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REGULAR ARTICLE

From coffee blend formulation until beverage production: Changes within fatty acids profile

Vânia Santos Ribeiro[1,2], Fernando Cebola Lidon[1]*, Paula Scotti Campos[2], Isabel P. Pais[2], António Eduardo Leitão[3], José Cochicho Ramalho[3]

[1]GeoBioTec, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, Campus da Caparica, 2829-516 Monte de Caparica, Portugal, [2]Unidade de Investigação em Biotecnologia e Recursos Genéticos, Instituto Nacional de Investigação Agrária e Veterinária, I.P., Quinta do Marquês, 2784-505 Oeiras, Portugal, [3]Department of Recursos Naturais, Ambiente e Território (DRAT), Instituto Superior de Agronomia (ISA), Universidade de Lisboa (ULisboa), Qta. Marquês, Av. República, 2784-505 Oeiras, Portugal

ABSTRACT

The extraction of fatty acids in coffee beverages entities sharply depends of the roasting of coffee beans, composition of the blend and extraction process. Accordingly, the definition of fatty acids profile from the blend formulation until beverage production prompted this work. A functional coffee blend (94% roasted Robusta and Arabica coffee powder, 70/30, w/w, 3% cocoa powder, 2% coffee silverskin and 1% golden coffee) was developed, followed by characterization of its components. The extracted coffee beverages obtained from the blend were also analyzed. It was concluded that total fat and fatty acids of the coffee blend prevailed in cocoa and in roasted coffee. The higher amounts of fatty acids in the components and on the milled coffee blend were C16:0, C18:0, C18:1, C18:2 and C20:0, but silverskin also revealed high contents of C20:2. In all the components of the coffee blend saturated fatty acids prevailed over unsaturated ones. No significant differences were found for the double bound index after extraction at 15 or 19 bar of water pressure. In conclusion, both total fat and TFAs were not affected by roasting in coffee alone or in the studied blend. Nevertheless, a higher extraction of total fat and total fatty acids was obtained to the beverage using a higher water pressure (19 bar). Still, this did not significantly change the profile related to the major FAs, as DBI in the beverage was similar with both extraction espresso machines.

Keywords: Cocoa; Coffee blend; Fatty acids; Golden coffee; Silverskin

INTRODUCTION

The consumption of coffee and its byproducts has proved to be a rich source of antioxidants in human diet (Ribeiro et al., 2014; Esquível and Jiménez, 2012; Murthy and Madhava-Naidu, 2012). It also provides other beneficial bioactive compounds (namely, caffeine, trigonelline and chlorogenic acids) for human health, being a potential functional food product (Ribeiro et al., 2014; Esquível and Jiménez, 2012; Murthy and Madhava-Naidu, 2012; Bonita et al., 2007; Dórea and da Costa, 2005). Yet, the formulation of a functional blend structured in a basic coffee beverage, depends on many factors, namely of species and coffee geographical origin, harvest and post-harvest operations and bean selection, storage conditions and roasting differentiation (Läderach et al., 2011; Farah et al., 2006).

A functional coffee blend was previously developed (Ribeiro et al., 2014) using roasted beans (*C. canephora* cv. Robusta and *C. arabica*) from different countries and geographical areas (Alonso-Salces et al., 2009; Bicho et al., 2011a,b, 2013a,b), golden coffee, coffee silverskin (from *C. canephora* cv. Robusta from Angola) and cocoa powder (from Ivory Coast). This coffee blend was developed considering caffeine positive effects on health (Ribeiro et al., 2014; Zhou et al., 2012). To maintain the amount of caffeine, trigonelline and chlorogenic acids and preserve the antioxidant properties of the blend, which decrease during roasting (Bicho et al., 2011a,b, 2013a,b), golden coffee of *C. canephora cv.* Robusta from Angola - Amboim was further included in the formulation due to the specific chlorogenic acids that occur solely in this genotype (Ribeiro et al., 2014; Clifford and Knight, 2004). Silverskin, which is a tegument of coffee beans obtained as a by-product

***Corresponding author:**
Fernando Cebola Lidon, GeoBioTec, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, Campus da Caparica, 2829-516 Monte de Caparica, Portugal. Phone/Fax: 00351212948574. E-mail: fjl@fct.unl.pt

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of the roasting process, was included in the coffee blend as a soluble dietary fiber with high antioxidant capacity (Mussatto et al., 2011). Cocoa powder was also added due to its antidepressant effects (Lipp et al., 2001). Additionally, cocoa is one of the highest polyphenol-containing foods (Shiina et al., 2009) and may lower LDL and cholesterol, further providing essential minerals such as calcium and potassium (Shiina et al., 2009; Lipp et al., 2001).

