Infrared Spectra of Green Arabica Coffee Extraction using Supercritical Carbon Dioxide and Soxhlet Technique

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The ATR-FTIR spectra of extraction oil from green arabica coffee was investigated. Coffee oil, caffeine and phenolic acids were extracted using supercritical CO_2, n-hexane and ethyl acetate. Assignments of the bands of the obtained FTIR spectra confirm the presence of lipids, polysaccharides, caffeine and phenolic acids in green coffee and coffee oil. The oil extracted by supercritical extraction is similar in composition to that obtained by Soxhlet extraction. The extraction yield using CO_2 supercritical is higher and requires much lower extraction time than the extraction with n-hexane. Using ethyl acetate solvent phenolic acids could be identified.

Keywords: supercritical extraction, coffee, coffee oil, caffeine, Attenuated Total Reflexion

The chemical composition of green coffee beans has been studied for over 140 years, but the greatest progress has been made during the last years due to the development of the modern separation and identification techniques. Many reviews on the chemical composition of coffee beans have been given by Streuli [1], Clifford [2], Farah [3], D. Pujol and col. [4] etc. The nonvolatile fraction of green Arabica coffee is composed primarily of water, carbohydrates (oligosaccharides and polysaccharides), proteins and free amino acids, lipids, minerals, organic acids, chlorogenic acids, trigonelline and caffeine. Of these compounds found in green coffee, chlorogenic acids, caffeine, trigonelline, soluble fiber, and diterpenes from the lipid fraction are most likely to be bioactive, and they may also be important contributors to the beverage flavor after roasting.

Lipids are major components of green coffee. The lipid fraction of coffee is composed mainly of triacylglycerols (approximately 75%), free fatty acids (1%), sterols (2.2% unesterified and 3.2% esterified with fatty acids), and tocopherols (0.05%), which are typically found in edible vegetable oils. This fraction also contains diterpenes of the kaurene family in proportions of up to 20% of the total lipid fraction [5, 6]. Fatty acids in coffee are found primarily in combined forms. Most fatty acids are esterified with glycerol in the triacylglycerol fraction, 20% esterified with diterpenes, and a small proportion in sterol esters. Fatty acids are not only important for health, but their integrity is important to keep coffee fresh and avoid the staleness caused by hydrolysis and oxidation of triacylglycerols [7].

The major categories of sterols in coffee are 4-desmethylsterols (accounting for approximately 93% of total sterols), 4-methylsterols (2%), and 4,4-dimethylsterols (5%). Sitosterol belongs to the first category and accounts for up to 54% of the sterol fraction; stigmasterol and campesterol each account for approximately 20% [8].

Caffeine

Caffeine (fig.1) is a methylxanthine with bitter characteristics; however, it is responsible for no more than 10% of the perceived bitterness of the coffee beverage [9]. Caffeine stimulates the central nervous system as an adenosine-receptor antagonist.

Phenolic acids

Caffeic acid and chlorogenic acid have an antioxidant activity due to dietary polyphenols who are thought to be beneficial for human health as antioxidants. Chlorogenic acid is the ester of caffeic acid. Chlorogenic acids confer astringency, bitterness, and acidity to the coffee brew [3]. Chlorogenic acids are precursors of phenols and cathecols which contribute to coffee flavour and have a potential biopharmacological importance of humans. Pharmacologic properties attributed to caffeoylquinic and dicaffeoylquinic acids include antiviral activity against adenovirus and herpes virus [10], hepatoprotective activity in an experimental model of liver injury [11], and immunostimulatory activity [12].

