

# Fractionated subcritical carbon dioxide extraction of triacylglycerides from *coffea arabica*

Jane Jesus da Silva Moreira<sup>1</sup>, Jair Sebastião da Silva Pinto<sup>2</sup>, Thaís Camarotto Oliveira<sup>3</sup>, Fernando Mauro Lanças<sup>3\*</sup>

<sup>1</sup>Departamento de Engenharia de Alimentos, Universidade Federal de Sergipe, Av. Marechal Rondon, s/n, 49100-000, São Cristóvão, SE, Brazil.

<sup>2</sup>Escola Superior de Agricultura Luiz de Queiroz-ESALQ/USP, 13418-900 Piracicaba-SP, Brazil.

<sup>3</sup> Universidade de São Paulo, Instituto de Química de São Carlos, 13560-970 São Carlos (SP), Brazil. Corresponding author: F.M. Lanças, [flancas@iqsc.usp.br](mailto:flancas@iqsc.usp.br)

## Resumo

Uma das variedades de café comercialmente importante é o *Coffea arabica* L., desde que muitas bebidas comerciais de café são feitas a partir de grãos de *arabica* ou suas misturas. Consequentemente, a variedade *arabica* tem preço mais alto. Oxidação de lipídeos em café armazenado por muito tempo tem sido reportada com a perda de qualidade do café. Embora o comportamento dos lipídeos do café durante o armazenamento não tenha sido sistematicamente examinado, poucos estudos têm sido reportados sobre a liberação de ácidos graxos. Análise química pode ser aplicada para o controle de qualidade da fração lipídica. Usando essa abordagem, um método para análise dos triacilglicerídeos (TAG) por cromatografia gasosa de alta resolução a alta temperatura com detector de ionização em chama (HT-HRGC/FID) depois da extração com fluido sub ou supercrítico (SFE/SbFE), foi desenvolvido. A extração foi executada com um extrator SFE, construído em nosso laboratório, com amostra de óleo de café *arabica*. A composição encontrada dos TAG do óleo de café *arabica* brasileiro foi de T<sub>52</sub> a T<sub>56</sub>.

**Palavras chaves:** Café *arabica*, Extração com fluido subcrítico, Química analítica verde (GAC)

## Abstract

A commercially essential coffee specie is *Coffea arabica* L. Most commercial coffee beverages are made from *arabica* beans or its blends. Consequently, the *arabica* variety has a higher price. Oxidation of lipids in coffee stored for a long time has been reported with the loss of coffee quality. Although the behavior of coffee lipids during storage has not been systematically examined, few studies have been reported on the release of fatty acids. Chemical analysis can then be applied to the quality control of the lipid fraction. Using this approach, a method for the analysis of triacylglycerides (TAGs) by high-temperature high-resolution gas chromatography with flame ionization detection (HT-HRGC/FID), after supercritical or subcritical fluid extraction (SFE/SbFE), was developed. The extraction was performed with a homemade SFE in the *Coffea arabica* L. oil sample. TAGs composition of Brazilian *Coffea arabica* oil was found to go from as T<sub>52</sub> to T<sub>56</sub>.

**Keywords:** SFE; HT-HRGC, *Coffea arabica* oil, Subcritical fluid extraction, Green Analytical Chemistry (GAC)

## 1. Introduction

The goal to reduce the use of hazardous organic solvents in analytical extractions has contributed in the last few years to the development of new technologies using less solvent than standard extraction procedures (1). Among these techniques for rapid, efficient, and cost-effective means of sample treatment, pressurized fluids have received particular attention. This class of techniques has received the broad name of Pressurized Fluid Extraction (PFE) (2). Including in this class of extraction methods are Supercritical Fluid Extraction (SFE), Sub-Critical Fluid Extraction (SbFE), and Accelerated Solvent Extraction, ASE.

An ideal extraction method should be fast, simple, and inexpensive to perform. It should yield quantitative recovery of the target analytes without loss or degradation, besides providing a sample that is immediately ready for analysis without additional concentration or class fractionation steps(3,4). Unfortunately, classical liquid solvent extraction techniques frequently fail to meet these goals. They often require several hours- or even days- to perform, result in a diluted extract, and may not result in a quantitative recovery of the target analytes. Recent concerns about the hazardous nature of many commonly used solvents, the costs and environmental dangers of waste solvent disposal, and the emission of hazardous solvents into the atmosphere have led to the development of alternative extraction methods(5). In this context, PFE, the technique based on the use of pressurized fluid as leaching agents, is a tool to overcome the difficulties of solid sample extraction (6,7).

