Research Paper

Insights into the physicochemical properties of coffee oil

Sonia Calligaris1, Marina Munari1, Gianmichele Arrighetti2 and Luisa Barba2

1 Dipartimento di Scienze degli Alimenti, University of Udine, Udine, Italy
2 Institute of Crystallography, National Council of Research, Trieste, Italy

The lipid fraction of roasted coffee is an interesting ingredient that could be used in a large number of food formulations. Coffee oil has peculiar flavouring as well as nutraceutical characteristics. The feasibility of the use of coffee oil as ingredient greatly depends not only on its chemical characteristics but also on its physical properties. The crystallisation and melting properties of the coffee oil extracted from Arabica roasted coffee powder were determined by using synchrotron X-ray diffraction coupled with differential scanning calorimetry. The fatty acid composition and the flavour profile were also assessed by using GC and GC-MS analyses, respectively. The main fatty acids found in coffee oil are linoleic and palmitic acid. Significant amounts of stearic and oleic acid are also present. These chemical characteristics are linked to the phase transition behaviour. The crystallisation of coffee oil occurs at 6.5 ± 0.3 °C, independently of the cooling rate applied (from 0.5 to 10 °C/min). A unique crystalline structure was identified: a double chain length (2L) β′ structure (55.29 Å). The sole formation of the β′ form indicates that this metastable crystal is the only one that one should expect in foods containing coffee oil stored below 7 °C.

Keywords: DSC/XRD / Oil / Physicochemical properties / Roasted coffee

Received: February 24, 2009; accepted: June 24, 2009
DOI 10.1002/ejlt.200900042

1 Introduction

Lipids in green coffee beans are mainly located in the endo-sperm while only a small amount is found in the outer layer. The lipid content ranges from 10 to 14%, depending on the coffee origin: in green Arabica coffee it averages some 15% on a dry basis, whilst in Robusta it is about 10% [1, 2]. Lipids extracted from coffee beans contain about 75% of triacylglycerols (TG) with a high percentage of unsaponifiables, including about 19% of total free and esterified diterpene alcohols, about 5% of total free and esterified sterols, and very low quantities of other substances such as tocopherols. Among the different diterpenes, cafestol and kahweol have been widely studied due to their potential anticarcinogenic effects [3–5]. It is well known that the chemical and physico-chemical characteristics of green coffee beans greatly change during the roasting process. The main changes are associated with the development of the Maillard reaction, which allows beverages with particular characteristics of flavour, colour and texture to be produced. In fact, the heat treatment at very high temperatures (around 200 °C) induces the formation of a number of volatile compounds with a wide range of functional groups [6, 7]. The majority of the components of the coffee aroma is liposoluble and can be extracted along with the lipids from the roasted coffee beans. Different extraction methods have been proposed: solvent extraction, supercritical carbon dioxide extraction, and mechanical extraction under pressure [8, 9]. As reported by Sarrazin et al. [8], aroma recovery greatly depends on the extraction methodology applied.

Even if the roasting process of green coffee induces dramatic changes in the coffee beans, the literature data evidence that the lipid fraction of coffee is stable just after processing has been completed and during storage [10, 11]. In particular, Anese et al. [10] evidenced that the roasting does not affect the oxidation level of the coffee lipid fraction. The stability of coffee oil was attributed to the presence of lipid-soluble dark-coloured Maillard reaction products. The latter are indicated as having strong antioxidant properties through different mechanisms, e.g. chain breaking, oxygen scavenging or metal chelating [12–16].

On the basis of these observations, roasted coffee oil could be considered an interesting matrix for a wide number of food formulations, in which it could be used as flavouring ingre-
dient (i.e. ice creams, ready-to-drink beverages, instant coffee) or as nutraceutical able to improve the health-protecting capacity of food products. The possibility of an efficient use of coffee oil as ingredient greatly depends not only on its chemical characteristics but also on its physicochemical properties. Knowledge of the phase transition behaviour of coffee oil is crucial to evaluate the feasibility of its use as ingredient in a complex food, which has to be stored under defined conditions. At the moment, very little literature data are available on the chemical characteristics of coffee oil, whereas no study provides information on its thermal and structural properties.