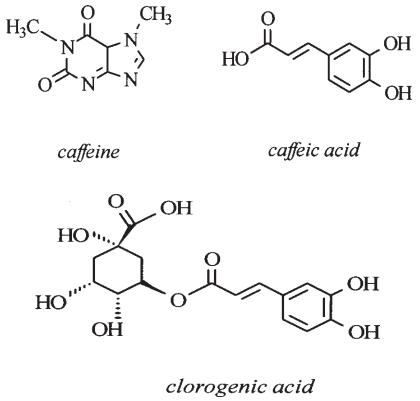

Fig. 1. Chemical structures of major coffee ingredients

Fourier transform infrared spectroscopy (FTIR) is a well established and constantly developing analytical technique, which allows for the rapid, high-throughput, non-destructive analysis of a wide range of sample types [13,14]. FTIR spectroscopy, a widely used technique in chemistry, biochemistry and biophysics, provides structural information of chemical and biological molecules including proteins, nucleic acids, carbohydrates, lipids etc. [15]. The resultant infrared spectrum can be described as an infrared 'fingerprint' characteristic of any chemical or biochemical substance [15-17].

This paper presents a FTIR study of green coffee extracts obtained by two techniques: extraction with supercritical fluid and extraction with conventional solvents.

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Supercritical CO_2 extraction is among the new emerging clean and environmental friendly technologies for the processing of food and pharmaceutical products [18]. The solubility of substances in supercritical CO_2 decreases with the increase in the number of polar functional groups (e.g. hydroxyl, carboxyl, amino and nitro). Thus the solubility of chlorogenic acid (main phenolic compound in coffee, an ester of *trans*-cinnamic acids) is expected to be low [19].

Experimental part

Green coffee oil (GCO) is extracted from Coffee arabica beans and first grounded in a mill.

Carbon dioxide was purchase from ICSI Rm Valcea.

n-Hexane and ethyl acetate are Fluka products.

Supercritical Fluid Extraction [SFE]

The Supercritical carbon dioxide extraction system and components were acquired from JASCO (Japan Spectroscopic Co.). Supercritical fluid extractor included the following: 250x 20 mm extraction vessel, column oven temperature (JASCO CO-2060), high-pressure pump (JASCO-PU-2080-CO_2), automated back pressure regulator (JASCO BP-2080). The experiments were carried out at 60°C and pressures of 25 MPa. Solvent mass flow rate was kept at 2 mL/min. At this flow rate it can be assumed that equilibrium concentration for the solvent and solute is achieved. Samples of 6 g of dried ground green coffee beans were placed in the extractor. The extracts were collected in one tube throughout the 150 min, and the yield was calculated.

Soxhlet extraction

Soxhlet extractor was used for coffee oil extraction. Oil was extracted from 50 g of green coffee grounds using 100 mL hexane solvent under reflux. The reaction time was 420 min. After the extraction had been accomplished, the extracts were left overnight so to allow separation of the oil phase from aqueous phase. Then, a vacuum distillation system at constant temperature was used to recover the oil from the extracting solvent using a rotary evaporator (Rotavapor, Heidolph) and a vacuum pump (Buchi V-700). The rotary evaporator was used for the efficient removal of n-hexane from samples by evaporation at 45°C.The amount of crude oil was determined from the original sample weight and the weight of the extraction cup before and after the extraction, i.e., by directly weighing the extracted crude oil [20].

Yields calculation in SFE and Soxhlet extraction

% crude oil extracted = $(W_2 - W_1)/W_3 \times 100$

Where W_1 = weight of the extraction cup , W_2 = weight of the extraction cup + extract, W_3 = weight of the coffee ground sample.

Extraction of ground coffee with ethyl acetate

50 g of green Arabica ground coffee was extracted with 300 mL of hot water. Extracts were immediately cooled on an ice bath and hydrophobic compounds were extracted with ethyl acetate. Subsequently the ethyl acetate phase was dried with magnesium sulphate and filtered. The ethyl acetate was evaporated using a rotary evaporator.

ATR-FTIR Analysis

The green Arabica coffee and the coffee extract were scanned using a FTIR Jasco 6300 spectrometer. An ATR accessory equipped with a diamond crystal (Pike Technologies) was used for sampling. The spectra were recorded in the region of 4000-400 cm^{-1}, detector TGS, apodization Cosine. The spectral data were processed with JASCO SpectraManager II software. Samples were scanned at 4 cm^{-1} resolution, accumulation: 100 scans.