The benefits of PFE, versus conventional techniques, result from the accelerated kinetic desorption processes for the analytes from the matrix. The rapid diffusion of analytes in the fluid and the fluid solvation power (8–10), has led to PFE being proposed as an alternative to conventional liquid solvent extractions such as Soxhlet and ultrasonic extractions.

The analytes are better extracted at lower temperature conditions, thus increasing the extraction selectivity. In this case, the subcritical fluid condition can be explored (11). This extraction condition offers some advantages over supercritical CO<sub>2</sub> conditions for selected compounds, especially for triacylglycerols (TAGs). The extraction is usually selective to TAGs in a complex matrix such as coffee oil. Brazil stands out as the world's largest coffee producer, with the Arabica species responsible for 75% of its production (12). Although coffee oil has commercial importance, few studies have been carried out on the undesirable changes in lipids due mainly to the hydrolysis of triacylglycerols during the storage of the seeds with the release of free fatty acids, in turn, are oxidized (13–15). Degradation of free amino acids, sugars, and oxidation of lipids in coffee stored for a long time has been reported along with the loss of Brazilian coffee quality. An increase in free fatty acid content was associated with enzymatic activity due to long storage periods in subtropical conditions (13).

The High-Temperature High-Resolution Gas Chromatography (HT-HRGC) used to analyze high molecular mass compounds is preferable than conventional Gas Chromatography (16). Among many other HT-HRGC/FID features, it does not require derivatization of the sample before the injection, permitting direct analysis of the TAGs extract(17,18).

In this context, this work aimed to study the feasibility of performing PFE using a home-made SFE instrument (19), for the extraction of TAGs from coffee oil, a complex matrix, using supercritical and subcritical CO<sub>2</sub> and further analysis of these extracts by HT-HRGC/FID.

## 2. Experimental

### 2.1. Solvents and Materials

All reagents and solvents were analytical grades. Carbon dioxide was from AGA (São Paulo, Brazil). Silica

gel G 60 and n-hexane from Merck (Rio de Janeiro, Brazil). FAMES and standard TAGs were from Sigma (St. Louis, MO, USA).

## 2.2. SFE/SbFE of coffee oil.

Pure and modified supercritical carbon dioxide are suitable extraction media for many lipid materials such as vegetable oils, fatty acids, fatty acid esters, phospholipids, triacylglycerol, and tocopherols (11).

The home-made SFE device used in this work consists of a high-pressure pump, extraction cell, restrictor, collection vial, tubes, and valves. The extraction cell has a volume of 2 mL, made from an empty stainless steel liquid chromatography column. A capillary fused silica restrictor

(30 cm x 0.05 mm i.d) was used to adjust the flow rate, maintain constant pressure, and transfer the extract into the collection device. To avoid the loss of volatile extracted analytes, the 10 mL collection vial containing n-hexane was maintained refrigerated with cooled water. The fluid used was CO<sub>2</sub> (carbon dioxide).

Sample of *Coffea arabica* L. oil, in triplicate, obtained from the toasted seeds was a donation from Dr. Suzana Nixdorf of the University of Londrina, Brazil, and was adsorbed in silica gel (20). In the extraction cell, 1 g of silica saturated with coffee oil sample is loaded. To optimize the PFE conditions, different temperatures and pressures were evaluated. The extraction was performed under both supercritical and subcritical conditions, as shown in Table 1, and the required extraction time was 25 min.

**Table 1.** Supercritical and subcritical fluid extraction conditions for triacylglycerides from coffee oil.

PSE condition	T (°C)	Pressure extraction (atm)	Density (g/L)
E-1	60	100	300
E-2	60	200	730
E-3	60	300	830
E-4	100	300	665
E-5	0	300	1050

## 2.3. Analysis of the extracts obtained through SFE and SbFE

After extraction, the contents of the vials were dried. The samples were prepared at 0.10 mg/mL of the oil in n-hexane as solvent and subjected to HT-HRGC/FID analysis.

