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Analytical Methods

Differential scanning calorimeter application to the detection of refined hazelnut oil in extra virgin olive oil

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Abstract

The potential application of differential scanning calorimetry (DSC) to verify adulteration of extra virgin olive oil with refined hazelnut oil was evaluated. Extra virgin olive oil and hazelnut oil were characterised by significantly different cooling and heating DSC thermal profiles. Addition of hazelnut oil significantly enhanced crystallisation enthalpy (at hazelnut oil $\geqslant 20\%$) and shifted the transition towards lower temperatures (at hazelnut oil $\geqslant 5\%$). Lineshape of heating thermograms of extra virgin olive oil was significantly altered by hazelnut oil addition: a characteristic exothermic event originated at -27 °C in extra virgin olive oil and progressively disappeared with increasing hazelnut oil content, while the major endothermic peak at -3.5 °C broadened (at hazelnut oil $\geqslant 40\%$) and the minor endothermic peak at 8 °C shifted toward lower temperatures (at hazelnut oil $\geqslant 5\%$). The preliminary results presented in this study suggest that DSC analysis may be a useful tool for detecting adulteration of extra virgin olive oil with refined hazelnut oil.

Keywords: Extra virgin olive oil; Refined hazelnut oil; Adulteration; DSC; Thermal analysis

1. Introduction

Extra virgin olive oil (EvoO) is a traditional Mediterranean food product, whose market has recently expanded to North Europe, USA, China and Japan, due to its highly appreciated organoleptic attributes and to its health and nutritional properties (Bendini et al., 2007; Harwood & Yaqoob, 2002).

Adulteration of EvoO with cheaper oils from other vegetable sources or seeds (hazelnut oil, in particular), as well as with lower quality olive oils (e.g. olive-pomace olive oil, virgin olive oil and gentle deodorised olive oil), is a serious concern for oil suppliers and consumers. European Commission and international institutions (e.g. International Olive Oil Council, national Customs and Excise Department) are actively involved in the prevention and detection of frauds in the extra virgin olive oil sector.

Hazelnut oil (HaO) is often used in EvoO adulteration, due to the similar chemical compositions of both major (i.e. fatty acid (FA), triacylglycerols (TAG)) and minor components (i.e. total sterols) of the two oils (European Union Research Committee, 2001). This resemblance makes it difficult to evince the presence of HaO in EvoO, especially at concentrations below 20%. In addition, genetic and climatic factors, as well as agricultural practices, largely influence the chemical composition of both oils, making it even more difficult to detect HaO in fraudulent admixtures.

Several methods have been proposed for detecting the presence of HaO in EvoO. Cold pressed HaO could be detected in virgin olive oil by on-line liquid chromatography–gas chromatography (LC–GC) identification of filbertone, (*E*)-5 methylhept-2-en-4-one, which is a typical volatile compound of HaO (Blanch, Caja, Herraiz, & del

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Castillo, 1999; Blanch, Caja, León, & Herraiz, 2000; del Castillo, Caja, Herraiz, & Blanch, 1998), or by reverse-phase high-performance liquid chromatography (RP-HPLC) analysis of the polar components (Gordon, Covell, & Kirsch, 2001; Zabaras & Gordon, 2004). TAG (Vichi, Pizzale, Toffano, Bortolomeazzi, & Conte, 2001), sterols (Mariani, Bellan, Morchio, & Pellegrino, 1999; Vichi et al., 2001) and tocopherols (Azadmard Damirchi, Savage, & Dutta, 2005; Morchio, Pellegrino, Mariani, & Bellan, 1999) composition have been also used as markers of HaO adulteration. Detection of both crude and refined HaO in virgin olive oil was recently achieved by means of direct coupling of headspace-mass spectrometry and multivariate regression techniques (Pena, Cárdenas, Gallego, & Valcárcel, 2005). The addition of refined HaO to olive oil (blend of refined and virgin olive oil) can also be assessed by determining esterified sterols by thin-layer chromatography (TLC-GC) (Cercaci, Rodriguez-Estrada, & Lercker, 2003).