On a dry weight basis, the average of lipids content in green coffee beans of Arabica and Robusta is about 15% and 10%, respectively (Speer and Kölling-Speer, 2001; Wilson et al., 1997). Lipids extracted from coffee beans contain about 75% of triacylglycerols with a high percentage of unsaponificables, including about 19% of total free and esterified diterpene alcohols, roughly 5% of total free and esterified sterols, and trace levels of other substances such as tocopherols. Among the different diterpenes, it was further found that cafestol and kahweol have potential anticarcinogenic effects (Huber et al., 2008; Ross et al., 2001; Cavin et al., 2002).

After roasting the lipid fraction of coffee beans remains highly stable, mostly due to the presence of lipid-soluble dark coloured Maillard reaction products (Speer and Kölling-Speer, 2006). In this context, only small changes usually occur in the fatty acids composition (Speer and Kölling-Speer, 2006), but the trans-fatty acid levels increase, specifically the contents of C18:2ct and C18:2tc (Speer and Kölling-Speer, 2006). In Arabica and Robusta coffee species, the prevailing fatty acids are C18:2 and C16:0, followed by C18:0, C18:1, C20:0 and C22:0 and minor traces of C14:0, C18:3 and C24:0 (Speer and Kölling-Speer, 2006). Moreover, while in Robusta coffees beans the proportion of stearic acid is noticeably smaller than that of oleic acid, the percentages of these two acids in the Arabica coffees are almost equal (Speer and Kölling-Speer, 2006).

The relevance of fatty acids for health and their contribution to the loss of coffee quality during storage, especially through oxidation and hydrolysis, are well known (Toci et al., 2008). This study aims to produce a fingerprint of the contents, composition and double bound index of fatty acids of coffee beverage resulting from previously developed coffee blend (Ribeiro et al., 2014), using the espresso machines with water pressure of 15 and 19 bar. Total fat and fatty acids in the coffee blend and components, as well as in the freeze-dried ground coffee and related extracted beverage are therefore characterized.

MATERIALS AND METHODS

Plant materials and blend preparation

Different roasting conditions from genotypes of the two main producing species were used: *C. canephora* cv. Robusta from Angola (light–medium roasted coffee beans), and *C. arabica* from Brazil, Honduras and Timor Lorosae (medium roasted coffee beans). Roasting conditions and blend preparation were performed exactly as described in (Ribeiro et al., 2014).

Golden coffee was obtained by drying the grinded green coffee in an oven (140°C, 10 min.). The coffee blend was performed as previously described (Ribeiro et al., 2014), and included: A) 94% roasted milled coffee (*C. canephora* cv. Robusta and *C. arabica*, 70/30, w/w), B) 3% cocoa powder (from Ivory Coast), C) 2% coffee silverskin (from *C. canephora* cv. Robusta from Angola), D) 1% golden coffee (*i.e.,* green coffee minimally processed of *C. canephora cv.* Robusta from Angola - Amboim).

Preparation of coffee beverages

The coffee blend was milled and 6.5 g was used to obtain a volume of 25 mL in 25 s of extraction by commercial espresso coffee machines Briel Riviera - ES45 (without capsules) and DQOOL, from Brasilia (with capsules), which allowed an extraction with water pressure of 15 and 19 bar, respectively, under 90 °C, as fully described in (Ribeiro et al., 2014).

Total fat and fatty acids profile

Coffee blend samples were analyzed with the characteristic particle size used for commercial blends. The raw materials of the coffee blend corresponding to the silverskin, golden coffee and roasted coffee were analyzed with a particle size lower than 0.425 mm, whereas cocoa had a particle size lower than 0.180 mm.

For extraction of total fat, 1-4 g of each sample was weighed and 20 mL of n-hexane were added (Lidon and Ramalho, 2011). After mixing in a vortex and thereafter in a stir plate (for 20 minutes), a centrifugation (4000 *g*, 20 min, 4°C) was carried out. The n-hexane containing the oil was collected in a weighed flask. Samples extraction was performed in triplicate. After evaporation of n-hexane in a rotary evaporator, the flask containing the fat was kept in an oven at 100 ± 5°C until constant weight. Total fat content was determined and thereafter ressuspended in a solution of ethanol: toluene (1:4). The same extraction procedure was carried out for total fat in the beverage after freeze-drying.