## 2.4. HT-HRGC/FID Analysis.

The chromatographic analyses were performed on a Hewlett-Packard 5890 Series II Gas Chromatograph with a split injector at 370 °C and a flame ionization detector (FID) at 380 °C. The injection volume was 1 µL, and the split ratio was 1:8. Hydrogen was employed as carrier gas at a pressure of 6 psi. A 9 m x 0.25 mm x 0.08 µm Chroma-5-HT (5% phenyl, 95% methyl polysiloxane bonded phase) capillary column, coated in-house, was used with

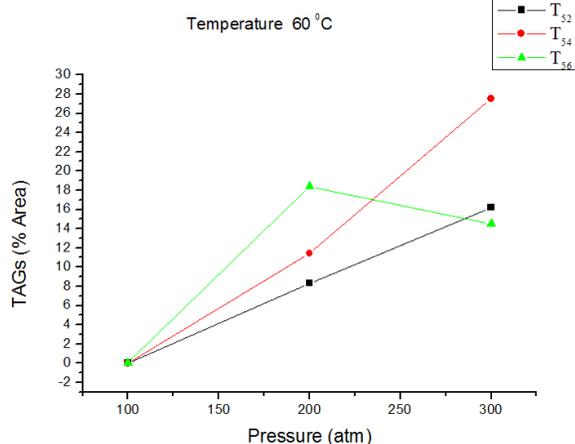
temperature programming from 280 °C for 1 min, then increased by 4 °C min<sup>-1</sup> to 380 °C, and maintained at this temperature for 5 min. The data were collected with an HP 5396 A integrator.

The identification of the TAGs was made by comparing the retention times of the components of interest with the retention time of the triolein (T54) analytical standard.

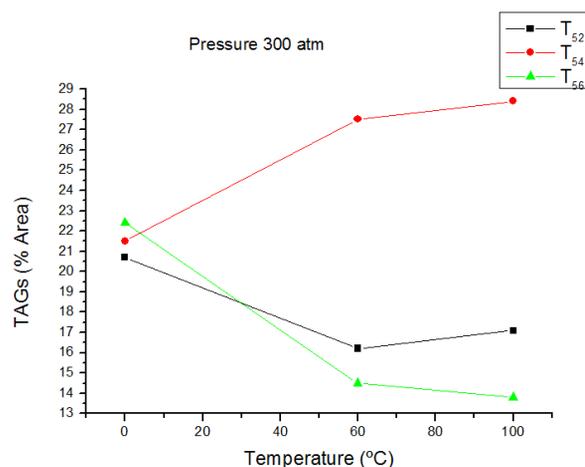
### 3. Results and Discussion

The SFE/SbFE was carried out with CO<sub>2</sub> in both supercritical conditions (60° C, and 100, 200 and 300 atm; 100° C and 300 atm) and subcritical conditions (0° C and 300 atm). Complete extraction could be achieved in less than 25 min.

The results obtained (Figure 1 and Figure 2), show that increasing the density of carbon dioxide results in increasing the solvation power of the extractant solvent, allowing a fractionated extraction to be conducted.

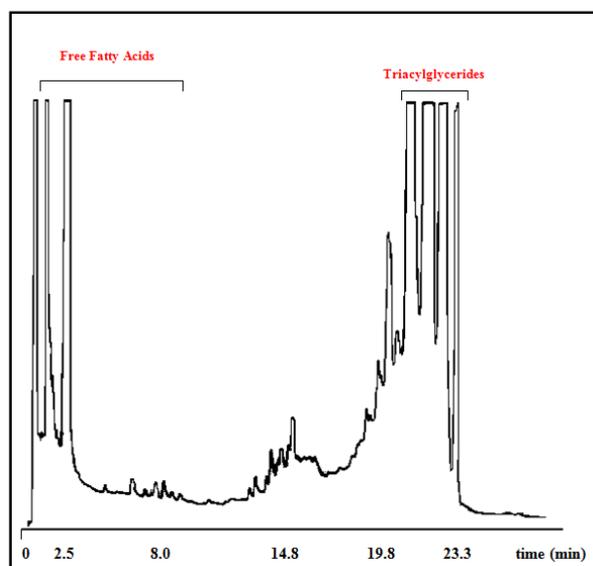


**Figure 1.** Fractions of TAGs obtained in PFE were carried out with CO<sub>2</sub> in supercritical conditions: temperature 60° C and 100, 200, and 300 atm at pressure.



**Figure 2.** Fractions of TAGs obtained in PFE were carried out with CO<sub>2</sub> in subcritical conditions: temperature 0° C and 300 atm at pressure.

In the literature, it is described that the obtainment of different classes of compounds is challenging due to the proximity of the extraction regions, producing, in many cases, a mix of the distinct phases (21).



**Figure 3.** HT-HRGC-FID chromatogram of a profile of coffee oil triglycerides by HT-HRGC-FID.

In our case, the complexity of the intact coffee oil sample can be observed in the chromatogram of Figure 3.