Most of these methods are expensive, time-consuming and have high environmental impact. Nowadays, alternative techniques are requested for evaluating EvoO adulterants, in particular HaO. Spectrofluorimetric methods, often coupled with multivariate statistical models, are emergent. Fluorescence spectroscopy has been recently employed to detect virgin and refined HaO in virgin olive oils (Sayago, Morales, & Aparicio, 2004). Refined HaO can be detected in refined and virgin olive oil by evaluating entire oils and/or the unsaponifiable fraction with Fourier Transformed-Raman (FT-Raman) and FT-Mid-Infrared (FT-MIR) spectroscopy (Baeten et al., 2005). FT-Infrared (FT-IR) has been found to be useful for detecting HaO in EvoO (Ozen & Mauer, 2002), while ^{1}H and ^{13}C nuclear magnetic resonance (NMR) techniques and artificial neural networks have been applied for its identification in both virgin olive oil and olive oil (García-González, Mannina, D'Imperio, Segre, & Aparicio, 2004).

DSC has some advantages over the more classical detection methods, as it is rapid and does not require sample preparation or solvent utilisation. The application of DSC to the analysis and characterisation of oils and fats for the determination of solid fat content, crystallisation and melting profiles, enthalpy of transitions and polymorphic forms, is well known and reviewed (Biliaderis, 1983). DSC application has also been suggested as a valuable tool for characterisation of oils from vegetable sources (Che Man & Tan, 2002; Tan & Che Man, 2002) and thermal parameters have been reported to correlate well with chemical parameters obtained with standard methods (Tan & Che Man, 2000). Thermal properties (measured both in cooling and heating regimes) of monovarietal EvoO samples were found to correlate well with the chemical composition (Chiavaro, Vittadini, Rodriguez-Estrada, Cerretani, & Bendini, 2008; Chiavaro et al., 2007).

Several works have evaluated DSC application to the detection of adulteration of edible oils, fats and fat-based products. In particular, DSC was able to detect the presence of pig and buffalo body fat in cow and buffalo ghees (Lambelet & Ganguli, 1983), tallow and margarine in butter (Aktaş & Kaya, 2001), lard and randomised lard or lipase-catalysed interesterified lard in refined-bleached-deodorised palm oil (Marikkar, Lai, Ghazali, & Che Man, 2001, 2002), and animal fat in butter (Coni, Di Pasquale, Coppolelli, & Bocca, 1994), canola oil (Marikkar, Ghazali, Che Man, & Lai, 2002) and palm olein (Marikkar, Ghazali, Che Man, & Lai, 2003).

Few reports are available in the literature on the application of DSC to assess EvoO adulteration with other vegetable or seed oils of lower quality and/or economic value. Jiménez Márquez (2003) evaluated DSC heating thermograms of admixtures of virgin olive oil with low-quality olive oils (i.e. refined crude olive oil and virgin olive oil obtained by second centrifugation of olives): melting transition enthalpy and peak temperatures discriminated virgin olive oil from other olive oils, as well as from their admixtures (the differences were attributed to the different TAG composition).

The objective of this preliminary work was to evaluate the potential use of DSC to detect adulteration of EvoO with refined HaO, by establishing possible relationships between the thermal properties of cooling and heating thermograms and the TAG and FA composition of the oils and their admixtures.

2. Materials and methods

2.1. Sampling

EvoO was supplied by Coppini Arte Olearia (Parma, Italy) and it was produced by cold pressing two types of olive cultivars (*Nocellara del Belice* and *Ogliarola Messinese*) harvested in 2006. Refined HaO was purchased at a local supermarket. One sample of each oil was analysed. Admixtures of EvoO:HaO were prepared at different ratios (60:40, 70:30, 80:20, 90:10 and 95:5, w/w). Samples were stored in dark bottles without headspace at room temperature before analysis.

2.2. Reagents, solvents and standards

All solvents used were of analytical or HPLC grade (Merck, Darmstadt, Germany). Reagents were purchased from Sigma–Aldrich (St. Louis, MO). The standard mixture of fatty acid methyl esters (GLC 463) was supplied by Nu-Chek (Elysian, MN).