Results and discussions

In order to estimate the oil extraction yield with supercritical CO_2 were performed conventional solvent extraction with n-hexane and ethyl acetate. The data reveal that the amounts of coffee oil extracted using supercritical CO_2 were higher than those obtained by Soxhlet extraction. Thus, with supercritical CO_2 extraction the yield in coffee oil is 8.5% in 150 min, using n-hexan in Soxhlet extraction the same yield is obtained over seven hours.

FTIR-based methods are fast, reliable, simple to perform and do not require sample pre-treatment. Such technique provides simple and reproducible means of handling food products with nondestructive analyses, with the sampling/ analysis procedure usually taking only a few minutes. Attenuated total reflexion (ATR) is now the most common sampling technique in FTIR spectroscopy. ATR technique involves the collection of radiation reflected from the interface between sample and a prism, in which evanescent wave penetrated from the prism in sample is absorbed by substances [21].

Infrared spectra of green Arabica ground coffee

Typical FTIR spectra obtained for Arabica green coffee samples are shown in figure 2.

The broad band about 3281 cm^{-1} is attributed to OH groups. The presence of methyl and methylene groups is confirmed by two sharp peaks at 2920.66 cm^{-1} and 2852. 2 cm^{-1} attributed to asymmetric and symmetric stretching of C-H bonds in aliphatic chain. These peaks identified in green coffee have been attributed to the presence of lipids. The same peaks have been attributed to the presence of caffeine [22].

Ground-coffee brand exhibited characteristic infrared bands between 1800 and 800 cm^{-1}. The 1800 to 800 cm^{-1} region contains absorbance bands attributed to the stretching vibrations of C=O groups. These bands can be ascribed to organoleptic vinyl esters, lactones, esters, aldehydes, ketones, and acids present in the brewed coffee. The sharp band at 1741 cm^{-1} is associated to the carbonyl vibration (C=O) in aliphatic esters, in triglycerides. Therefore, this band can be attributed to lipids. The low intensity bands at 1644 cm^{-1} and 1550.49 cm^{-1} are due to C=C vibration of lipids and fatty acids, and C=C vibrations of aromatic rings from lignin moieties, respectively [23]. The band at 1455.03 corresponds to C-H bending of CH_3 groups.

The bands appearing below 1400 cm^{-1} are referred to as the fingerprint region, as they are difficult to assign to specific functional groups. However, in this case, the strong absorptions between 1200 and 900 cm^{-1} can be assigned to C-O-H and C-O-C groups stemming from carbohydrate absorptions (for example, cellulose) [22].

For polifenolic acids (caffeic, chlorogenis acids) there are characteristic bands at 1376.96, 1241.93, 1153.22, 1024.98 cm^{-1}. FTIR spectroscopy cannot distinguish the bands corresponding to chlorogenic acids from polysaccharides. A chromatographic analysis should be applied to detect the presence of chlorogenic acids. Some studies have shown that chlorogenic acids were only extracted when isopropyl alcohol was used as a cosolvent [24].

