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A PRELIMINARY STUDY ON THE EFFECTS OF SUPERCRITICAL CO2 EXTRACTION ON CHEMICAL COMPOSITION OF MAQUI (ARISTOTELIA CHILENSIS [MOL] STUNTZ) BERRIES

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ABSTRACT

There is a growing interest to develop formulations with integrated berries extracts as a source of bioactive compounds with high antioxidant capacity, destined to the pharmaceutical and food industries. These bioactive compounds, such as flavonoids, phenolic acids and phenolic compounds, in fact, have demonstrated significant effects in reducing the risk of cardiovascular diseases and cancer.

Among these red/blue berries the Maqui fruits (Aristotelia chilensis (Mol.) Stuntz) have shown significant interest. Maqui berries were classified as novel food, ''food that has not been used for human consumption to a significant degree in the EU before 15 May 1997'' (Regulation (EC) No. 258/1997).

In this preliminary study supercritical carbon dioxide ($SCCO_2$) extraction was applied for the extraction of both bioactive compounds molecules and oil of Maqui. Early experimental results, at constant temperature and extraction time, showed that the extraction of oils was favored by higher values of P (in the studied range of 100-200 bar). The best oil yield was about 12 % and the different pressure values operated in $SCCO_2$ extraction were significant in the qualitative e quantitative profile of fatty acids.

As regarding phenolic compounds the yields were in the range of 3-35 % and the main class of compound in the extracts were anthocyanins such as delphinidin-3-sambudioside-5-glucoside, cyanidin-3-sambudioside-5-glucoside, delphinidin-3-sambudioside, delphinidin-3-glucoside, cyanidin-3-sambudioside, cyanidin-3-glucoside and delphinidin-3,5-diglucoside.

Keywords: Supercritical Carbon Dioxide, Extraction, Maqui, Oil, Fatty acid, Phenolic compounds.

1. INTRODUCTION

In the general classification system of the vegetable kingdom the Aristotelia Chilensis is a 3-4 meter high evergreen bush, native to Chile as well as western Argentina (Patagonian region), commonly called Maqui, which belongs to the Elaeocar paceae family. This dioecious plant produces small edible purple/black berries, with a diameter of 4-6 mm, that are eaten fresh or used for juice or jams: they ripen from December to January, the summer season in the southern hemisphere, and have a taste similar to blackberries. The A. Chilensis was classified for the first time, in 1844, by Claude Gay, a french botanist and naturalist, in his monumental work "Physical Atlas of History and Politics of Chile". Maqui is the Mapuche indigenous name of Aristotelia Chilensis: the local Mapuche people has for centuries known about the remarkable properties of the Maqui berry and used it to give them energy and remedy for dysentery, whereas the leaves were used to treat wounds. The Maqui berries, in fact, contain more polyphenols, in particular the flavonoid subgroup called anthocyanins, than any other known berry. Anthocyanins are responsible for the very dark purple color of the Maqui berries, also known from the dark color of red wines. Maqui cultivations exist also in Spain and in the more humid parts of Great Britain. A recent interesting study reports how the polyphenol and anthocyanin concentrations of the Maqui fruit are associated with different ripening stages [1].

In literature, many authors confirm the rich phenolic content and the high antioxidant capacity of the A. Chilensis fruits. They indicate the Maqui consumption related to health benefits such as anti-diabetic, anti-inflammatory effects and cardioprotective activities [2-8].

Generally, the isolation of natural compounds with functional properties from natural substrates is a very crucial step. Since these active compounds are present in low concentrations, in literature several works aimed on the development of more effective and selective extraction methods for their recovery from the raw materials. Therefore, extraction processes become essentials when they operated in the preparation of food additives, nutraceuticals, pharmaceutical, cosmetic products and so on. Supercritical fluid extraction (SFE) could be a "green" alternative to the conventional organic solvent extraction of these compounds, being the SFE processes fast, selective and with extracts free of residual solvents.

It is well known that supercritical carbon dioxide ($SFCO_2$) is the ideal solvent for the extraction of non-polar materials because its low critical conditions and GRAS status. For this reason, pure carbon dioxide is not usually employed for the extraction of hydrophilic phenolic compounds: several experimental articles are reported concerning the use of small amount of a polar co-solvent to increase the extraction power of CO_2 [9]. The most studied entrainers in the SFE extraction of phenolic compounds are methanol and ethanol, preferring the latter for applications in food and pharmaceuticals. [10-14]. Nevertheless, pure $SFCO_2$ can be used, in pre-treatment processes, for the removal of lipophilic compounds of the natural raw materials, giving to the hydrophilic polyphenols higher accessibility for further extraction steps [9, 15].