2.3. DSC

Samples of oil (8–10 mg) were weighed into aluminium pans, covers were sealed into place and analysed with a DSC Q100 (TA Instruments, New Castle, DE). Indium (melting temperature 156.6 °C, $\Delta H_f = 28.45$ J/g) and *n*-dodecane (melting temperature −9.65 °C, $\Delta H_f = 216.73$ J/g) were used to calibrate the instrument

and an empty pan was used as reference. Oil samples were equilibrated at 30 °C for 3 min and then cooled at −80 °C at the rate of 2 °C/min, equilibrated at −80 °C for 3 min and then heated from −80 °C to 30 °C at 2 °C/min. Dry nitrogen was purged in the DSC cell at 50 cm^3/min. Thermograms were analysed with Universal Analysis Software (Version 3.9A, TA Instruments) to obtain enthalpy (ΔH, J/g), T_{on} (°C) and T_{off} (°C) of the transitions (intersection of baseline and tangent at the transition) and peak height (H_p W/g). Range of the transitions was calculated as temperature difference between T_{on} and T_{off}. At least triplicate analyses were carried out per sample.

2.4. GC determination of total FA

Total FA were determined as suggested by Bendini, Cerretani, Vecchi, Carrasco-Pancorbo, and Lercker (2006). The results were expressed as area normalisation in percent (%). Three replicates were prepared and analysed per sample.

2.5. Free acidity

Free acidity was determined only on pure oils, following the NGD C-10 official method published by Norme Grassi e Derivati (NGD) (1979). Three replicates were analysed per sample.

2.6. Iodine value

Iodine value was measured only on pure oils, following the NGD Ba IV-15 official method published by Norme Grassi e Derivati (1979). Three replicates were analysed per sample.

2.7. HPLC determination of TAG

HPLC analyses were carried out using an HP 1100 Series instrument (Agilent Technologies, Palo Alto, CA), coupled to a 5-μm Luna™ C18 (Phenomenex, Torrance, CA) column (25 cm × 3.0 mm ID) and equipped with a binary pump delivery system, a degasser, an autosampler, diode-array (DAD) and mass spectrometry (MSD) detectors. A C18 precolumn filter (Phenomenex, Torrance, CA) was used. All solvents were filtered through a 0.45 μm nylon filter disk (Lida Manufacturing Corp., Kenosha, WI) prior to use. Samples were prepared by dissolving the oil at 3% in a mixture of 2-propanol/*n*-hexane/acetonitrile (2:1:2, v/v/v). The injection volume was 10 μl. All the analyses were carried out at room temperature. The gradient elution was performed by using 2-propanol and acetonitrile as mobile phases, A and B, respectively. The linear gradient elution system was: from 0 to 40 min held at 70% A; from 40 to 51 min, decreased to 45% A; from 51 to 60 min, decreased to 0% A; from 60 to 65 min, increased to 100% A; from 65 to 70 min, decreased to 60% A; from 70 to 75 min, increased to 70% A, as post-run. The flow rate was 0.4 ml/min from 0 to 51 min, 0.5 ml/min from 51 to 55 min, 0.6 ml/min from 55 to 60 min and 0.4 ml/min until the end of the HPLC run. The effluent was monitored with both DAD and MSD. The wavelength was set at 205 nm. MS detection was performed by using an atmospheric pressure chemical ionisation (APCI) interface in the positive mode, according to the following conditions: drying gas flow, 3.0 l/min, nebuliser pressure, 60 psi, drying gas temperature, 350 °C, vaporiser temperature, 450 °C, capillary voltage, 3000 V, corona current, 4 μA, and fragmentor voltage, 70 V. These compounds were tentatively identified by their UV–vis and mass spectra obtained by HPLC-APCI-MSD and literature data (Nagy et al., 2005). The following TAG were identified: dilinoleoyl-linolenoyl-glycerol (LLLn), trilinolein (LLL), dilinoleoyl-palmitoleoyl-glycerol (LLPo), oleoyl-linoleoyl-linolenoyl-glycerol (OLLn), dilinoleoyl-oleoyl-glycerol (OLL), palmitoleoyl-oleoyl-linoleoyl-glycerol (OLPo), dilinoleoyl-palmitoyl-glycerol (LLP), dioleoyl-linoleoyl-glycerol (OLO), palmitoyl-oleoyl-linoleoyl-glycerol (OLP), palmitoyl-palmitoleoyl-oleoyl-glycerol (POPo), triolein (OOO), dioleoyl-palmitoyl-glycerol (OOP), stearoyl-oleoyl-linoleoyl-glycerol (SLO), dipalmitoyl-oleoyl-glycerol (POP), dioleoyl-stearoyl-glycerol (SOO) and palmitoyl-stearoyl-oleoyl-glycerol (SOP). Three replicates were prepared and analysed per sample.