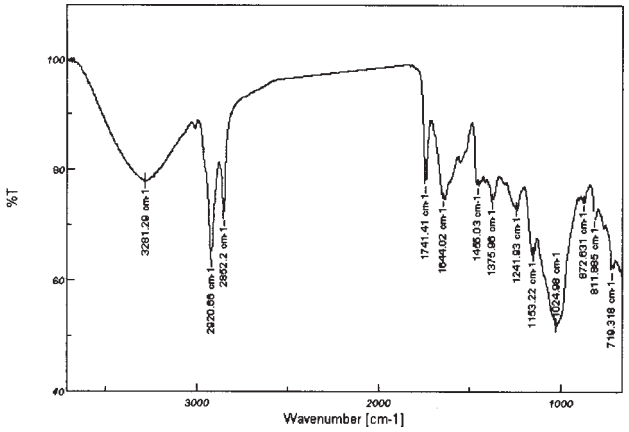

Fig.2. FTIR spectrum of green coffee

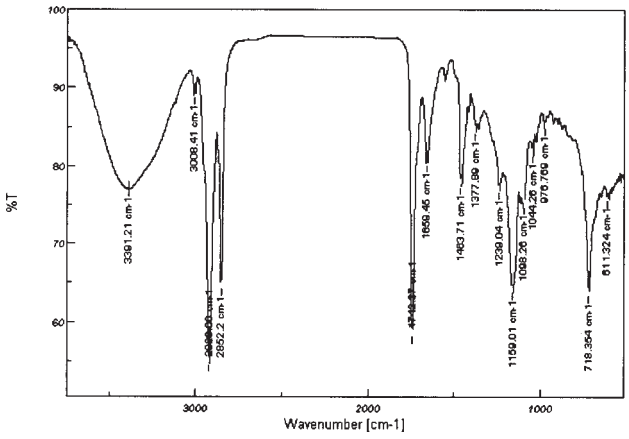

Fig.3. FTIR spectrum for oil extracted by using supercritical CO_2

Infrared spectra of green Arabica coffee oil by supercritical CO_2 extraction

Figure 3 shows the FTIR spectrum for the crude oil from green coffee obtained by supercritical CO_2 extraction.

In figure 3 the functional groups observed were methyl, methylene, amine and carbonyl groups associated to lipids (fatty acids, sterols, glycerides) and caffeine. A broad absorbance between 3000 and 3550 cm^{-1} was attributed to OH from water. The methyl group is shown by the presence of bands: 2852.2 cm^{-1} for the C-H stretch and 1463.7 cm^{-1} for the C-H bend. Also, C-H bend, C-H stretch and C-H rocking were observed at 2920, 1463.7 and 718.35 cm^{-1} respectively for methylene group. Other band observed for carbonyl group at 1742.37 cm^{-1} and secondary amine C-N stretch at 1159.01cm^{-1}.

Figure 4 presented the FTIR spectra of green ground coffee before and after extraction (decaffeinated coffee) with supercritical CO_2.

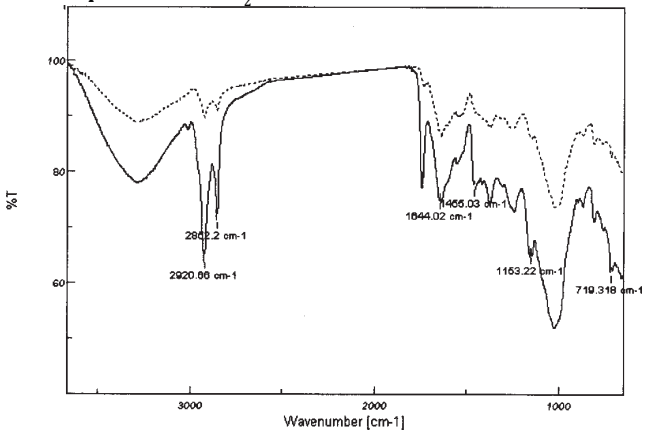

Fig.4. Comparison of caffeinated (–) and decaffeinated (···) Arabica green coffee

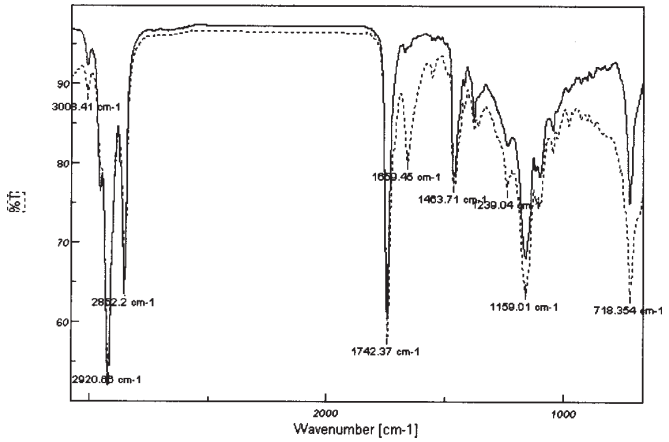

Fig.5. FTIR spectra of oil extracted by supercritical CO_2 (···) and by Soxhlet extraction with n-hexane (–)