The crystallisation behaviour of complex lipids can be studied by combining thermo-analytical techniques, among which differential scanning calorimetry (DSC) is the most widely used, and diffraction techniques such as X-ray diffraction (XRD) [17]. The latter is the most direct technique to study polymorphism arising from the different lateral packing of fatty acid chains and of longitudinal stacking of molecules in lamellae [18]. The two levels of organisation are easily identifiable from the short and long spacing observed by XRD in lamellae [18]. The two levels of organisation are easily identifiable from the short and long spacing observed by XRD, respectively. In particular, the use of synchrotron radiation, which provides an X-ray flux $10^3$–$10^6$ times more intense than that generated by usual X-ray sources, allows step-by-step recordings as a function of temperature [17, 19].

The aim of this paper was to study the crystallisation and melting properties of the coffee oil extracted from Arabica roasted coffee powder. In particular, the phase transition behaviour of TG in coffee oil was evaluated by synchrotron XRD coupled with DSC. The fatty acid composition and the flavour profile of coffee oil were also assessed.

2 Materials and methods

2.1 Coffee oil preparation

The lipid fraction of commercial roasted coffee powder (100% *Coffea Arabica*) was obtained by solid-liquid extraction using chloroform/methanol (Carlo Erba, Milan, Italy) mixtures (2 : 1 wt/wt) by stirring at room temperature for 3 h. The ratio between the coffee and solvent mixture was 1 : 6 on weight basis. After filtration through filter paper (Whatman No. 1), the oil was separated from the solvent by evaporation with a Rotavapor (mod. 4001; Heidolph Instruments, Milan, Italy) at 40 °C.

2.2 Analytical determinations

2.2.1 Fatty acid content

Analysis of the fatty acid composition of the coffee oil was carried out according to the European Official Methods of Analysis [20].

2.2.2 Headspace solid-phase micro-extraction sampling

The manual holder and the solid-phase micro-extraction (SPME) fibre Sableflex 2 cm-50/30 μm DVB/CAR/PDMS film were purchased from Supelco (Bellefonte, PA, USA). Before sampling, the fibre was reconditioned for 30 min in the GC injection port at 240 °C. Aliquots of 3 g of coffee oil were inserted in 10-mL capacity vials, immediately sealed with butyl septa and metallic caps. Vials were equilibrated at 60 °C in a thermostatic bath for 30 min. An optimisation of the experimental conditions had previously been realised. The SPME fibre was exposed to the coffee oil headspace for 5 min.

2.2.3 GC-MS

The SPME coating containing the headspace volatile compounds was immediately inserted into the GC injection port, pushed out of its housing, and thermally desorbed for 5 min at 250 °C. A HGRG Mega 2 Series gas chromatograph (Fisons Instruments, Milan, Italy) and a thermal conductivity detector (Fisons HWD Control; Fisons Instruments) were used. The separation was done by using a capillary column (CP Wax 52 CB, 50 m × 0.32 mm × 0.40 μm film thickness; Chrompack, Middelburg, The Netherlands). The injector temperature was set at 200 °C and helium (1.7 mL/min linear speed) was the carrier gas. The oven temperature was maintained at 60 °C for 6 min and then raised at 5 °C/min up to 200 °C. The chromatograms were integrated using Chromcard (Ver. 1.18, 1996; CE Instrument, Milan, Italy) chromatography data system software.

The MS analysis was performed using a Varian Saturn mass spectrometer (ion trap detector) (Varian, Palo Alto, CA, USA) operated in the electron impact ionisation mode (70 eV). The ion source temperature was set at 250 °C. Each sample was analysed in triplicate.

2.2.4 Identification of volatile compounds

The identification of volatile compounds was carried out by comparison of their mass spectra with those of pure reference compounds and the Wiley library, and also by comparing their retention times with those of standard compounds and data from the literature.