2.8. Statistical analysis

Means and standard deviations (SD) were calculated with SPSS (Version 13.0, SPSS Inc., Chicago, IL) statistical software. SPSS was used to perform one-way analysis of variance and Tukey's honest significant difference test at a 95% confidence level ($p < 0.05$) to identify differences among groups.

3. Results and discussion

3.1. Chemical composition of oils and their admixtures

Chemical compositions of EvoO and HaO used in this study were characterised in terms of free acidity and iodine value, TAG and FA. EvoO showed an iodine value equal to 89.2 ± 0.4 (g I_2/100 g oil) and free acidity of 0.37 ± 0.03 (g oleic acid/100 g oil), below the limit set by the EC Regulation (European Community, 2003). A refined HaO with an iodine value equal to 92.1 ± 0.2 (g I_2/100 g oil) and free acidity of 0.47 ± 0.02 (g oleic acid/100 g oil) was utilised in this study to prepare the EvoO:HaO admixtures; similar iodine values have been reported by Crews et al. (2005) for HaO, while refined vegetable oils commonly show low acidity values.

Table 1 shows the TAG composition of EvoO, HaO and the admixtures (5–40% HaO in EvoO). Thirteen TAG were detected in EvoO, whereas only 11 TAG were identified in HaO. The data show that TAG compositions of EvoO and

Table 1
TAG composition of EvoO, HaO and their admixtures[A] (% of total TAG)

TAG	100% EvoO	5% HaO	10% HaO	20% HaO	30% HaO	40% HaO	100% HaO
LLLn	nd[B]	nd	nd	nd	nd	nd	3.20 (0.01)
LLL+LLPo	2.23 (0.22)^{d}	2.69 (0.07)^{c}	2.72 (0.18)^{c}	2.97 (0.16)^{c}	3.33 (0.14)^{b}	3.41 (0.15)^{b}	8.61 (0.06)^{a}
OLLn	1.10 (0.15)^{a}	0.81 (0.10)^{b}	0.79 (0.12)^{b}	0.81 (0.14)^{b}	0.92 (0.14)^{ab}	0.92 (0.14)^{ab}	0.77 (0.01)^{c}
OLL+OLPo	12.38 (0.32)^{d}	13.06 (0.46)^{c}	13.37 (0.44)^{c}	13.75 (0.22)^{bc}	14.20 (0.42)^{ab}	14.98 (0.30)^{a}	14.80 (0.24)^{ab}
LLP	3.61 (0.28)^{a}	3.80 (0.43)^{a}	4.00 (0.28)^{a}	3.79 (0.23)^{a}	3.49 (0.50)^{a}	3.60 (0.05)^{a}	3.87 (0.01)^{a}
OLO	21.44 (0.27)^{d}	22.17 (0.20)^{b}	21.93 (0.09)^{bcd}	22.05 (0.29)^{bc}	21.64 (0.48)^{bcd}	21.72 (0.16)^{bcd}	24.67 (0.46)^{a}
OLP	12.15 (0.42)^{a}	11.42 (0.31)^{b}	11.59 (0.14)^{ab}	11.55 (0.29)^{ab}	11.62 (0.37)^{ab}	11.81 (0.35)^{ab}	7.53 (0.18)^{c}
POPo	1.65 (0.26)^{a}	1.18 (0.18)^{b}	1.23 (0.08)^{b}	1.37 (0.07)^{ab}	1.20 (0.21)^{b}	1.16 (0.18)^{b}	nd
OOO	25.33 (0.64)^{b}	26.01 (0.57)^{ab}	26.00 (0.44)^{ab}	25.26 (0.37)^{b}	25.48 (0.40)^{b}	25.03 (0.54)^{b}	26.17 (0.31)^{a}
OOP+SLO	13.51 (0.04)^{a}	12.51 (0.25)^{b}	12.47 (0.67)^{b}	12.28 (0.07)^{bc}	12.29 (0.21)^{bc}	11.70 (0.26)^{c}	7.26 (0.47)^{d}
POP	1.58 (0.14)^{a}	1.41 (0.90)^{ab}	1.36 (0.27)^{ab}	1.34 (0.23)^{ab}	1.30 (0.10)^{ab}	1.11 (0.30)^{b}	nd
SOO	4.18 (0.49)^{a}	3.64 (0.46)^{ab}	3.79 (0.28)^{ab}	3.83 (0.62)^{ab}	3.66 (0.15)^{ab}	3.62 (0.33)^{ab}	3.45 (0.29)^{b}
SOP	0.84 (0.15)^{a}	0.97 (0.14)^{a}	0.83 (0.11)^{a}	1.00 (0.22)^{a}	0.88 (0.34)^{a}	0.95 (0.30)^{a}	0.37 (0.09)^{b}
DSTAG	2.41 (0.29)^{a}	2.41 (0.11)^{a}	2.20 (0.38)^{a}	2.33 (0.45)^{a}	2.18 (0.45)^{a}	2.06 (0.60)^{a}	0.37 (0.11)^{b}
MSTAG	35.11 (1.49)^{a}	32.62 (0.67)^{b}	33.09 (1.44)^{b}	32.83 (1.29)^{b}	32.25 (1.43)^{bc}	31.89 (1.17)^{c}	22.11 (0.63)^{d}
TUTAG	62.48 (1.60)^{c}	64.97 (0.79)^{bc}	64.71 (1.22)^{bc}	64.84 (1.13)^{bc}	65.57 (1.37)^{b}	66.05 (1.28)^{b}	77.52 (0.83)^{a}