For total fatty acids analysis, an aliquot of the total fat extract was saponified according to (Metcalfe et al., 1966), with minor modifications (Partelli et al., 2011). Each sample of total lipids (100 µL) was homogenized with 4 mL of sodium hydroxide solution in methanol. The homogenate was shaken (in a vortex system) and heated in a water bath, at 65°C, for 15 min, to induce lipids saponification. After cooling, it was added 100 µg of internal standard

(heptadecanoic acid), and the sample was methylated (with 2 mL of boron trifluoride-methanol), shaked, heated (in a water bath, at 65°C, for 15 min), and finally cooled. For removal of methylated fatty acids, 10 mL of pentane and 2 mL of deionized water were added. The sample was then shaken, and thereafter decanted for 1 h to allow phase separation; the upper phase containing the methylated fatty acids was collected and dried in a water bath at 40°C, under nitrogen. The residue was redissolved in 600 µL of ethanol: toluene (1:4) and stored at -30°C. The sample was dried under nitrogen flow and ressuspended in 100 µL of n-hexane for analysis by gas chromatography.

The fatty acid methyl esters were analyzed with a GC-FID chromatograph (CP-3380, Varian, Palo Alto, CA, USA). Separation was performed using a fused silica capillary column (30 m, 0.25 mm i.d., 0.25 µm Carbowax coating, DB-Wax, J&W Scientific, USA). The column temperature was programmed to rise from 80 to 200°C (12°C min^{-1}), after 2 min at the initial temperature. Injector and detector temperatures were, respectively, 200°C and 250°C. The carrier gas was hydrogen at a flow rate of 1 mL min^{-1}, and a split ratio of 1:50 of the sample. The fatty acids were identified by comparison to known standards (Restek and Supelco). Total fatty acids corresponded to the sum of individual fatty acids. Chromatograms (Fig. 1) were analyzed through Star Chromatography Workstation software Version 6:41. The unsaturation index (Double Bound Index, DBI) was determined from the percentage of fatty acids (Mazliak, 1983), according to the following equation: DBI = [(% monoenes + 2 x % dienes + 3 x % trienes)/% saturated fatty acids].

Statistical analysis

The various measured parameters were analyzed using a one-way ANOVA ($P \leq 0.05$), followed by a Tukey test for mean comparison (95% confidence level). Different letters indicate significant differences in a multiple range analysis for 95% confidence level.

RESULTS AND DISCUSSION

The diversity of cocoa composition, one of the components of the blend under study, is usually found within a narrow concentration range of total fat and total fatty acids, almost irrespective of the different geographical growing regions and breeding lines (Lipp et al., 2001). In green coffee beans total fat content, mainly located in the endosperm and in small amounts in the outer layer (Speer and Kölling-Speer, 2001), is lower than that of cocoa, showing mean values of 15% and 10% for Arabica and Robusta type of coffees, respectively. Besides, the lipid fraction remains quite stable during roasting, after processing has been completed and during storage (Vila et al., 2005; Anese et al., 2000). In our study, roasting temperatures ranging between 170 - 200°C, associated to a relative humidity of 14% (v/w), were tested considering two roasting times (15 and 40 min.) for *C. canephora* cv. Robusta and *C. arabica*, in order to obtain roasted beans with acceptable sensory properties of taste and aroma. Indeed, the Maillard and caramelization reactions are influenced by the rate of roasting process, and therefore for the browning of beans and formation of the aroma. In this context, the characteristic roasted (light, medium or dark) taste, which also depends on the level of moisture content in the bean, is further developed.

Among the components of the coffee blend, total fat prevailed in cocoa, while the lowest level (about 8 fold) was found in coffee silverskin (Fig. 2). Furthermore, the amounts of fat in roasted and golden coffees fell within previous reported values (Sivetz and Desrosier, 1979; Folstar, 1985; Speer and Kölling-Speer, 2001), with cocoa further showing contents similar to those previously described (Lipp et al., 2001; Vesela et al., 2007). Total fatty acids followed a similar

