Study Of The Supercritical Carbon Dioxide Extraction Conditions Of PUFA From Perilla Seeds

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ABSTRACT

The limited amount of fish oils available has led to extensive search for alternative sources of oils rich in long-chain n-3 polyunsaturated fatty acids (PUFA).
Evidence from a large number of epidemiological studies, clinical studies and intervention trials have established the protective effect of omega 3 PUFA against several pathologies such as cardiopathies, diabetes and cancer.
The fish oil remains the principal source of omega 3 even if a variety of alternative PUFA sources, such as bacteria, fungi, plants and microalgae are currently being explored for commercial production.
The present study reports the extraction of perilla frutescens var frutescens seeds oil by means of supercritical carbon dioxide technique. Composition of the obtained extracts was analyzed by different techniques allowing a careful comparison with oil obtained from mechanical press extraction.
Moreover, this plant of Asian origin, has been cultivated and bred in Italy.

Keywords: Perilla frutescens var frutescens, supercritical fluids extraction, quali-quantitative composition, PUFA.

INTRODUCTION

Perilla L. is a genus of annual herb in the Lamiaceae family. The most common species is Perilla frutescens. It is a common TCM plant in China. Its fresh leaves are used as vegetables in China and commonly used for seasoning pickles or as garnish for raw fish dishes in Japan. It is a popular leafy vegetable in Korea, which is generally consumed as a pickle or wrapping with roasted meats. The seeds are ground and added to soup for seasoning in Korea. Perilla has significant antioxidant, anti-inflammatory, antimicrobial, antidepressive, anxiolytic, chemopreventive and strong antitumor-promoting activities [1-3]. It is useful in the treatment of asthma, colds, cough, vomiting, abdominal pain, poisonings with seafood and allergic reactions [4].
The seed oil is rich in polyunsaturated fatty acids (PUFA) and in particular the C18:3 is particularly expressed making it an interesting source of this even more required compound.
Perilla seeds oil is also rich in flavors components such as perillaldeide that is the more expressed components. This components is the main base of many perfumes.

The perilla seeds oil is mainly extracted by two classical techniques that are those of solvent and mechanical press, but recently, supercritical carbon dioxide is successfully applied. The use of supercritical technique increase the extraction yield respect to the other two approaches.
In this paper, is presented the extraction of perilla seeds oil by means of supercritical carbon dioxide. For the first time, Perilla fruttescens var fruttescens has been planted in Italy and precisely in the hills of the province of Belluno (north east) where it was grown healthy and robust, meanwhile the strong climate condition affecting the zone.
The quali-quantitative results were compared to those obtained by mechanical press extraction.

MATERIAL AND METHODS

Material

Perilla seeds were supplied by De Bona's Azienda Agricola (Belluno- Italy). CO_2 purity 99% was purchased by Siad (Trieste- Italy). All the solvent were of analytical grade and supplied by Sigma Aldrich.

Method

Roasting perilla seeds

Dry perilla seed were roasted at 150 °C for 20' in an electric air oven. Then, the perilla seeds were cooled at room temperature.

Mechanical press

For the mechanical press extraction, roasted perilla seeds (30 g) were transferred into a hydraulic oil press machine (Sifradent, Germany). The maximum pressure for mechanical hydraulic press extraction was 5 Tons and held at the pressure for 20 min. The obtained oils were filtered through clean filter clothes to remove any particles. The oil was kept overnight at 4 °C.