Abbreviations: FA, fatty acids; EvoO, extra virgin olive oil; HaO, hazelnut oil; LLLn, dilinoleoyl-linolenoyl-glycerol; LLL, trilinolein; LLPo, dilinoleoyl-palmitoleoyl-glycerol; OLLn, oleoyl-linoleoyl-linolenoyl-glycerol; OLL, dilinoleoyl-oleoyl-glycerol; OLPo, palmitoleoyl-oleoyl-linoleoyl-glycerol; LLP, dilinoleoyl-palmitoyl-glycerol; OLO, dioleoyl-linoleoyl-glycerol; OLP, palmitoyl-oleoyl-linoleoyl-glycerol; POPo, palmitoyl-palmitoleoyl-oleoyl-glycerol; OOO, triolein; OOP, dioleoyl-palmitoyl-glycerol; SLO, stearoyl-oleoyl-linoleoyl-glycerol; POP, dipalmitoyl-oleoyl-glycerol; SOO, dioleoyl-stearoyl-glycerol; SOP, palmitoyl-stearoyl-oleoyl-glycerol; DSTAG, disaturated triacylglycerides; MSTAG, monosaturated triacylglycerides; TUTAG, triunsaturated triacylglycerides.

[A] Same letters within each column do not significantly differ; SD is given in parenthesis ($n = 3$, $p < 0.05$).

[B] nd, not detected.

HaO are quite similar, making it difficult to differentiate between the oils and, even more challenging, to identify the presence of HaO in EvoO. Although LLLn was only found in HaO, it is not a suitable marker of HaO, even in 40% admixtures, due to its scarce occurrence (3.2% in HaO). If TAG are grouped according to the type of FA bonded to the glycerol structure, it can be observed that disaturated triacylglycerols (DSTAG) remain constant in the admixtures with increasing HaO, while monosaturated triacylglycerols (MSTAG) and triunsaturated triacylglycerols (TUTAG) significantly increase at the 5% and 30% of HaO, respectively. However, in the samples analysed, LLL + LLPo, OLL + OLPo, POPo and OOP + SLO significantly differ at the lowest level of HaO addition (5% HaO).