In this work pure $SFCO_2$ is used for the extraction of fatty acids (FA) from commercial lyophilized Maqui berries.
The influence of pressure on the process, at constant temperature and time values, is analyzed with attention to extraction yield and chemical composition of obtained fractions.

2. MATERIALS & METHODS

Materials

Lyophilized Chilean Maqui berries have been provided, in packages of 125 g, by Nutrisslim (Vrhnika - Slovenia), CO_2 (99% purity) was purchased by SIAD (Trieste – Italy).
Methylpendatecanoate, Dichloromethane, sulfuric acid, Methanol, ethanol, n-hexane, diethyl ether, sodium sulphate of analytical grade were provided by Sigma Aldrich.

Methods

Material preparation

Due to the non-homogeneity of the starting material, eight packages of lyophilized Maqui berries (about 1000 g) have been blending. Then the material was kept in a darkplace to avoid any possible degradation.

Supercritical extraction

The extraction process was operated using a continuous flow apparatus described previously [15] and reported in figure 1. The extraction of the samples with SCO_2 was conducted on a heated Separex SFE 20 system, equipped with a Lewa EKM210V1 high pressure pump and maintained at predetermined pressure by a Tescom 26-1000 valve (BPR1 of figure 1). In each experiment about 15 g of lyophilized Maqui berries were loaded into a 100 cc stainless steel extraction vessel. During the extraction process any sample was soaked in SCO_2 for 30 min and then extracted with a flow of 2 L/min, measured by a wet gas-meter at room conditions. The extract was recovered in a collecting chamber at 50 bar (by needle valve BPR2), to avoid the volatilization of the more volatile substances.

At the end, each extract was accurately collected and weighed using a balance (Sartorius BP3100 S) after a gradual and slow CO_2 releasing. Maqui oil yields were then calculated using the following equation:

$$Y = \frac{\text{weight of the extracted oil}}{\text{starting material weight}} \cdot 100 \qquad (1)$$

GC-MS analysis

GC-MS analyses on extracted oils were executed with a Varian 3800 gas chromatograph, equipped with auto-sampler (model 9800) and a Saturn 2100 Ion Trap Mass spectrometer. Mobile phase was Helium with a ion trap temperature of 210 °C. Injector was set at 220°C and mass spectra were acquired in the range 40-650 Da. Fatty Acid Methyl Ester (FAME) were prepared from extracted oils with the protocol of Indarti et al [16] with few modifications. Triplicate oil samples of 50 mg, placed in round bottomed flasks, were accurately weighed using a

balance (Mettler Toledo Classic AB204-S). Methylpendatecanoate solution in hexane (10 mg/mL) was introduced as internal standard. Dichloromethane, methanol and sulfuric acid (10: 30: 0,5 v/v) were then added and the mixture was refluxed for 30 minutes. Then, the resulting liquid was transferred to flasks kept in ice bath and containing water and diethyl ether. Flasks were vigorously shaken and the upper layer was collected, dried on anidrous sodium sulphate and used for GC-MS analysis. Agilent HP-88 (0,25 x 60m) and HP-5 (0,25 x 30 m) columns were used for fatty acid assays. For quantitative purposes, calibration curves were obtained using methylpentadecanoate as internal standard. Identification of fatty acids was achieved by standard references compounds and by comparison of the MS spectra obtained with NIST 2012 library of the instrument.

HPLC-MS analysis

The analysis of the original Maqui dried liophylized powder and of two SCO_2 raffinates (samples 2 and 3 obtained by runs 2 and 3 as above described), with ethanol/water as mobile phase, were obtained both in positive ion mode (for antocyanin derivatives) and in negative ion mode (for flavonoids, phenolic acid constituents). The liquid samples were directly used for HPLC analysis (after filtration 0,45 micron) the solid sample was exactly weighted (50-100mg) and extracted with methanol water for polyphenol analysis or with diluted HCl (0,5M) for antocyanin analysis. The negative ion mode allowed the detection of phenolic constituents that were characterized on the basis of their MS spectra and fragmentations. Assignments allowed the identification in the sample (Powder, sample 2 and 3) of several myricetin and quercetin derivatives, as well as phenylpropanoids and other flavonoid constituents.

