lino.

Introduction

Flax (*Linum usitatissimum* L.) is an annual plant of the Linacee family, and its seeds are a rich source of lipids, proteins and dietary fibre (1). The composition of the flaxseeds is approximately 40 g of fat, 30 g of fiber, 20 g of protein, and the energetic value of flaxseeds is 1900 kJ for 100 g of product (2).

Research results indicate several possible human health benefits associated with consumption of flaxseeds and flaxseed oil (3). Flaxseeds are recognized to a be functional food because deliver a health boost beyond that might be expected from its nutrient content (4, 5); they are rich for almost the 20% of alpha-linolenic acid (ALA), and phytochemicals.

In fact, recent studies confirm the role of flaxseeds consumption on improvement of cardiovascular and skeletal diseases (6), inflammatory conditions such as rheumatoid arthritis, psoriasis, and ulcerative colitis (7).

The flaxseed oil consumption has significant effects on lowering blood pressure, particularly in middle age dyslipidaemia (8), and on reducing the risk of myocardial infarction in type 2 diabetic patients, since it can slow bleeding time (9). Besides, it provides significant improvement in attention deficit and hyperactivity disorder in children (10).

Flaxseed oil differs from other oilseeds, such as soybeans, cottonseed, corn and sunflowers, which are lower in ω-3 content and higher in ω-6 polyunsaturated linoleic acids content. Additionally, the amount of saturated fatty acids in flaxseeds is lower than soybeans, cottonseed and corn (11).

Human feeding is currently characterized by a high use of saturated, ω-6 poliunsaturated fatty acids, and therefore by chronic inadequacy of ω-3 poliunsaturated fatty acids. Then, if the recommended optimal ratio between ω-6 and ω-3 poliunsaturated fatty acids intake is 5:1 according the FAO and WHO (2), the actual real ratio is 13:1. In this context, introducing flaxseed oil in the human diets can help the approach to an equilibrate diet (12).

Moreover, flaxseeds are rich of lignans, important phenolic compounds with activity through a variety of mechanisms including phytoestrogenic and antioxidant effects verified in vitro and in vivo studies (13-17). Phytoestrogens have potential uses also in hormone replacement therapies and cancer prevention (18).

Secoisolariciresinol diglucoside (SDG) is the principal lignan in flaxseeds (19, 20). SDG is metabolized by the colon microflora to enterodiol, which can then be oxidized to enterolactone (20).

The flaxseeds lignans and their

mammalian metabolites have been reported to exert protective effects against diet-related chronic diseases, as heart disease, both lupus and polycystic kidney disease models, in type I and II diabetes models, and flaxseeds have also been reported to be hepatoprotective and able to contrast carcinogenesis (8, 9, 17, 21-26).

Currently, the most common method of oil extraction from seeds is hexane extraction. The process is very efficient, but hexane elimination after extraction represents a problem. An alternative method is the mechanical extraction. This method offers a considerable reduction of environmental impact but it gives a lower extraction yield in comparison with solvent extraction (27).

Therefore, some authors have proposed the substitution of the traditional process with the use of Supercritical fluid extraction adopting CO_2 as a solvent (SC-CO_2) to extract oil from seeds (28-32).

CO_2 is a good solvent for extracting non polar compounds, such as glycerides (33). In fact, its critical point is at the temperature of 31.1 °C, and it is neither inflammable nor toxic. Unfortunately, many nutraceuticals, like phenols, alkaloids and glucoside compounds are less soluble in CO_2 and, therefore, they cannot be extracted (34).

The addition of polar co-solvents to SC-CO_2 is known to increase the solubility of polar compounds also. Methanol, ethanol, acetonitrile, acetone, water, ethyl ether, and dichloromethane, are studied as co-solvent; methanol is miscible with CO_2 up to 20% but, when mixed with CO_2, it necessitates of a high temperature to reach the supercritical state and this can damage natural matrices; moreover, the methanol has a high toxicity for human diets. Therefore, ethanol can be a better choice also because of its low toxicity (35, 36).