Table 2 shows the main FA of pure oils and their admixtures, as well as the three fatty acid classes (according to their unsaturation degree). The fatty acid composition of HaO showed significantly higher amounts of oleic and linoleic acids than did EvoO (Crews et al., 2005), whereas the latter had significantly higher contents of palmitic, stearic and palmitoleic acids. All FA significantly differed at the lowest level of HaO addition (5%), except for linolenic acid content that becomes significantly different in admixtures with $\geqslant$20% HaO. The different fatty acid classes (polyunsaturated (PUFA), monounsaturated (MUFA) and saturated fatty acids (SFA)) exhibit trends similar to most FA, as they significantly changed in admixtures with $\geqslant$5% HaO.

Table 2
Percentage composition of main FA and their classes of EvoO, HaO and their admixtures[A] (% of total FA)

FA	100% EvoO	5% HaO	10% HaO	20% HaO	30% HaO	40% HaO	100% HaO
Palmitic acid	11.81 (0.18)^{a}	10.16 (0.14)^{b}	10.18 (0.18)^{b}	9.96 (0.12)^{b}	9.44 (0.10)^{c}	8.89 (0.12)^{d}	5.61 (0.06)^{e}
Palmitoleic acid	0.81 (0.02)^{a}	0.51 (0.01)^{b}	0.55 (0.01)^{b}	0.56 (0.01)^{b}	0.52 (0.01)^{b}	0.38 (0.01)^{c}	0.15 (0.04)^{d}
Stearic acid	4.08 (0.04)^{a}	3.89 (0.02)^{c}	3.72 (0.02)^{c}	3.83 (0.00)^{b}	3.83 (0.01)^{b}	3.74 (0.02)^{c}	3.44 (0.02)^{d}
Oleic acid	72.12 (0.14)^{d}	73.90 (0.32)^{c}	73.95 (0.26)^{c}	73.98 (0.25)^{c}	74.54 (0.13)^{bc}	75.10 (0.31)^{b}	77.23 (0.27)^{a}
Linoleic acid	6.86 (0.05)^{g}	7.42 (0.02)^{f}	7.50 (0.02)^{e}	7.72 (0.02)^{d}	8.05 (0.01)^{c}	8.40 (0.03)^{b}	11.39 (0.03)^{a}
Linolenic acid	0.69 (0.02)^{a}	0.68 (0.02)^{a}	0.66 (0.01)^{a}	0.61 (0.01)^{b}	0.56 (0.01)^{c}	0.52 (0.01)^{d}	0.30 (0.01)^{e}
SFA	16.84 (0.20)^{a}	14.99 (0.11)^{b}	14.80 (0.17)^{bc}	14.63 (0.13)^{c}	14.04 (0.10)^{d}	13.31 (0.15)^{e}	9.33 (0.08)^{f}
MUFA	75.62 (0.24)^{e}	76.91 (0.11)^{d}	77.04 (0.17)^{cd}	77.04 (0.13)^{cd}	77.35 (0.09)^{bc}	77.77 (0.17)^{b}	78.99 (0.10)^{a}
PUFA	7.55 (0.01)^{g}	8.10 (0.01)^{f}	8.16 (0.01)^{e}	8.33 (0.01)^{d}	8.61 (0.01)^{c}	8.91 (0.02)^{b}	11.69 (0.02)^{a}

FA, fatty acids; EvoO, extra virgin olive oil; HaO, hazelnut oil; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

[A] Same letters within each column do not significantly differ, SD is given in parenthesis ($n = 3$, $p < 0.05$).