2.2.5 DSC

Calorimetric analyses were made using a TA4000 differential scanning calorimeter (Mettler-Toledo, Greifensee, Switzerland) connected to GraphWare software TAT72.2/5 (Mettler-Toledo). Heat flow calibration was achieved using indium (heat of fusion 28.45 J/g). Temperature calibration was carried out using hexane (m.p. −93.5 °C), water (m.p. 0.0 °C) and indium (m.p. 156.6 °C). Samples were prepared by carefully weighing 10–15 mg of the coffee oil in 160-μL alumi-
mium DSC pans, which were closed without hermetic sealing. An empty pan was used as reference. Samples were heated under nitrogen flow (0.5 mL/min) at 40 °C for 10 min to destroy the crystallisation memory, cooled to ~30 °C and then heated from ~30 to 40 °C. The scanning rate was 0.5, 2, 5 and 10 °C/min. The start and the end of the melting transition were taken as onset (T_{on}) and offset (T_{off}) points of transition, which are the points at which the extrapolated baseline intersects the extrapolated tangent of the calorimetric peak in the transition state. Results were normalised to account for the weight variation of the samples. Total peak enthalpy was obtained by integration. The programme STAR ever. 8.10 (Mettler-Toledo) was used to plot and analyse the thermal data.

### 2.2.6 XRD analysis

XRD patterns were recorded at the XRD beam-line at the Elettra storage ring in Trieste. The X-ray beam emitted by the wiggler source on the Elettra 2 GeV electron storage ring was monochromatised by an Si(111) double crystal monochromator, focused on the sample and collimated by a double set of slits, giving a spot size of 0.2 x 0.2 mm. The sample consisted of a drop of oil kept in the photon flux by means of a nylon loop of 0.7 mm. The temperature of the sample was varied by means of a 700 series cryocooler (Oxford Cryosystems, Oxford, UK) with an accuracy of ~1 °C. The temperature profile was the same as that of the DSC experiments (heating at 40 °C for 10 min, cooling to ~30 °C and then heating to 20 °C at a scanning rate of 2 °C/min). In order to collect data under the best analytical conditions at both the wide- and small-angle signal, experiments were carried out at two different photon energies and sample distances from the detector (1.41 Å, 93.3 mm and 0.85 Å, 300 mm). A MarResearch 165 mm CCD detector assembly was used. As the intensity of the synchrotron radiation beam decreases in time, the dose of photons absorbed by the sample was the same for every step. Several hundreds of bi-dimensional patterns collected with the CCD were calibrated and integrated using the software FIT2D [21], resulting in two series of powder-like patterns. The high-brilliance source allowed to record weak structures not otherwise detectable, which helped in the process of indexing the patterns.

### 2.3 Data analysis

Each coffee oil sample was analysed in triplicate. All results are shown as mean and standard deviation. The indexing of the XRD patterns obtained by the two crystalline phases was performed using the programmes Winplotr [22] and Checkcell [23].

### 3 Results and discussion

Coffee oil extracted from coffee powder is a brown viscous liquid. The colour of the product is mainly due to the presence of liposoluble Maillard reaction products separated during the oil extraction. Table 1 shows the fatty acid composition of the coffee oil. The main fatty acid is linoleic acid (L), followed by palmitic acid (P). Significant amounts of stearic (S) and oleic acid (O) are also present, whereas the percentages of linolenic (L) and arachidonic (A) acid are about 1–2%. Finally, gadoleic (G) and behenic (B) acid are found only in traces. It should be remembered that the roasting process is indicated to cause only slight changes in the fatty acid composition [11]. The results are in agreement with previous literature data on crude green coffee [2]. As reported by Fölster [24], the fatty acids of the coffee oil are organised predominantly in two TG: PLP (about 28.1%) and PLL (about 27.5%). Significant quantities of SLP (about 8.6%), LLL (about 6.7%), POP (5.9%) and SLL (4.2%) are also present.

In accordance with literature data, the oil extracted from the roasted coffee powder is rich in aroma compounds [8]. Table 2 shows the volatile compounds identified in the headspace of the coffee oil, including aldehydes, ketones, furans, pyroles, pyrazines, pyridine and phenolic compounds. All these compounds are typical of roasted coffee flavour [25, 26].