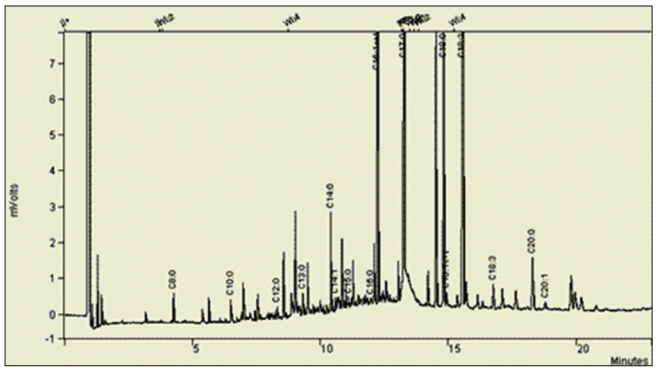

Fig 1. Example of a chromatogram of fatty acids from the coffee blend (C8:0 = Caprylic; C10:0 = Capric; C12:0 = Lauric; C13:0 = Tridecanoic; C14:0 = Myristic; C14:1 = Myristoleic; C15:0 = Pentadecanoic; C16:0 = Palmitic; C16:1 = Palmitoleic; C18:0 = Stearic; C18:1 = Oleic; C18:2 = Linoleic; C18:3 = Linolenic; C20:0 = Arachidic; C22:0 = Behenic; C20:1 = Gadoleic; C20:2 = Eicosadienoic).

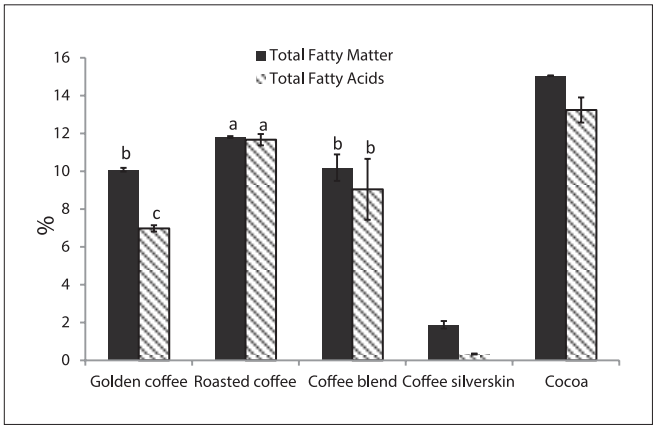

Fig 2. Total fat and total fatty acids of the coffee blend and respective components. Each value is the mean ± SE (n=3 for total fat; n = 6 for total fatty acids). Different letters (a-c) indicate significant differences ($p \leq 0.05$) among coffee samples.

trend, with the lower value found in silverskin (Fig. 2). Moreover, in opposition to (Calligaris et al., 2009), roasted coffee displayed significant higher values of total fat and total fatty acids (11.76% and 67.26% increases, respectively) than golden coffee (a minimally processed bean) (Fig. 2). Indeed, it is well known that during roasting the chemical and physicochemical characteristics of green coffee beans greatly change, mostly due to the Maillard reaction (Bicho et al., 2011a,b; 2013a,b; Ribeiro et al., 2014). However, the oxidation level of the coffee fat fraction might not be affected due to the presence of lipid-soluble dark coloured Maillard reaction products, which have strong antioxidant properties through different mechanisms, *e.g.* chain breaking, oxygen scavenging or metal chelating (Calligaris et al., 2009). The final coffee blend showed total fat and total fatty acids contents of *ca.* 10% and 9.1%, respectively (Fig. 2).

Weight ratio between the dry product and the volume of water used for coffee beverages production is controlled by national or local taste, and also depends of the nature and origin of the coffee. Although these factors might influence the ratio between soluble and insoluble substances in the ground coffee, the different pressure applied (15 and 19 bar) also influenced the total fat and total fatty acids values in the beverage (Fig. 3). Indeed, after beverage production, the coffee ground of the blend revealed that the use of a water pressure of 19 bar (DQOOL) had a significant higher efficiency to extract total fat and total fatty acids than that of 15 bars (Briel). Coffee grounds obtained after application of water pressure of about 19 bar, only contained 15.5% and 9.3% of the initial amount of total fat and total fatty acids, respectively, whereas by the application of water pressure of 15 bar, 21.4% and 10.25% of these compounds were still present (Fig. 3).