The first extract fraction (E-1, 100 atm, and 60 °C), shown in Table 1, corresponds to a 300 g/L of carbon dioxide density. This region presents lower solvation power; on the other hand, high selectivity (21) produces an extraction of the essential oil with high volatility. Increasing the pressure to 200 atm (E-2) at a constant temperature (60 °C), the carbon dioxide density increasing to 730 g/L, allowing an extraction with a higher potential of solvation. The extraction condition E-3 (300 atm, 60 °C, 830 g/L) and E-4 (300 atm, 100 °C, 665 g/L) presented similar lipid composition presented free fatty acids and monoacylglycerides, without TAGs selectivity. This similarity between E-3 and E-4 was due to a little difference in carbon dioxide's solvation power.

The E-5 condition (300 atm, 0 °C, 1050 g/L) was a better experimental point (Figure 4). Due to the soft temperature condition, lower vapor pressure, and high selectivity to TAGs compounds, the extract obtained presented TAGs as a majority of compounds and traces of

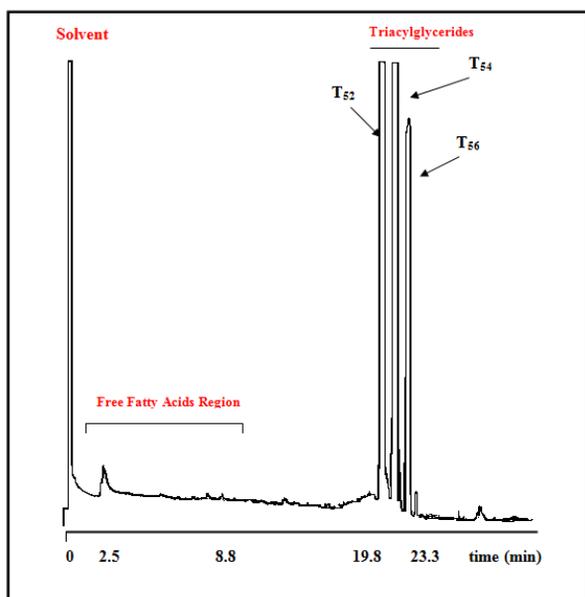
the free fatty acids mono- and possible diacylglycerides, preserving the sample of the probable thermal degradation during the analysis.

The HT-HRGC E-5 fraction analysis shows an average area of  $21.8 \pm 1.1\%$  in the three TAGs groups ( $T_{52}$  at  $T_{56}$ ). The advantage of PFE in subcritical conditions with a temperature of 0 °C was the reduction of the degradation products. In the case of coffee oils, especially, the degradation by exposure at high temperature for an extended period is a critical point and HT-HRGC it supplies an excellent method of analysis. This occurs because of a change in the original composition of the oil, reducing the concentration of the same compounds as free fatty acids. In extract E-5, obtained under subcritical conditions, the lipid content was determined using both TGAs obtained by HT-HRGC/FID and the sum of all fatty acids, expressed as triacylglycerides. They were obtained from the gas chromatographic (GC) profiles of the fatty acid methyl esters (FAME) analysis, being the result compared with those reported in the literature (13,17,18). TAGs composition of Brazilian *Coffea arabica* oil was found to be mainly  $T_{52}$  to  $T_{56}$ . The main components of the triacylglycerides comprised an average of  $64.3 \pm 3.2\%$ . We were able to analyze the triacylglycerides molecular species by HT-HRGC/FID without derivatization.

The crude coffee oil matrix, in addition to TAGs, presents a large variety of other compounds that might degrade when submitted to the high injector temperature (370 °C) and the use of the SbFE before analysis by HT-HRGC/FID, eliminating the interferences.

#### 4. Conclusion

In conclusion, SbFE combined with HT-HRGC proved to be a viable alternative for the analysis of coffee oil samples. The proposed approach maintain the intact structure of the TAGs', keeping the apparent profile, while providing a reduction in the amounts of solvents and



**Figure 4.** HT-HRGC-FID chromatogram of a coffee oil triglycerides extracted at 0 °C and 300 atm, with carbon dioxide. T54-triglycerides with 54 carbons.

reagents used, and short extraction time, becoming a good example of a Green Analytical Chemistry (GC) method.

support provided for this research and to our laboratory. The donation of the coffee samples by Dr. Suzana Nixdorf is also appreciated.