Supercritical extraction

Roasted perilla seeds were milled with a blade mill (Moulineux) for 30 seconds. The supercritical fluid extraction experiments were performed using a column type extraction vessel (Separex-France, Autoclave A21 model) depicted in Figure 1. It consists of an extraction vessel of 100 mL

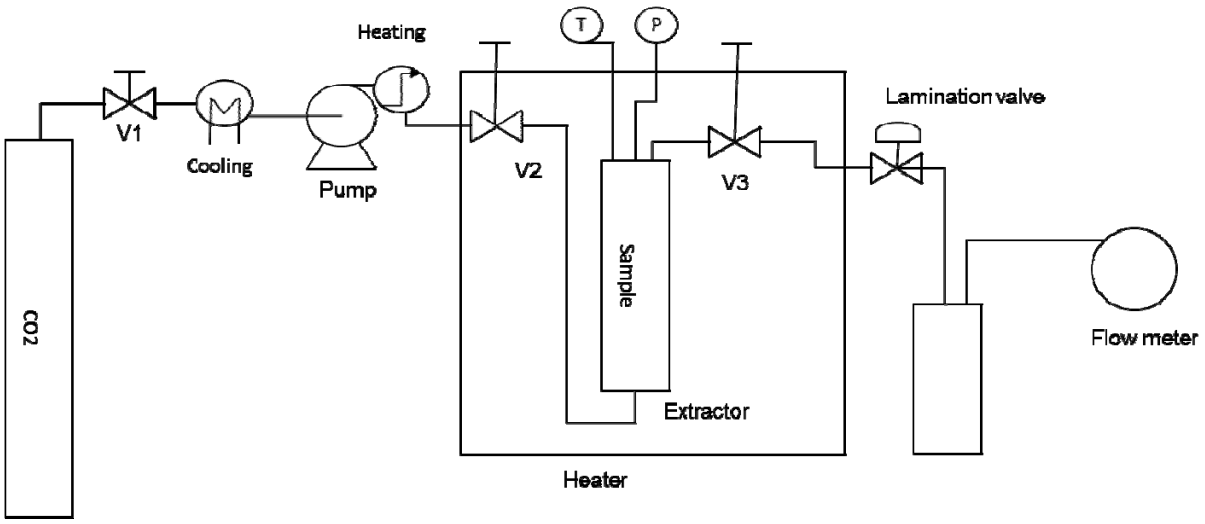


Figure 1: Schematic diagram of SFC extractor. V1, V2 and V3 are stopping valves; T, P are temperature and pressure meters, respectively.

filled within 30 g of milled perilla seeds. The extraction pressure was controlled by means of a back pressure regulator valve. In order to prevent sample plugging during experiment, the restricting point was warmed electrically. The gas flow-rate and total mass of CO_2 consumed in the experiment were measured with a gas flow meter at room conditions. The extracts were also precipitated and collected into a glass trap, immersed in an ice bath.
After extraction the samples were weighted (Sartorius BL 210 S) in order to calculate the extraction yield (y):

$$y = \frac{mass\ extract}{starting\ material\ mass}\ 100 \qquad (1)$$

Chemical analysis

The quali-quantitative evaluation of phytoconstituents was achieved using H- NMR (Bruker AMX-300 spectrometer) and different HPLC analysis. An Agilent 1260 RR-HPLC equipped with auto sampler, column oven and Diode Array detector was used for HPLC DAD analysis. The HPLC-MS system was formed by a Varian 212 binary liquid chromatograph equipped with Prostar 430 autosampler and an Ion Trap 500 Mass spectrometer. For H-NMR analysis the samples were solubilized in deuterated chloroform; for GC-MS analysis a Varian GC 3800 with a Satum ion Trap Mass spectrophotometer was used. Oils were hydrolyzed and derivatized to methyl esters, while for HPLC-MS analysis the samples were solubilised in DMSO.

RESULT AND DISCUSSIONS

The supercritical extraction of roasted perilla seeds were conducted at 300 bar, 50 °C for 4 hours showing an extraction yield of 45 % while the extraction conduced with mechanical press shows only the 36 %.
The extracted oil appears as limpid yellow liquid associated to a fresh characteristic odor.