The ultimate source of carbon for lipid synthesis comes from photosynthesis. In seeds and oil-accumulating fruits, photosynthate has to be translocated from leaf tissue *via* sucrose or mannose. Final steps of glycolysis generate acetyl-CoA which is carboxylated to malonyl-CoA through acetyl-CoA carboxylase and carboxyl transferase. Long chain fatty acids (C12:0 upwards) are synthesized from smaller precursor molecules, having as end products the saturated fatty acids palmitic (C16:0) and stearic (C18:0), which predominate due to the nature of the fatty acid synthase of most plants. These are invariably even carbon numbered because of their route of synthesis, resulting from elongases activity which adds two-carbon units from malonyl-CoA to the precursor molecule (Harwood, 1997). In each component of the coffee blend, once the long chain acids have been produced, desaturation and further modifications may occur giving rise to other fatty acids namely C18:1, C18:2, C20:0 and C20:2 (Fig. 4). Fatty acids are present in lipid extracts either free or mainly esterified by glycerol or diterpenic alcohols (Martin et al., 2001; Lipp et al., 2001; Vesela et al., 2007). In roasted and golden coffees the relative proportions of the major fatty acids showed similar profiles, being C18:2 > C16:0 > C18:1

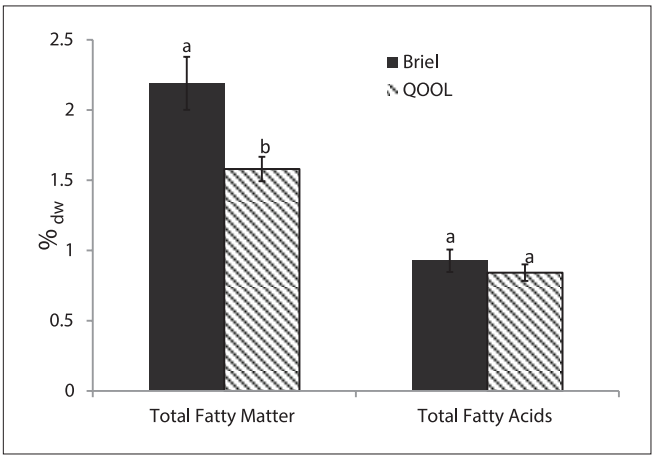

Fig 3. Total fat and total fatty acids of the freeze-dried extract, after beverage production from the coffee blend, by the espresso machines Briel and DQOOL (using 15 and 19 bar pressure, respectively). Each value is the mean ± SE (n=3 for total fatty matter; n=6 for total fatty acids). Different letters (a, b) indicate significant differences ($p \leq 0.05$) between coffee samples.

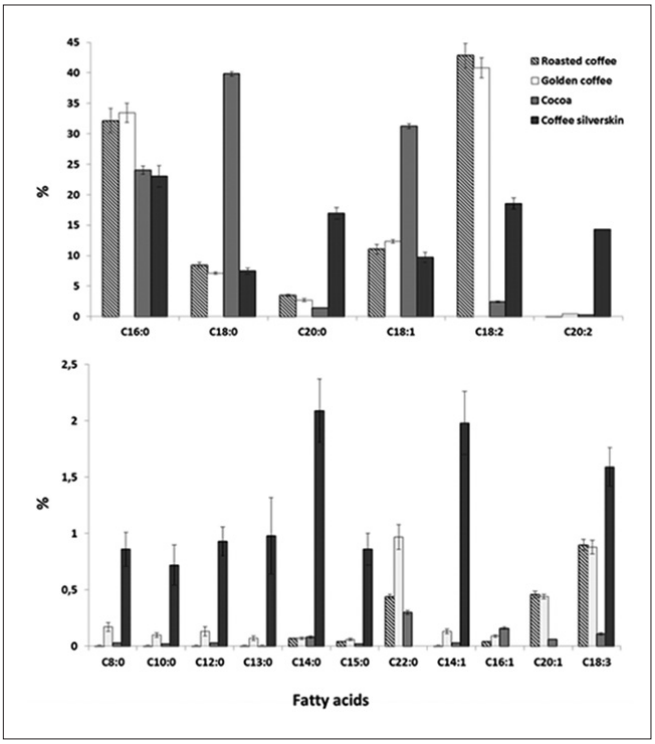

Fig 4. Fatty acids profile of each component of the coffee blend (C8:0 = Caprylic; C10:0 = Capric; C12:0 = Lauric; C13:0 = Tridecanoic; C14:0 = Myristic; C14:1 = Myristoleic; C15:0 = Pentadecanoic; C16:0 = Palmitic; C16:1 = Palmitoleic; C18:0 = Stearic; C18:1 = Oleic; C18:2 = Linoleic; C18:3 = Linolenic; C20:0 = Arachidic; C22:0 = Behenic; C20:1 = Gadoleic; C20:2 = Eicosadienoic). Each value is the mean ± SE (n=6). Different letters (a-d) indicate significant differences ($p \leq 0.05$) for each fatty acid between Roasted coffee, Golden Coffee, Cocoa and Coffee Silverskin samples.

