Mild technologies and flaxseed oil

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Recent literature shows the healthy and nutritional properties of food derivates of flaxseed (*Linum usitatissimum* L.). Flaxseeds are a rich source of Alpha-Linolenic Acid (ALA, an essential ω-3 fatty acid) and lignans (phytoestrogens that can have hormone-like effects in the human body). Organic solvents are generally used to obtain a high extraction yield of flaxseed oil. An alternative method is the mechanical extraction that offers a remarkable reduction of environmental impact but with lower extraction yield in comparison with these solvents.
The first aim of this research activity was a focus on the mild extraction with SC-CO_2 SFE (Supercritical Fluid Extraction) for a comparison with the solvent extraction at lab scale. Therefore, extraction tests with SC-CO_2 were carried out, testing the operating conditions concurring to a different extraction in terms of extraction yield and/or implementation of the quality of the extracted oils in terms of fatty acids composition. The oils extracted with the different systems were analyzed in order to estimate the fatty acids composition, the oxidation stability and the phenolic composition. Another purpose of the research was to improve the quality of the flaxseed oil currently produced by mechanical extraction. Therefore, research was conducted to obtain useful information on the chemical composition of flaxseed oil and to modify this composition by fractionation with SC-CO_2. SC-CO_2 alone does not allow great extraction of lignans (important secondary components in flax seeds). Therefore, because of lignans polarity, the use of a polar co-solvent that enhances the extraction of these compounds was conducted too. The results obtained confirmed the potential ability of SC-CO_2 to obtain high quality products and by-products for agri-food chain.

INTRODUCTION

Flax (*Linum usitatissimum* L.) is an annual plant of the family of *Lineacee* [1], and its seeds are a rich source of lipids, proteins and dietary fibre [2]. Flaxseeds are also a very important source of Alpha-Linolenic Acid (ALA, an essential ω-3 fatty acid).
Moreover, the acidic composition of flaxseed oil presents the highest concentration of Poli-Unsatured Fatty Acids (PUFA) in the vegetal kingdom and linolenic acid and linoleic acid are the principal constituents of the total fatty acid component of flaxseed oil.
Finally, flaxseeds are an important source of lignans. The lignans are diphenolic compounds with a 2,3-dibenzylbutane skeleton [3]. Lignans are described also as phytoestrogens that have hormone-like effects in the human body. In fact, they are important for their anti-cancer properties and for their anti-oxidant power [4]. In flaxseed oil, Secoisolariciresinol-Di-Glucoside (SDG) and Matairesinol are the lignans mainly present. In humans digestion, SDG is converted in its agliconic form and, then, in enterolactone [5].
Flaxseed contains the highest concentration of SDG of the vegetal kingdom, and this presence is determined by the variety, pedo-climatic environment, year and the sowing period.
Flax and its derivatives consumption seem to have a fundamental role in the prevention of many pathologies [5], [6]:

- cardiovascular diseases;
- cancer forms; studies show how the assumption of flax derivates, thanks to the presence of ALA, can reduce the release of eicosanoids, primary font of growth of cancer cells, with particular effects on:
  1) Breast cancer: flax flour and flax seed oil can inhibit its development and growth. Lignans reduce the total neo-formed tumor mass of the 50%.
  2) Colon cancer: it is just the organ in which aglyconic and enterolactonic forms of lignans are formed.
- Diabetes; SDG, fibers and ALA are compounds of flaxseed that can reduce glycaemia.

Organic solvents are generally used to obtain a high extraction yield of flaxseed oil. An alternative method is the mechanical extraction, which offers a remarkable reduction of environmental impact but with lower extraction yield in comparison with these solvents [7].
The unquestioned high nutritional value of Essential Fatty Acids (EFAs) has moved studies to further concentration of EFAs in the diet oil [8].
The use of SC-CO_2 is the technology with the aim to achieve such efforts, by the use of a solvent with reduced environmental impact [9].
The first aim of this research activity was a focus on the mild extraction by SC-CO_2 (Supercritical Fluid Extraction, SFE) to be compared with the solvent extraction at lab scale. Therefore, extraction tests with SC-CO_2 were carried out, testing the operating conditions concurring to extraction yield and/or implementation of the quality of the extracted oils regard to the fatty acids composition.
The oils extracted by the different systems were analyzed in order to estimate the fatty acids composition, the oxidative stability and the phenolic composition.
Another purpose of the research was to improve the quality of the flaxseed oil currently produced by mechanical extraction. Therefore, a research to obtain useful information on the chemical composition of flaxseed oil and to, modify this composition by fractionation with SC-CO_2 was conducted.
Neat SC-CO_2 does not allow great extraction of lignans (important secondary components in flaxseeds). Because of lignans polarity [10], therefore the use of a polar co-solvent that enhances the extraction of these compounds was conducted too.