NMR analysis of the obtained products

Considering triacylglycerol base structure, the relative positions are marked in the spectrum. The alpha-linolenic acid and some other fatty acids are the main groups of the triacylglycerols and diacylglycerols present in the perilla oil.
The spectrum in figure 2 shows the signals of the main fatty acids groups and those of esterified glycerol.
The integral values of the main identified groups (aliphatic protons, glycerol signals, olefinic protons) appear very similar in both samples extracted by supercritical fluids and mechanical press. This data reveals a substantial similarity about the composition of all the fractions analyzed.

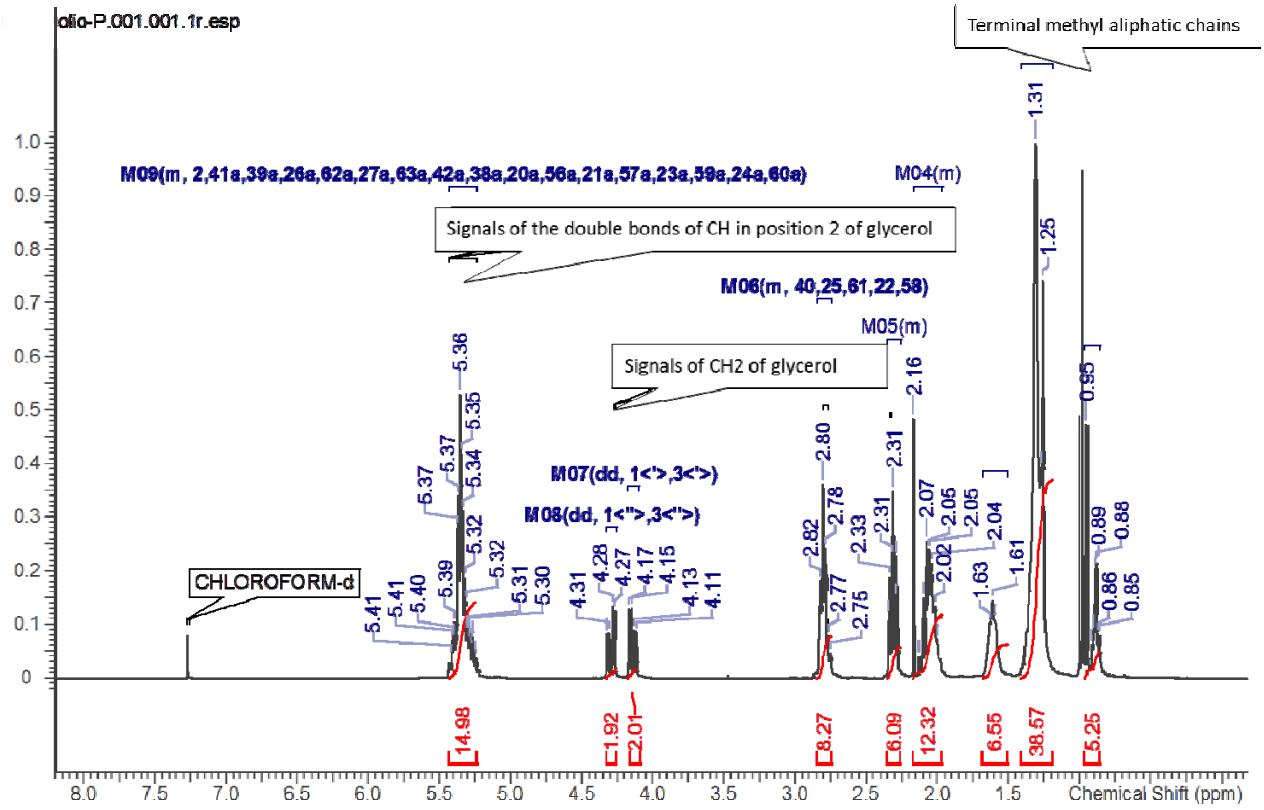


Figure 2: Perilla seeds oil H-NMR spectra

GC-MS analysis of methyl ester derivatives

In order to have more accurate information about the different fatty acids in the oil a GC-MS analysis was performed.
Fatty acids methyl esters identified and quantified are reported in table 1. The compositions of the different fractions of both samples extracted by supercritical fluids and mechanical press appeared very similar.