## Acknowledgment

The authors are grateful to the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior—Brasil (CAPES)—Finance Code 001; to FAPESP (Grants 2019/22724-7 2017/02147-0, 2019/26263-4, 2015/15462-5, 2014/03765-0 and 2014/07347-9); and to CNPq (307293/2014-9 and 308843/2019-3) for the financial

## References

- [1] Armenta S, Garrigues S, Esteve-Turrillas FA, de la Guardia M. Green extraction techniques in green analytical chemistry. *TrAC - Trends Anal Chem.* 2019;116:248–53.
- [2] Sheibani A, Ghaziaskar HS. Pressurized fluid extraction of pistachio oil using a modified supercritical fluid extractor and factorial design for optimization. *LWT - Food Sci Technol.* 2008;41(8):1472–7.
- [3] Silva RPF, Rocha-Santos TAP, Duarte AC. Supercritical fluid extraction of bioactive compounds. *TrAC Trends Anal Chem.* 2016;76:40–51.
- [4] Bahruddin S, Basheer C. Sample Preparation Methods for the Analysis of Biogenic Amines. In: *Biogenic Amines in Food: Analysis, Occurrence and Toxicity.* Royal Society of Chemistry; 2019. p. 212–38.
- [5] Płotka-Wasyłka J, Rutkowska M, Owczarek K, Tobiszewski M, Namieśnik J. Extraction with environmentally friendly solvents. *TrAC Trends Anal Chem.* 2017;91:12–25.
- [6] Pronyk C, Mazza G. Design and scale-up of pressurized fluid extractors for food and bioproducts. *J Food Eng.* 2009;95(2):215–26.
- [7] Khezeli T, Ghaedi M, Bahrani S, Daneshfar A. Supercritical fluid extraction in separation and preconcentration of organic and inorganic species. In: *Soylak M, Yilmaz EBT, editors. New Generation Green Solvents for Separation and Preconcentration of Organic and Inorganic Species.* Elsevier; 2020. p. 425–51.
- [8] Pinto JSS, Lanças FM. Design, construction and evaluation of a simple pressurized solvent extraction system. *J Braz Chem Soc.* 2009;20(5):913–7.
- [9] Montoya MF, Sánchez FA, Pereda S. Equilibrium in Pressurized Systems (Sub and Supercritical). In: *Pereira CGBT-T of PE in FE, editor. Thermodynamics of Phase Equilibria in Food Engineering.* Academic Press; 2019. p. 385–418.
- [10] McHug M, Krukonic V. *Supercritical Fluid Extraction: Principles and Practice.* 2nd ed. Butterworth-Heinemann; 1994.
- [11] Hamdan S, Daood HG, Toth-Markus M, Illés V. Extraction of cardamom oil by supercritical carbon dioxide and sub-critical propane. *J Supercrit Fluids.* 2008;44(1):25–30.
- [12] CONAB. Acompanhamento da Safra Brasileira de Café. *Monit Agrícola.* 2020;6(1):1–62.
- [13] Nikolova-Damyanova B, Velikova R, Jham GN. Lipid classes, fatty acid composition and triacylglycerol molecular species in crude coffee beans harvested in Brazil. *Food Res Int.* 1998;31(6):479–86.
- [14] Jham GN, Muller HV, Cecon P. Triacylglycerol molecular species variation in stored coffee beans determined by reverse-high-performance liquid chromatography/refractive index detector. *J Stored Prod Res.* 2008;44(1):82–9.
- [15] Toci AT, Neto VJMF, Torres AG, Farah A. Changes in triacylglycerols and free fatty acids composition during storage of roasted coffee. *LWT - Food Sci Technol.* 2013;50(2):581–90.
- [16] Pereira AS, Padilha MC, Neto FRA. Two decades of high temperature gas chromatography (1983–2003): what's next? *Microchem J.* 2004;77(2):141–9.
- [17] Pinto JSS, Lanças FM. Hydrolysis of corn oil using subcritical water. *J Braz Chem Soc.* 2006;17(1):85–9.
- [18] Pinto JS, Lanças FM. Hydrolysis of azadirachta indica oil using subcritical water and determination of triacylglycerides and fat acids by HT-HRGC-FID and HRGC-MS. *Quim Nova.* 2010;33(2):394–7.
- [19] Pinto JS, Esmeraldo A, Lanças FM. Design and Construction of an on-line SPE-SFE-CGC System. *J Braz Chem Soc.* 2001;12(2):192–5.
- [20] King JW, Holliday RL, List GR. Hydrolysis of soybean oil . in a subcritical water flow reactor. *Green Chem.* 1999;1(6):261–4.
- [21] Brogle H. CO<sub>2</sub> as a solvent: Its properties and applications. *Chem Ind.* 1982;12:385–90.