> C18:0 > C20:0 > C20:2. Other fatty acids occurred in trace levels and followed different trends (Fig. 4). In roasted coffee, their relative abundance was C18:3 > C20:1 > C22:0 > C14:0 > C16:1 = C15:0 > C14:1 = C13:0 = C12:0 = C10:0 = C8:0, while in golden coffee proportions followed the sequence C22:0 > C18:3 > C20:1 > C8:0 > C12:0 = C14:1 > C10:0 > C16:1 > C14:0 = C13:0 > C15:0 (Fig. 4). Additionally, fatty acids profile in silverskin was C16:0 > C18:2 > C20:2 > C20:0 > C18:1 > C18:0 > C14:0 > C14:1 > C18:3 > C13:0 > C12:0 > C15:0 = C8:0 > C10:0, whereas in cocoa the pattern was C18:0 > C18:1 > C16:0 > C18:2 > C20:0 > C22:0 > C20:2 > C16:1 > C18:3 > C14:0 > C20:1 > C14:1 = C12:0 = C8:0 > C15:0 = C10:0 > C13:0 (Fig. 4). In this context, it was found that in all the components of the coffee blend saturated fatty acids overcame (Fig. 4) and, therefore, relatively high melting points prevailed. Regarding the monounsaturated oleic acid (C18:1), silverskin, roasted and golden coffees showed close contents, well below the amount found in cocoa that reached ca. 31%, in agreement with previous results reported for cocoa (Elkhori et al., 2007). Additionally, for roasted and golden coffee, the levels of saturated, monounsaturated and polyunsaturated fatty acids remained quite similar, whereas in cocoa the contents of saturated fatty acids represented *ca.* 24 and 2 higher fold than the amounts of polyunsaturated and monounsaturated fatty acids, respectively (Fig. 5). Saturated fatty acids of silverskin were also 1.57 and 4.61 fold higher than polyunsaturated and monounsaturated fatty acids, respectively. Accordingly, whereas unsaturated fatty acids are susceptible to oxidation (the more double bonds, the greater the susceptibility), in all the components of the coffee blend, the high amounts of saturated fatty acids determines its stability. Following this background, the double bound index did not vary significantly between roasted and golden coffees, but in silverskin and cocoa different values were observed (Fig. 6).

The amounts of saturated, monounsaturated and polyunsaturated fatty acids of the milled coffee blend and of roasted and golden coffee kept a similar relative proportion, as also denoted by the same value of double bound index (Figs. 5 and 6). In the milled coffee blend, the pattern of the major fatty acids were C18:2 > C16:0 > C18:1 > C18:0 > C20:0, while the profile of the remaining fatty acids was C18:3 > C20:2 > C20:1 > C8:0 = C12:0 = C14:0 = C13:0 = C10:0 = C14:1 = C16:1 = C15:0 (Fig. 7).

Fatty acids are poorly soluble in water in their undissociated (acidic) form. Thus, the actual water solubility, particularly of longer-chain acids, is often very difficult to determine since it is markedly influenced by pH, temperature and also because fatty acids have a tendency to associate, leading to the formation of monolayers or micelles that might rapidly change in physical properties over a limited range of concentration. Nevertheless, in spite of the different water pressure applied for the extraction of the coffee beverages, only minor changes (*ca.* 1%) were found between the relative amounts of saturated and polyunsaturated fatty acids (Fig. 5) and no significant differences were found for the double bound index (Fig. 6). Additionally, it is well known that the influence of a fatty acid's structure on its melting point is such that branched chains and *cis* double bonds will lower the melting point compared with that of equivalent