3. RESULTS & CONCLUSION

Supercritical extraction

In table 1 the planned run conditions and the obtained Maqui oil yields, are reported. The results represent the average of at least three separate supercritical extractions. The maximum deviation between the measurements was of ± 5% giving a good indication of the expected accuracy of these preliminary results. It can be observed the strong correlation of the increasing oil yields with the pressure.

GC-MS analysis

In table 2 the compositions of the Maqui oil extracts (%w/w), obtained by GC-MS analysis, are reported. A notable difference is evident in total fatty acid content in the sample of extracted 1 (100 bar) if compared with samples 2 and 3 extracted at 150 and 200 bar respectively.

The linoleic acid is the component most quantitatively present in the extracts. It is an essential fatty acid that belongs to the ω6 group highlighting remarkable properties for the prevention and the treatment of cardiovascular disease (heart attack, atherosclerosis) and metabolic diseases (type II diabetes).
Figure 2 reports the gas chromatographic analysis of the fatty acids content in the SCO_2 extract of Run 2.

HPLC-MS analysis

In tables 3 and 4 the analyses of the original Maqui dried liophylized powder and of the two SCO_2 raffinate samples (1 and 3 of table 1) are compared. The results represent the average of five separate measurements. Also in this case pressure increases lead to higher contents in raffinates 1 and 3 of total phenolic compounds and anthocyanins.

The adopted extraction method appears to be very promising in order to extract and characterize many different compounds present in the analyzed material. There are several advantages in these extraction procedure, included the absence of the use of chemical solvents. Both gas chromatographic and HPLC analysis of Maqui estracts, even in this preliminary study, have confirmed that this product is a natural source of components as insatured fatty acids and phenolic compounds (especially anthocyanins). These molecules are believed to possess beneficial effects on the human health. Therefore the work is in progress for obtain more data in a larger number of samples and in order to optimize the extraction conditions.

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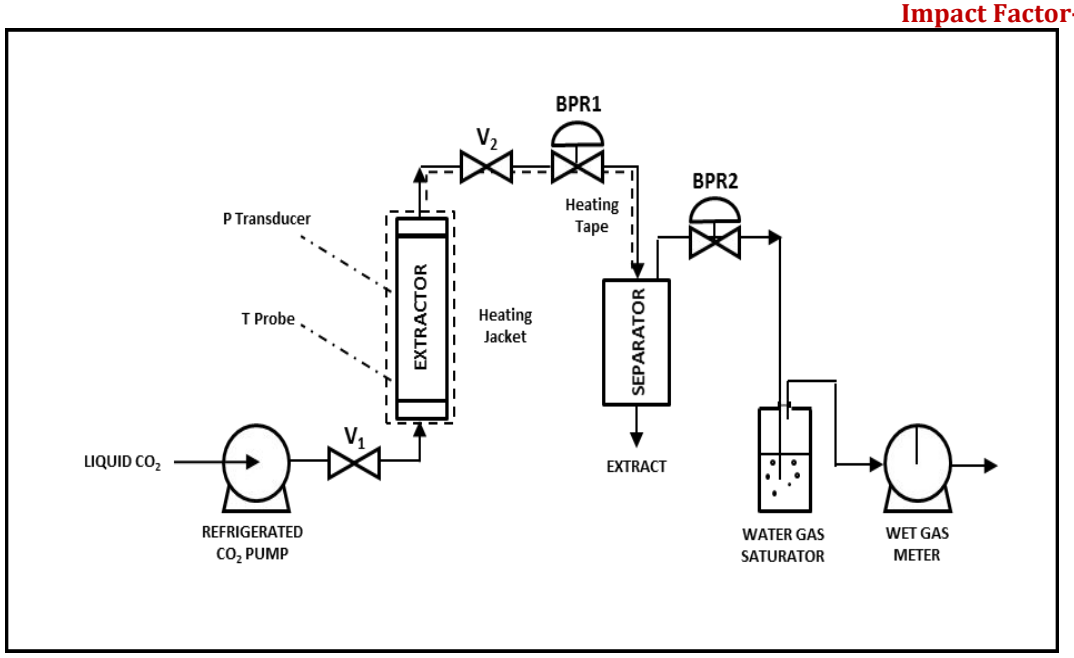

Figure 1: The SFE system: V_i = on-off Valves; BPRi = Back pressure regulators.