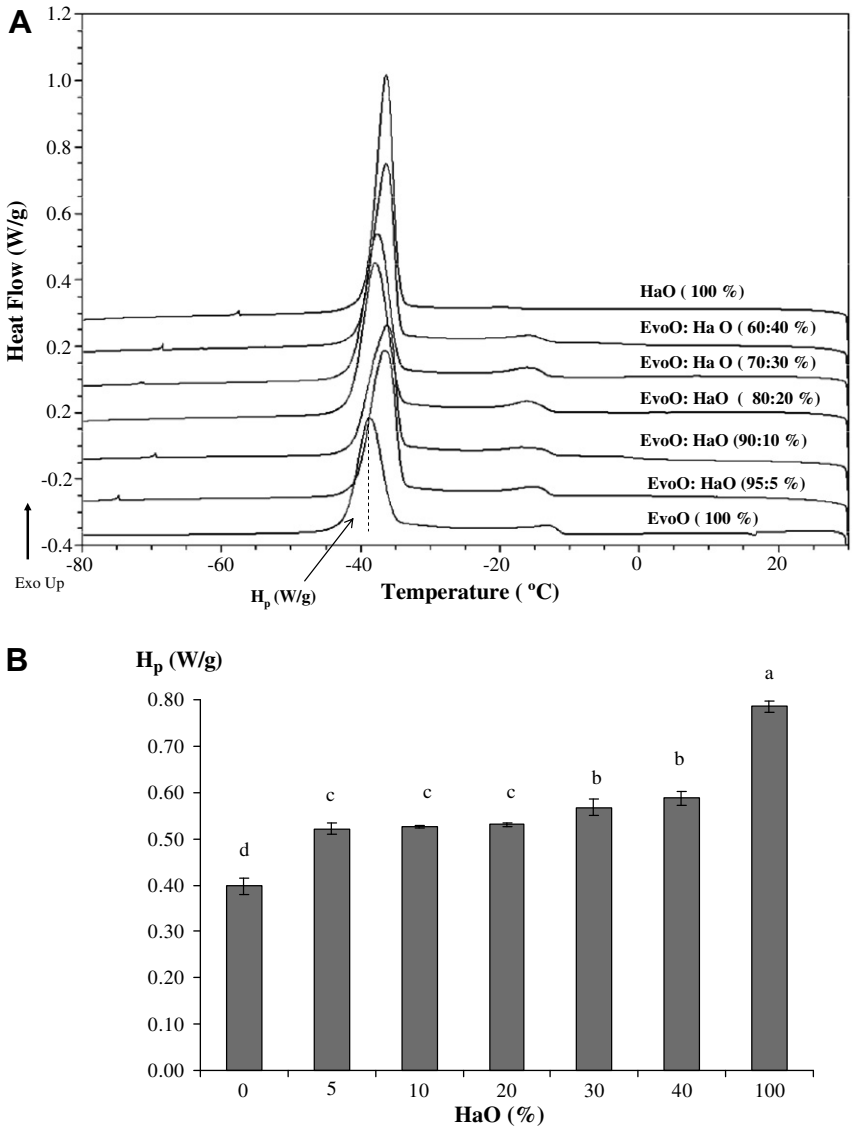

Fig. 1. (A) Representative DSC cooling thermograms of EvoO and HaO and their admixtures. (B) Peak height (W/g) of the major exothermic event (dotted line A) of the cooling thermograms at different HaO percentages added. Error bars represent ±1SD ($n = 3$). Bars with the same letters are not significantly different ($p < 0.05$). *Abbreviations*: EvoO, extra virgin olive oil; HaO, hazelnut oil.

3.2. DSC analysis of cooling thermograms

DSC cooling thermograms obtained for EvoO, HaO and their admixtures (5–40% HaO in EvoO) are shown in Fig. 1A. Cooling lineshapes of EvoO 100% samples were similar to those previously reported by Chiavaro et al. (2007), where lipid crystallisation started at −11.0 °C and developed over a 35–40 °C range. Two well defined exothermic events were observed, a minor and a major peaking at −13° and −38 °C, respectively. The major peak was previously associated with the crystallisation of highly unsaturated TAG, while the minor one was attributed to the crystallisation of more saturated TAG fractions of EvoO (Chiavaro et al., 2007; Tan & Che Man, 2000).

HaO cooling thermograms showed two well defined exothermic events, as previously reported (Tan & Che Man, 2000): a first, less defined shoulder peak, onsetting at −15 °C, followed by a more important event peaking at −37 °C. The entire crystallisation process developed over a ~30 °C range, narrower than that observed in EvoO. The formation of a more ordered crystal structure (i.e. TGA chains more compactly associated) attributed to a highly cooperative phase transition in HaO, resulted in a taller and sharper crystallisation peak, which developed over a narrower temperature range. This was possibly associated with the higher TUTAG, MUFA and PUFA contents (e.g. OOO, OLL, LLL, oleic and linoleic acid) of HaO in comparison with EvoO (Table 1). The major exothermic event was previously associated with the

co-crystallisation of TUTAG that contained oleic, linoleic and linolenic acids (mainly OOO, OOL and OLL), whereas the small shoulder peak was related to the crystallisation of MSTAG and/or DSTAG (Che Man & Tan, 2002).