The possibility to use coffee oil as flavouring ingredient in foods greatly depends on its physicochemical properties. The latter have been assessed by applying DSC and synchrotron XRD analysis. Figures 1 and 2 show the crystallisation and melting curves of coffee oil obtained during cooling and subsequent heating at 0.5, 2, 5 and 10 °C/min from 20 to ~40 °C and vice versa. It can be noted that a single exothermic event was recorded during cooling, and a single endothermic one was observed during heating. Tables 3 and 4 show the onset temperature (T_{on}), the offset temperature (T_{off}) and the enthalpy (∆H) associated with crystallisation and melting of coffee oil. T_{on} of the crystallisation and melting curves is not affected by the scanning rate. On the contrary, slight differences in T_{off} of crystallisation and melting are observed as a function of the scanning rate. These changes take place concomitantly with a decrease in the transition enthalpy as the scanning rate increases. This result is attributable to the fact
that a lower portion of oil crystallised at the higher cooling rates, probably because the TG under such conditions do not have enough time to organise [27].

These results allow coming to the hypothesis that one single crystal form develops in the coffee oil during crystallisation, independently of the cooling rate applied. This indicates that the main TG molecules, which have long fatty acid chains from 16 to 18 carbons, crystallise together in a unique crystal structure during a unique thermal event.

To study the polymorphic structure of the coffee oil crystals, synchrotron XRD analysis was performed at both small and wide angles. Figures 3 and 4 show the patterns recorded at small and wide angles, respectively, during the diffraction experiment performed at a wavelength of 0.85 Å. The results are reported as a function of temperature during cooling and heating at 2 °C/min. In order to evidence the correspondence between DSC and XRD events, white lines at temperatures corresponding to the DSC thermal events ($T_{on}$, $T_{off}$ and temperature corresponding to the peak) are also reported in Figs 3 and 4.

From 60 to 6.5 °C, two bumps at 4.69 and 23.61 Å are observed. These bumps can be associated with the short-range organisation of the TG molecules in the liquid phase, as previously reported by other authors [27, 28].

At about 6.5 °C, in agreement with the DSC data, the crystallisation of coffee oil is put in evidence by the appearance of a number of diffraction peaks. In particular, wide-angle diffraction peaks emerge at 4.17, 3.73 and 2.51 Å concomitantly with small-angle peaks at 54.80, 27.60, 18.41, 13.81 and 9.22 Å. The intensity of these peaks increases progressively. Once the temperature reaches –40 °C, after a 10-min pause, the samples was heated at 2 °C/min. At –15 °C, in correspondence with the endothermic DSC peak, the intensity of all the XRD peaks decreases, indicating that the oil starts to melt. The peaks disappear at about 6.5 °C ($T_{off}$ of the DSC exothermic peak). It is evident that no polymorphic transformation is observed during the heating of the samples.

The interplanar distances at 4.17 and 3.73 Å are typical of the organisation of the acylglycerol chain in an orthorhombic perpendicular β' subcell [15]. The small-angle diffraction peaks (54.80, 27.60, 18.41, 13.81 and 9.22 Å), along with the wide-angle peak, 2.51 Å, correspond to a double chain length organisation, named 2L, with a parameter $c$ of 55.29 Å.

The refining of the reticular parameter $c$ against the five peak positions at low resolution, performed by using the software Checkcell, converged to a value that agreed very well ($Δθ = 0.0009^\circ$) with the position of the high-resolution peak, purposely excluded from the refinement in order to validate the value chosen as $c$ parameter.

Since the occurrence of the β' polymorph can be expected when one of the three fatty acid chains of a TG is somehow different from the other two [18], the formation of the β' crystal in coffee oil could be related to the predominance of this type of TG (i.e. PLP, PLL, SLL, POP, SLL). In addition, considering the fatty acid composition of coffee oil, 18 can be considered as the mean number of carbon atoms in the fatty acid chains. Since 1.52 Å is reported as an average carbon-carbon distance in the zigzag plane of the acylglycerol chain [29], the mean length of one fatty acid chain is 1.52 x 18 = 27.36 Å. Thus, the length corresponding to the two fatty acid chains is 54.72 Å. This value is an excellent confirmation of the experimental data, indicating that the β' L2 structure is mainly constituted by palmitic (16:0), stearic (18:0), oleic (18:1) and linoleic acid (18:2).