MATERIALS AND METHODS

Samples. For this research, commercial oils extracted by pressure and commercial flaxseeds of two cultivars (Barbara and Niagara) were used. The crops were of years 2005, 2006 and 2007 (for this year, cultivars Niagara of both winter and spring sowing were used;).
Reagents. Petroleum ether, 80% methanol solution, ethyl alcohol – ethyl ether 1:1 (v/v) solution, acetic acid-chloroform 3:2 (v/v) solution, water, n-hexane, acetonitrile, formic acid, all HPLC grade, were used. Nitrogen, purchased by Linde Gas Tecnici (Perugia, Italy), was of analytical grade and all solvents were purchased from Carlo Erba (Milano, Italy). For the determinations of free acidity, peroxide number, total polyphenols and total lipids, the AOAC official methods were used [11].
For the extraction of SDG from flaxseed oil, the method of Montedoro et al. [12] slightly modified was used.
HPLC/DAD analysis. High-Performance Liquid Chromatography (HPLC) equipment with a UV-VIS Diode Array Detector (DAD) 1100 Series (Agilent Technologies, Germany) was

used. A reversed phase HPLC was performed by a gradient elution with 100% of methanol and methanol/water. The detector was set up at 283 nm.

Supercritical Fluid Extraction (SFE) and SFE with co-solvent. The flaxseeds were stored at 15°C until their use. The experiments were conducted using a "Spe-ed-SFE" pilot plant of Applied Separation (Lehigh, PA-USA). 30 g of ground flaxseed were placed into an extractor vessel of 50 mL. The extractions were conduced at the pressure of 40 and 65MPa, at 70°C. The extraction consists of a static phase for 10 min. and a dynamic phase for 120 min. with CO_2 flow rate of 1.97 g $min.^{-1}$).

For the SFE with co-solvent, ethanol in percentage of 10% (w/w) on CO_2 (total 11,5 mL) was added at the moment of the sample loading in the vessel.

Supercritical fluid fractionation (SFF). The study was conducted on flaxseed oil extracted by pressure. The experiments were conducted using a pilot plant (Muller Extract Company GmbH, Koburg, Germany) equipped with a three stages column (total length 3 m and 30 mm i.d.) with internal volume of 2 L, and packed with stainless steel Raschig rings (10mm×10 mm); each column stage was individually thermostated. The 120 min runs were conducted using different pressures (10, 14, 15, 30 and 33 MPa) and CO_2 flow rate of 1.39 g $min.^{-1}$, setting the temperatures of the three column sections at 40, 50 and 60 °C, respectively, starting from the bottom. More details of the equipment are reported in Perretti et al, 2007 [13]

The lignans content (HPLC/DAD analysis) and the fatty acid profile with chromatographic analyses GC were conduced on the oils obtained by the selective extraction (fractionation).

RESULTS

The two samples of flax seed oil obtained by pressure (Barbara and Niagara 2005) were analyzed and lead the results shown in **table 1**.

Table 1: Quality parameters flaxseed oils obtained by pressure extraction (n=2).

Sample	**Peroxide number** (meq O_2/kg)	**Free acidity** (% oleic acid)	**Total Polyfhenols** (mg/Kg gallic acid)
Barbara 2005	6.20 ± 0.20	0.21 ± 0.01	12.7 ± 2.41
Niagara 2005	3.40 ± 0.20	0.42 ± 0.03	19.2 ± 3.93

In the two samples extracted by pressure, it is possible to see how the two seeds are in good conservation state, taking into account the parameters for other vegetable oil (Reg. 2568/91/CEE, modified by Reg. 1989/03 CE). The content of total polyphenols (less than 19.20 ± 3.93) resulted lower than that of other vegetable oils.

The extraction yields are reported in **table 2**.