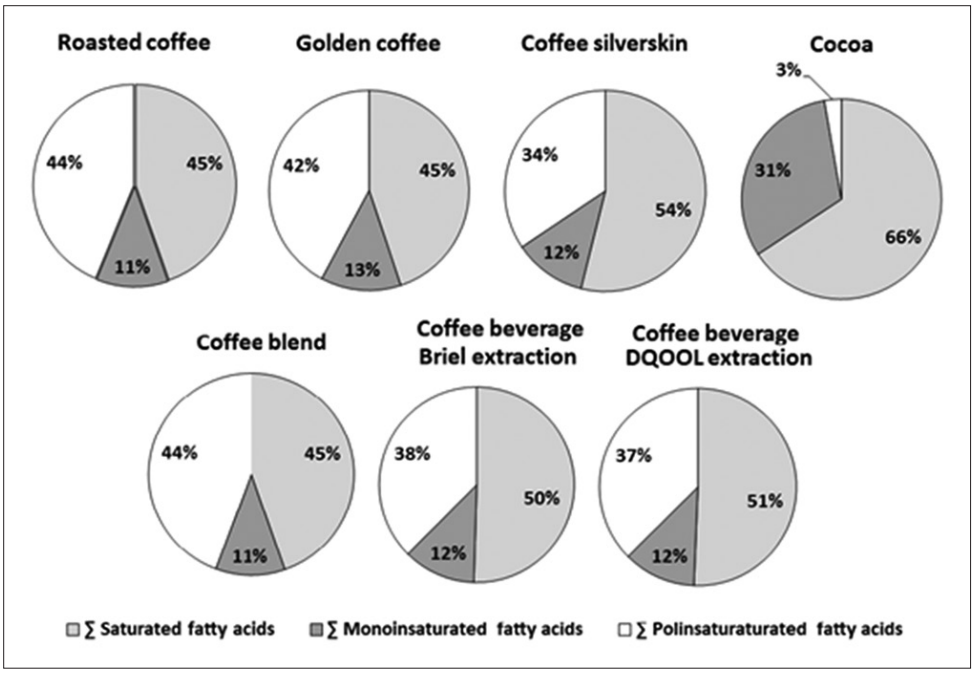

Fig 5. Relative abundance of saturated, monounsaturated and polyunsaturated fatty acids of the coffee blend, in each component and in the coffee beverages extracted with the expresso machines Briel and DQOOL (using 15 and 19 bar pressure).

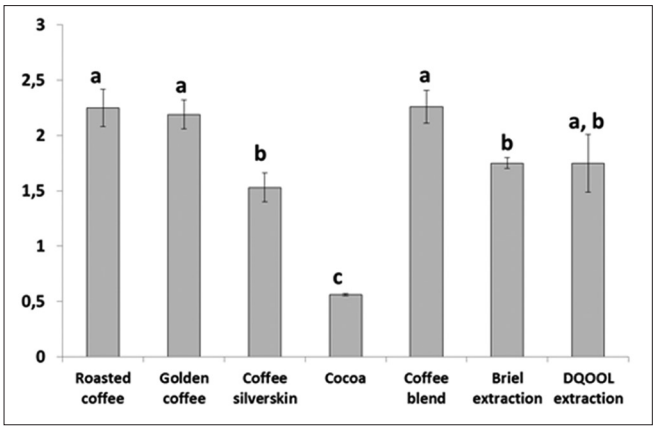

Fig 6. Double bound index of fatty acids in the coffee blend, in each component and in coffee beverages extracted with the expresso machines Briel and DQOOL (using 15 and 19 bar pressure). Each value is the mean ± SE (n=6). Different letters (a-c) indicate significant differences ($p \leq 0.05$) among samples.