It should be noted that the same crystalline structure was the only one identified even after a flash freezing of the coffee oil (data not shown). As is well known, flash freezing of the lipid matrix is generally applied to induce the formation of the less thermostable crystal form ($α$ form) [15]. Under our experimental conditions, the sole presence of the β' form...
Figure 1. DSC crystallisation curves of coffee oil at scanning rates of –0.5, –2, –5 and –10 °C/min.

Figure 2. DSC melting curves of coffee oil at scanning rates of 0.5, 2, 5 and 10 °C/min.
indicates that this metastable crystal is the only one that should be expected in foods containing coffee oil stored below 7 °C.

In conclusion, the use of DSC analysis in combination with a high-flux X-ray source for the XRD technique allows the identification and the characterisation of the crystalline structures formed during the crystallisation of coffee oil. In particular, the TG organise in a double chain length structure with an orthorhombic perpendicular subcell: β’ 2L (55.29 Å). From a technological point of view, coffee oil crystals could be found in products stored at the temperatures normally applied for chilled (4 °C) and frozen (−18 °C) storage. Under such conditions, coffee oil is not completely crystallised and part of it is in the amorphous state, as shown by the presence of the amorphous signal during the whole XRD experiment. In addition, the crystallised fraction is expected not to undergo polymorphic transformation during storage below the phase transition temperature. It is interesting to note
Table 3. Onset temperature \( (T_{on}) \), offset temperature \( (T_{off}) \), and enthalpy \( (\Delta H) \) associated with the crystallisation of coffee oil as a function of the scanning rate.

<table>
<thead>
<tr>
<th>Scanning rate ([\text{C/min}])</th>
<th>( T_{on} ) ([\text{[C]}) crystallisation</th>
<th>( T_{off} ) ([\text{[C]}) crystallisation</th>
<th>( \Delta H ) ([\text{[J/g]}]</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>6.8 ± 0.6(^a)</td>
<td>−14.6 ± 0.9(^a)</td>
<td>49.4 ± 2.1(^a)</td>
</tr>
<tr>
<td>2</td>
<td>6.6 ± 0.4(^a)</td>
<td>−15.4 ± 0.5(^a)</td>
<td>49.1 ± 1.5(^a)</td>
</tr>
<tr>
<td>5</td>
<td>6.5 ± 0.3(^a)</td>
<td>−16.6 ± 0.4(^a)</td>
<td>45.0 ± 1.2(^a)</td>
</tr>
<tr>
<td>10</td>
<td>6.5 ± 0.4(^a)</td>
<td>−21.6 ± 1.2(^a)</td>
<td>41.0 ± 1.9(^a)</td>
</tr>
</tbody>
</table>

\(^a\) Data with different letters in the same column are significantly different \((p < 0.05)\).

Table 4. Onset temperature \( (T_{on}) \), offset temperature \( (T_{off}) \), and enthalpy \( (\Delta H) \) associated with the melting of coffee oil as a function of the scanning rate.

<table>
<thead>
<tr>
<th>Scanning rate ([\text{C/min}])</th>
<th>( T_{on} ) ([\text{[C]}) melting</th>
<th>( T_{off} ) ([\text{[C]}) melting</th>
<th>( \Delta H ) ([\text{[J/g]}]</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>−15.1 ± 0.2(^a)</td>
<td>5.71 ± 0.7(^a)</td>
<td>49.1 ± 1.1(^a)</td>
</tr>
<tr>
<td>2</td>
<td>−15.6 ± 0.6(^a)</td>
<td>7.6 ± 0.5(^a)</td>
<td>51.1 ± 1.3(^a)</td>
</tr>
<tr>
<td>5</td>
<td>−14.8 ± 0.4(^a)</td>
<td>9.36 ± 0.9(^a)</td>
<td>45.8 ± 1.2(^a)</td>
</tr>
<tr>
<td>10</td>
<td>−15.0 ± 0.3(^a)</td>
<td>10.7 ± 1.0(^a)</td>
<td>44.0 ± 1.6(^a)</td>
</tr>
</tbody>
</table>

\(^a\) Data with different letters in the same column are significantly different \((p < 0.05)\).

The conflict of interest statement

The authors have declared no conflict of interest.

References