Table 2: Extraction yields for oils from the two cultivars, for the different extraction methods (n=2).

Extraction method	***Barbara 2005***	***Niagara 2005***
Pressure	10.45 ± 1.34 %	12.77 ± 0.33 %
SC-CO_2 40 MPa	10.59 ± 1.6 %	6.85 ± 3.39 %
SC-CO_2 65 MPa	18.97 ± 0.08 %	20.51 ± 2.33 %
n-hexane	24.00 ± 0.68 %	30.40 ± 0.90 %

Using hexane as solvent (Soxhlet method) it was possible to obtain the higher yield values, followed by those obtained from extraction with SC-CO_2 at 65 MPa. Lower yields were obtained with SC-CO_2 at 40 MPa and in oils obtained by pressure. SC-CO_2 at 15 MPa was tested without extraction effect, because of the limited density of the solvent. The fatty acid compositions were analyzed to have a marker to test the quality of the oils extracted and to obtain preliminary information. In **table 3**, the percentage values of the most representative fatty acids, the percentage value of the fatty acids grouped for the length of the carbon chain, and for the unsaturation level, of the sample of oil extracted for pressure, with hexane and with SC-CO_2 at 40 and 65 MPa are reported.

Table 3: fatty acids in the oils extracted by pressure, SC-CO_2 and n-hexane (n=1)

Barbara 2005	**Pressure**	**n-hexane**	**SC-CO_2 40 MPa**	**SC-CO_2 65 MPa**
Palmitic acid C 16:0	5.84	5.83	6.01	7.13
Stearic acid C 18:0	5.18	5.22	5.11	4.66
Oleic acid C 18:1	19.13	19.18	19.20	19.12
Linoleic acid C 18:2	11.25	11.51	11.48	11.64
Linolenic acid C 18:3	57.66	57.34	57.27	56.57
C 14	0.04	0.04	0.04	0.07
C 16	5.92	5.94	6.11	7.23
C 17	0.09	0.08	0.08	0.09
C 18	93.46	93.30	93.29	92.23
C 20	0.25	0.21	0.21	0.2
C 22	0.16	0.17	0.19	0.12
C 24	0.08	0.06	0.08	0.04
Saturated Fatty Acids	11.50	11.70	11.62	12.22
Unsaturated Fatty Acids	88.5	88.3	88.38	87.78
Mono-Unsaturated FA	19.35	19.40	19.40	19.33
Di-Unsaturated FA	11.28	11.54	11.51	11.68
Poly-Unsaturated FA	57.87	57.36	57.47	56.77
Niagara 2005	**Pressure**	**n-hexane**	**SC-CO_2 40 MPa**	**SC-CO_2 65 MPa**
Palmitic acid C 16:0	4.34	4.47	4.84	4.61
Stearic acid C 18:0	5.09	4.92	4.58	4.67
Oleic acid C 18:1	20.25	20.07	20.06	19.82
Linoleic acid C 18:2	15.40	15.37	15.73	15.61
Linolenic acid C 18:3	53.99	54.4	53.96	54.47
C 14	0.02	0.03	0.03	0.03
C 16	4.41	4.52	4.91	4.68
C 17	0.10	0.07	0.09	0.08
C 18	94.87	94.96	94.56	94.79
C 20	0.28	0.18	0.21	0.21
C 22	0.15	0.15	0.13	0.14
C 24	0.08	0.06	0.04	0.06
Saturated Fatty Acids	9.87	9.78	9.78	9.67
Unsaturated Fatty Acids	90.13	90.22	90.22	90.33
Mono-Unsaturated FA	20.51	20.25	20.30	20.03
Di-Unsaturated FA	15.44	15.40	15.77	15.64
Poly-Unsaturated FA	54.18	54.57	54.15	54.66

It is possible to observe that there are not significant differences, because glycerides are not easy to be extracted selectively as in the case of free fatty acids. The chemical characterizations of the fatty acidic compositions, and the antioxidant contents of the oils obtained by SC-CO_2 and n-hexane were realized for the flaxseed samples of 2006 and 2007. Such comparisons were not done with the oils obtained by pressure, given the impossibility of reproduction of a continuous pressure extraction system for laboratory similar the continuous industrial presses. The results of the analysis of the flaxseed oils extracted with SC-CO_2 are shown in **table 4**.