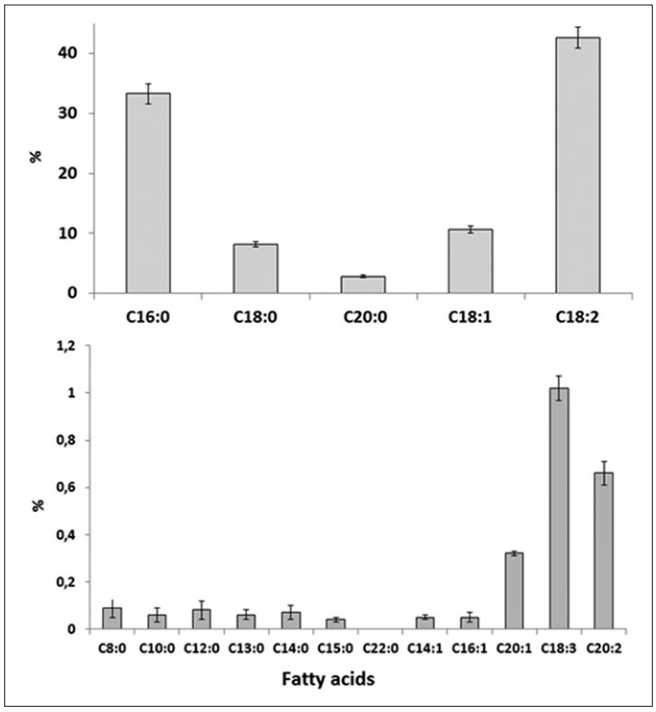

Fig 7. Fatty acids composition of the milled coffee blend (C8:0 = Caprylic; C10:0 = Capric; C12:0 = Lauric; C13:0 = Tridecanoic; C14:0 = Myristic; C14:1 = Myristoleic; C15:0 = Pentadecanoic; C16:0 = Palmitic; C16:1 = Palmitoleic; C18:0 = Stearic; C18:1 = Oleic; C18:2 = Linoleic; C18:3 = Linolenic; C20:0 = Arachidic; C22:0 = Behenic; C20:1 = Gadoleic; C20:2 = Eicosadienoic). Each value is the mean ± SE (n=6). Different letters (a-h) indicate significant differences ($p \leq 0.05$) between individual fatty acids.

saturated chains. Coffee beverages extracted at 90 ºC with the application of 15 and 19 bar showed similar extractions for the major fatty acids (C16:0, C18:0, C18:1, C18:2, C20:0), although for the minor fatty acids (except C8:0) it was observed a tendency to a lower extraction under 19 bar pressure, significantly only for C12:0 and C14:0 (Fig. 8).

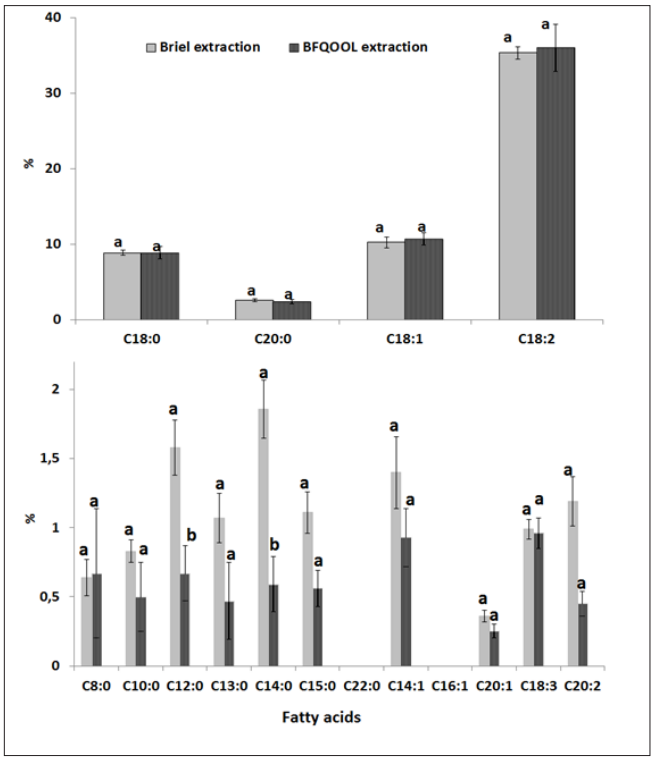

Fig 8. Fatty acids composition of beverages extracted from the coffee blend by the espresso machines Briel and DQOOL (using 15 and 19 bar pressure, respectively) (C8:0 = Caprylic; C10:0 = Capric; C12:0 = Lauric; C13:0 = Tridecanoic; C14:0 = Myristic; C14:1 = Myristoleic; C15:0 = Pentadecanoic; C16:0 = Palmitic; C16:1 = Palmitoleic; C18:0 = Stearic; C18:1 = Oleic; C18:2 = Linoleic; C18:3 = Linolenic; C20:0 = Arachidic; C22:0 = Behenic; C20:1 = Gadoleic; C20:2 = Eicosadienoic). Each value is the mean ± SE (n=6). Different letters (a, b) indicate significant differences ($p \leq 0.05$) between Briel extraction and DQOOL extraction machines for each individual fatty acid.

In conclusion, both total fat and TFAs were not significantly affected by roasting in both coffee and in our blend. However, the extraction with a higher water pressure (19 bar) influenced the total fat and total fatty acids values in the obtained beverage, although without a significant profile change related to the major FAs, as DBI in the beverage was similar with both extraction espresso machines.

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Author's contributions

All authors of the paper contributed equally to the writing of the paper and were involved in the overall planning and supervision of the work.

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