The aim of this work was to add value, in terms of yields and quality properties, to the flaxseed oils obtained with SC-CO_2 extraction by the use of ethanol as co-solvent. The purpose was achieved by the determination of the presence of important active molecules, like the polyphenols in the extracted oils, compared with the extraction by the use of neat SC-CO_2.

Materials and methods

Samples

Flaxseeds of the commercial cultivar Barbara of 2008 crop were used. The flaxseed samples were ground with a pilot hammer mill (Siemens Electra S.p.A., Milano, Italy). The output size range was of 3.5 mm of diameter and the sample was ground twice. The whole samples were stored at 14°C before grounding, and at 4°C before extraction.

Reagents

n-Hexane, ethanol, were all HPLC grade and purchased from Carlo Erba (Milano, Italy). Nitrogen and carbon dioxide were of analytical grade, and purchased by Linde Gas Tecnici (Perugia, Italy). The free acidity of the extracted oils was measured in accordance with the European Official Methods (EU 1989/2003 modifying the EEC 2568/91) (37); for the determination of the total polyphenols, the method of Montedoro et al. (38) was used.

Supercritical Fluid Extraction (SFE)

The experiments were conducted using a "Spe-ed-SFE" laboratory-scale from Applied Separation (Lehigh, PA, USA). Thirty g of ground flaxseed were placed into a 50 mL extractor vessel. The extractions were conducted at the pressure of 65 MPa, at 70°C. The extraction run consisted of a 10 min static phase and a 120 min dynamic phase; CO_2 flow rate was 1.97 g min^{-1}. These operative parameters of SFE were chosen after our preliminary tests (data not reported) based on the study of literature (28, 39).

For the SFE with modifier, 5% (w/w) ethanol was added to the vessel after the sample loading in the extractor, before the CO_2 input. For the determination of the SFE with modifier, the ethanol was removed by rotary evaporation at 50°C for 1 hour, and dried in oven at 105°C for 1 hour.

Table 1 - Extraction yields of flaxseed oil obtained by SC-CO_2, SC-CO_2 with ethanol, and n-hexane ($n \geq 3$)

Extraction method	Yield %	Standard Deviation
SC-CO_2	18.05 [a]	0.88
SC-CO_2 + ethanol	23.73 [b]	1.50
n-Hexane	26.05 [b]	0.40

The value in the same column followed by the same superscript letters are not statistically different ($P < 0.05$). Data presented at the 9th Conference on Supercritical Fluids and their applications, September 2010, Sorrento (SA), Italy

Results and discussion

The results of the quantitative and qualitative analysis of the extracted oils are here reported. The results take into account the effect of SC-CO_2 extraction in terms of extraction yield, and the quality parameters of the extracted oil.

Table 2 - Qualitative analysis of the flaxseed oils extracted by SC-CO_2 and SC-CO_2 with ethanol (n=3)

Extraction method	Total Polyphenols (mg gallic acid/kg oil)	Free Acidity (% oleic acid)
SC-CO_2	154.75 ± 4.92	6.29 ± 0.12
SC-CO_2 + ethanol	390.01 ± 26.87	6.19 ± 0.12

Data presented at the 9th Conference on Supercritical Fluids and their applications, September 2010, Sorrento (SA), Italy

Table 1 reported that the extraction of flaxseed oil by SC-CO_2 with ethanol is more efficient than neat SC-CO_2 with statistically significant difference ($P < 0.05$), confirming what is reported in literature (40). The increase of extraction yield by SC-CO_2 with ethanol is comparable to the extraction with hexane. The increase of extraction yield realized by the SFE with co-solvent in comparison with neat SC-CO_2 is due to the higher capacity to extract polar lipids also (34, 35).

To explore the quality of the oil obtained without the use of organic solvents, chemical analyses were conducted, and the results are shown in the table 2.