Table 4: Quality parameters for the oils obtained by SC-CO_2 at 65 MPa.

Sample	**Peroxide nr.** (meq O_2/kg)	**Free acidity** (% oleic acid)
Barbara 2005	6.20 ± 0.25	0.21 ± 0.01
Barbara 2006	2.49 ± 0.05	1.72 ± 0.30
Barbara 2007	7.42 ± 0.04	0.50 ± 0.01
Niagara 2005	2.15 ± 0.47	1.14 ± 0.40
Niagara 2007 (spring)	4.63 ±0.24	1.24 ±0.02
Niagara 2007 (autumn)	2.82 ±0.23	1.81 ±0.02

It is possible to ascertain that the oils obtained by SC-CO_2 extraction are generally in a good conservation state, with a moderate variability. To verify conservation conditions, Kreis test was conducted too, with results under 127.5, typical of the well conserved oils. [14]. Total polyphenols analysis was done, as a marker, to verify the quality of the extracted oil (data not reported). In **table 5** the extraction yields are reported for flaxseed form different cultivars and harvests to verify the effect of SC-Co2 and hexane extractions.

Table 5: Extraction yields obtained from different varieties with different technologies.

Sample	**SC-CO_2 65 MPa (n>3)**	**n-hexane (n=2)**
Barbara 2005	18.97 ± 0.08 %	24.00 ± 0.68 %
Barbara 2006	12.94 ± 0.78 %	-
Barbara 2007	11.53 ± 1.42 %	17.45 ± 1.08 %
Niagara 2005	20.51 ± 2.33 %	30.40 ± 0.90 %
Niagara 2007(spring)	14.02 ± 1.52 %	24.13 ± 0.18 %
Niagara 2007 (autumn)	11.54 ± 1.12 %	18.38 ± 0.74 %

It is evident how n-hexane yields are higher than those of SC-CO_2; the SC-CO_2 yields are lower is in a range between 21% (Barbara 2005) and 42% (spring Niagara 2007) in comparison to the n-hexane extractions. The presence of minor compounds with healthy activity (lignans) was observed. The quantities are reported in **table 6**.

Table 6: Lignans in the analyzed samples.

Sample	**SDG** (mg/Kg)
Barbara 2005	1.68 ± 0.04
Barbara 2006	2.08 ± 0.10
Barbara 2007	n.q.
Niagara 2005	0.76 ± 0.10
Niagara 2006	n.q.
Niagara 2007	n.q.

(n.q. = not quantifiable)

Finally, with the aim to assay the enrichment of ω-3 fatty acids in flaxseed oil by the SC-CO_2 fractionation, numerous tests were done with a counter-current Pilot Plant. The tests have been realized in three different series. In terms of capacity to modify the fatty acid composition of flax seed oil, the best results are obtained by pressure of 33 MPa, with SC-CO_2 temperature of 40°C and gradient of temperature from the 40°C in the lower level of the column, till 60° of the higher level. Finally, for what regards the co-solvent extraction, the actual dates (non reported) indicate that co-solvent extraction can be considered suitable for further consideration.

CONCLUSIONS

It was observed how the SC-CO_2 technology can extract the glycerides with fatty acids of the ω-3 and ω-6 series, with extraction yields close to those of traditional solvents. The use of hexane as solvent (Soxhlet method) assures indeed yields higher than those of SC-CO_2 extraction at 65 MPa. Niagara is the studied cultivar with higher oil yields, but where lower quantities of SDG were detected after SFE. It was seen how the oil yields are in relation with the different years of harvesting. The extractive capacities of SC-CO_2 and its capacity to obtain interesting products by the point of view of quality were consolidated. The comparison with the other technologies confirmed their lower capacity to extract minor compounds important for their healthy power. At the actual state of the research, the minimal differences in terms of selectivity of fatty acids are not considerable. The amounts of lignans in the oils extracted by SC-CO_2 are lower in comparison with the amount of SDG in seeds (0.2-0.3%) described in literature [15]. Neat SC-CO_2 does not allow great extraction of those polarity compounds, therefore, the use of a polar co-solvent that enhances the extraction of these compounds was conducted too. At the moment, the results relative to the co-solvent extraction are in preliminary state. When also these additional results will be available, the properties of SC-CO_2 and its capacity to enrich selectivelly in lignans will be finally clarified.

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