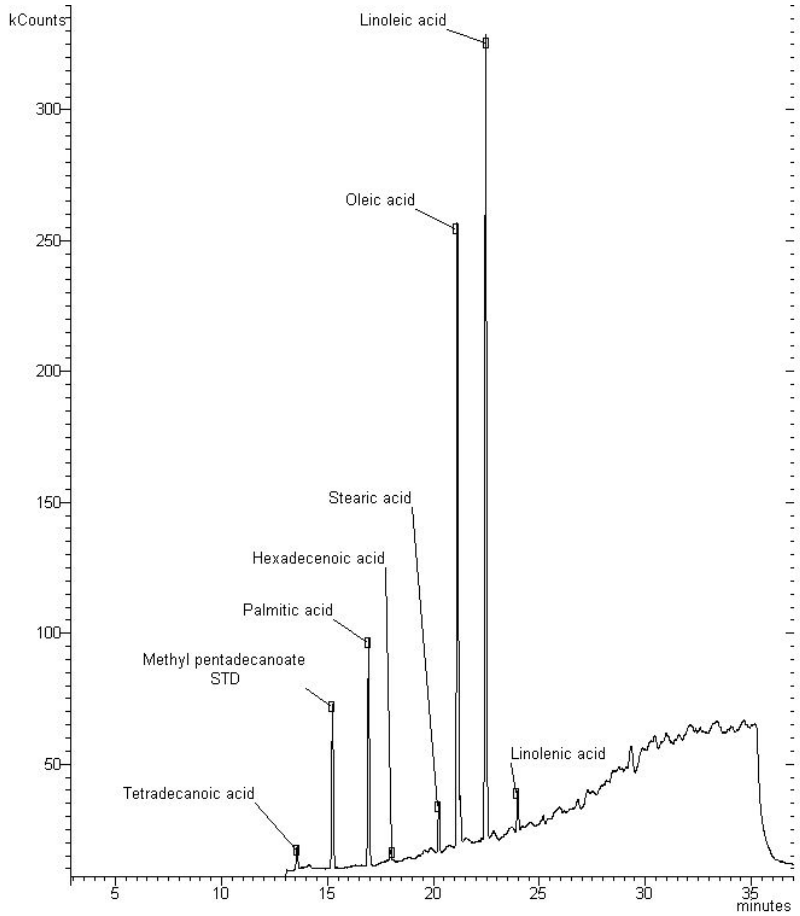

Fig 2: Fatty acids content in the SCO_2 extract of Run 2.

Table 1: Planned runs and obtained Maqui oil yields at 45,0°C and 90,0 min of extraction time.

Run	P (bar)	Y oil yield (%)
1	100,0	1,99
2	150,0	6,26
3	200,0	11,96

Table 2: Fatty acids (FA) amounts (%w/w) in the SCO_2 extracted oils.

Run	Tetradecanoic acid	Palmitic acid	Hexadecenoic acid	Stearic acid	Oleic acid	Linoleic acid	Linolenic acid	Total FA %
1	1,44	4,57	0,15	0,96	14,42	10,89	0,77	33,20
2	1,18	10,39	0,49	2,30	28,68	30,93	1,98	75,95
3	0,91	9,63	0,48	2,20	28,67	33,95	1,31	77,14

Table 3: Main MS identified Fragments.

Compound	M/z	Fragments	Powder	Sample 2	Sample 3
4-caffeoyl quinic acid	353	175-111	+	+	+
Chlorogenic acid (5-caffeoyl quinic acid)	353	191-173-127-111	+	+	+
Myricetin-rhamnoside	463	315-300-282-271-163	+	+	+
Myricetin-glucoside	479	315-300-271	+	+	+
Myricetin-galactoside	479	315-300-271	+	+	+
Myricetin galloyl hexoside	631	479-315-300-271	+	+	+
Quercetin glucoside	463	301-271	+	+	+
Dimentoxy quercetin	329	315-300-271	+	+	+
Ellagic acid	301	229-284-185	+	+	+
Quercetin-O-galloyl glucoside	615	463-301-271	+	+	+
Quercetin xyloside	433	301-271	+	+	+
Luteolin glucoside	447	285-257	+	+	+
Luteolin galactoside	447	285-257	+	+	+
Quercetin	301	271	+	+	+

Table 4: Comparison between total phenolics and anthocyanins contents in original Maqui powder and SCO_2 raffinates.

Compounds	Powder (mg/g)	Sample 1 (mg/g)	Sample 3 (mg/g)
Total phenolics	340,99 ± 1,76	365,50 ± 1,05	388,17 ± 12,98
Total anthocyanins	9,70 ± 0,42	10,57 ± 0,31	12,01 ± 0,13