The oil extracted by SC-CO_2 with ethanol shows a total polyphenols content 100% higher than oil extracted by SC-CO_2. This difference confirms the higher extractive ability of ethanol as co-solvent for the SC-CO_2 towards partially hydrophilic molecules in comparison with neat SC-CO_2 (41).

The total polyphenols in the extracted oils, expressed as mg of gallic acid/kg of oil, resulted comparable with the range of content for total polyphenols in another important vegetable oil, like the olive oil. For olive oil normal contents of polyphenols can be in a range of 100-200 mg/kg (42). This result suggests to explore more deeply the presence of polyphenols, also with repetition of these experiments with other varieties and the exploration of the identification of the specific fractions and molecules among the polyphenols in the flaxseed oil obtained by SFE. Moreover, the extraction conditions optimized in this study for higher extraction yields could be studied to improve the enrichment of phenolic compounds.

The total polyphenols content found in the flaxseed oils of this study indicates an interesting result, that suggest a high quality, considering the useful properties of this class of molecules for the human health (42).

Data regarding the free acidity are

similar for both extraction methods. Therefore there is not significative selectivity by co-solvent to extract a higher quantity of free fatty acids in comparison with neat SC-CO_2. The high level of free acidity, in both methods, emphasize bad storage conditions of the seeds, taking into account that the extraction by SC-CO_2 is conducted with an inert solvent and in absence of light (40). Flaxseeds are a grain commodity dried during ripening and harvesting, and therefore prone to be stored for several months without microbial spoilage. Nevertheless, the enzymatic activity of lipase lead to increase in high levels of free acidity in comparison with other oils. Actually, the flaxseed oils sold on the market are not governed by specific standards or specifications for the food sector (41), but it is possible to do a comparison with another vegetable oil typical of the Mediterranean diet: the extra virgin olive oil. For this product, the European Regulation CEE n. 2568/91 and following modifications, indicate the maximum content of free acidity expressed in 0.8 g of oleic acid for 100 g of oil for its commercialization. Considering that the seeds used for this study are of commercial origin, it is possible to find flaxseed oils in the market with the conditions similar to those of this study. To exploit the useful properties of this high quality oil, it is important to preserve the flaxseeds from degradation, and for this aim, the low temperature of storage is probably needed to reduce lipase activity (43).

Conclusions

From a nutritional point of view, in recent years the flaxseeds are gaining more value, and are considered as functional food. In fact, they contain a high concentration of ω-3 fatty acids, fiber and lignans, essential nutrients for maintaining the health of the organism.

For the flaxseed oil of this study, the extraction yields of SC-CO_2 are lower by about 20% to those obtained by extraction with hexane. But the use of SC-CO_2 with ethanol as co-solvent allows to get an extraction yield higher than neat SC-CO_2, comparable with the hexane extraction, and allows to obtain a product of high quality. The analysis of the oils extracted by SC-CO_2 with ethanol shows a higher extraction of total polyphenols, molecules potentially useful for their antioxidant activity, and for the prevention and reduction of many chronic-degenerative diseases. The polyphenols content of the extracted oil is 100% higher than the oil extracted with co-solvent, in comparison with neat SC-CO_2. The high level of free fatty acids for both neat SC-CO_2 and SC-CO_2 with ethanol demonstrate the importance of storage conditions for flaxseeds as a raw material, and the not selective effect of the co-solvent studied for extraction of free fatty acids.

Because of the lipophilic nature of SC-CO_2, the application of SC-CO_2 for industrial scale flaxseed oil extraction can be explored, but its implementation is currently very limited, due to high costs of the industrial SC-CO_2 plants. When the technology cost could be reduced, the industrial application of oil extraction could be feasible also for flaxseeds.

This work suggests to investigate the content of lignans also because they could be concentrated in flaxseed oils obtained with SC-CO_2 mixed with ethanol. This hypothesis arise because the chemical structure and its polarity could influence its selective extraction.

This information would be important to confirm the previously validated high quality of flaxseed oil, also considering the contribution from the dietary intake of the lignans for maintaining of the health status, the prevention and reduction of diseases in the modern society.

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