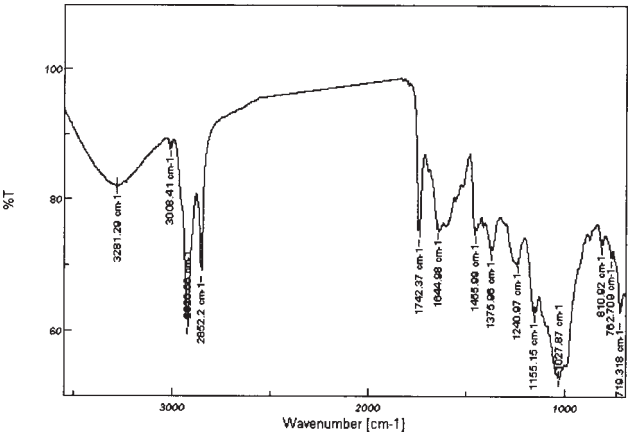

Fig.6. FTIR spectrum of oil extracted using ethyl acetate

The identification of the bands associated to caffeine in green coffee are: 2929.66, 2852.2, 1644.02, 1153.22 and 719.31 cm^{-1} (the last are attributed for C-H deformation in caffeine).

In the samples of green coffee the peaks attributed to caffeine displays a higher absorption. There was a significant decrease in peak intensity with the decrease in caffeine concentration.

Infrared spectra of green Arabica coffee oils extracted using methanol and ethyl acetate

The oil produced by different extraction solvents was also analyzed.

The FTIR results for the crude oil in hexane by Soxhlet extraction are presented in figure 5.

The functional groups observed in oil with n-hexane extraction were methyl, methylene (2920.66, 2852.2, 1463.7 and 718.35cm^{-1}), amine (1159.01cm^{-1}) and carbonyl groups (1744.45, 1669.45 cm^{-1}). It was found that there is no difference in the functional groups observed for the two samples.

The oil obtained through ethyl acetate extracts and analyzed by FTIR spectroscopy (fig. 6) revealed the spectral regions with mainly bands at 2920.66 and 2852.2 cm^{-1} due to asymmetrical and symmetrical methyl stretching, 1742.3 cm^{-1} due to carbonyl stretching vibrations, 1647cm^{1} for α,β-unsaturated C=O, 1455 cm^{-1} for aromatic C=C (from caffeic and chlorogenic acids).

Conclusions

The FTIR study revealed that Coffee oil extracted with Supercritical CO_2 and n-hexane led to the oil-like composition (fatty acids, sterols, caffeine). The CO_2

decaffeination process is superior to other decaffeination methods with other solvents because in first of all the CO_2 is a green solvent, is non-flammable, exhibits a relatively low toxicity and is naturally abundant, it removes caffeine while leaving the coffee's flavour compounds intact. Supercritical CO_2 removes caffeine from coffee; it does not affect the carbohydrate (sugar, starch) and peptides (protein), which are ultimately responsible for the flavour and aroma of brewed coffee. Since carbohydrates and peptides are large polar molecules, nonpolar CO_2 extracts only the caffeine molecules, which are small nonpolar molecules.

Using polar solvent such as ethyl acetate can be extracted phenolic acids (caffeic, chlorogenic, quinic acids). In such case, the spectra were relatively more complex than those from CO_2 supercritical and n-hexane extracts.

Natural phenolic compounds, caffeine from green coffee shows antiviral effects, thus the CO_2 extractions will be used in subsequent studies.

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