%	SCO2 extract	Mechanical press
Palmitic acid	6,89	6,00
Methyl isostearate	2,08	2,17
Vaccenic acid	1,45	0.5
Oleic acid	14,92	16,95
Linoleic acid	18,34	18,72
α –linolenic acid	56,32	56,15
α -cadinol	traces	

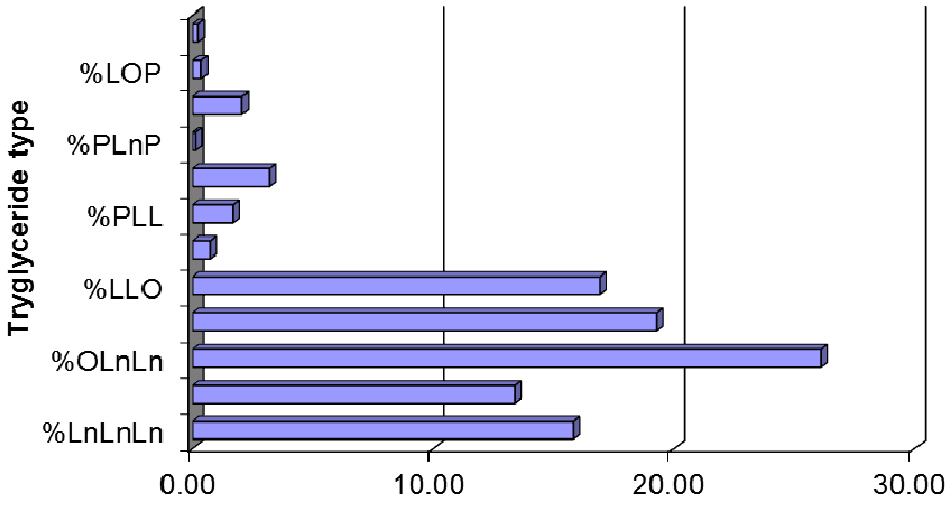
Table 1: qualitative composition of perilla seed oil obtained by supercritical extraction and

mechanical press

The main compound of perilla oil is the α-linolenic acid (56 %). The SCO_2 extract reveals traces of α-cadinol, a monoterpene described as a volatile component of the essential oil from perilla leaves. Moreover, higher amount of vaccenic acid was found in the SCO2 extract than that obtained by mechanical press.

HPLC-MS analysis

HPLC-MS analysis was performed in order to evaluate the possible changes in the native form of the lipids constituting the oils. Fragmentation patterns allow to discriminate the major fatty acids of triglycerides. As reported in literature [5] the main constituents in perilla oils are triglyceides containing α-linolenic acid. About the 80% of the lipids in the oils contain at least one fatty acid residue of linolenic acid. The relative amount of the detected species is reported in the table 4.



	%LnLnLn	%LLnLn	%OLnLn	%LnLO	%LLO	%LPS	%PLL	%LnPLn	%PLnP	%LnLP	%LOP	%LnLnSt
Serie1	15.8	13.3	26.0	19.2	16.9	0.72	1.68	3.19	0.13	2.05	0.35	0.23

Figure 4: % relative abundance of tryglycerides formed by the combination of the following fatty acids: L=linoleic; Ln=linolenic; O=oleic; P=palmitic; S=stearic; St=stearidonic

CONCLUSION

The obtained data revealed that the CO_2 treatment does not cause significant changes in triglyceide composition compared to the sample obtained by mechanical press. Nevertheless, the possibility to use SCO_2 for perilla oil processing appear to be interesting due to the fact that the composition of the product is similar to the one that can be obtained with conventional techniques but the supercritical treatment can be obtained in mild conditions of temperature and in large excess of CO2 thus limiting the possibility of oxidation and or oil modifications.

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