HaO addition to EvoO altered the overall lineshape of all cooling thermograms in comparison with pure EvoO, as shown in Fig. 1A. Two main thermal events were always present, but both minor and major peaks showed some important changes in their characterising thermal properties. The minor peak slightly shifted towards lower temperatures, peaking at −14.6, −16, and −16.8 °C upon addition of 5–10%, 20–30% and 40% HaO, respectively. Peak heights of the major crystallisation event of pure oils (dotted line in Fig. 1A) and their admixtures were measured and their values are shown in Fig. 1B as a function of HaO addition. Peak height significantly increased upon addition of HaO to pure EvoO (Fig. 1B), even at the lowest HaO level (5%). A 30% HaO substitution had to be reached to notice a further significant increase in peak height that may be associated with the significant increase of TUTAG observed for oil admixtures at the same percentage of HaO added (Table 1). Pure HaO (100%) showed the highest peak height value among all samples.

Table 3 summarises the thermal properties (enthalpy, T_{on} and range of the transition) that characterise the cooling transitions of pure oils and their admixtures. Crystallisation enthalpy of EvoO was significantly increased by HaO $\geqslant$20% (Table 3). Crystallisation T_{on} and temperature range of transition of oil admixtures were, respectively, significantly lower (at HaO $\geqslant$ 5%) and narrower (at HaO $\geqslant$ 10%) than those of pure EvoO. A more ordered crystal structure, having TAG chains more compactly associated, could be formed as a result of HaO addition to EvoO, as reflected by the higher crystallisation enthalpy. The shift of crystallisation toward lower temperatures may be due to the addition of more unsaturated lipid fractions of HaO in EvoO, since lower crystallisation temperature onset and significantly narrowed transition range were previously reported for oils with higher degree of unsaturation (Tan & Che Man, 2000).

Table 3
DSC data obtained from the cooling thermograms of EvoO, HaO and their admixtures[A]

	ΔH (J/g)	T_{on} (°C)	Range (°C)[B]
EvoO	65.2 (0.2)^{c}	−10.9 (0.4)^{a}	35.2 (0.3)^{a}
5% HaO	65.3 (0.3)^{c}	−12.1 (0.4)^{b}	35.0 (0.3)^{a}
10% HaO	64.9 (0.8)^{c}	−12.1 (0.4)^{b}	33.1 (0.3)^{b}
20% HaO	68.5 (2.4)^{b}	−12.6 (0.4)^{bc}	33.2 (0.3)^{b}
30% HaO	68.6 (3.5)^{b}	−12.6 (0.5)^{bc}	32.1 (0.5)^{c}
40% HaO	68.3 (0.2)^{b}	−13.0 (0.3)^{c}	31.8 (0.4)^{c}
100% HaO	70.3 (0.1)^{a}	−15.8 (0.2)^{d}	29.4 (0.4)^{d}

Abbreviations: EvoO, extra virgin olive oil; HaO, hazelnut oil.
[A] Same letters within each column do not significantly differ; SD is given in parenthesis ($n = 3$, $p < 0.05$).
[B] Temperature difference between T_{on} and T_{off}.

3.3. DSC analysis of heating thermograms

DSC heating thermograms obtained for EvoO, HaO and their admixtures (5–40%) are shown in Fig. 2A. EvoO exhibited heating lineshapes similar to those reported in previous works (Chiavaro et al., 2008; Jiménez Márquez & Beltrán Maza, 2003).

Multiple endothermic transitions were detected: first an exothermic event, followed by two endothermic events. The exothermic event originated at −27 °C and may be related to the crystallisation of the TAG fraction that did not solidify under the cooling regime used in this study and/or to a rearrangement of polymorphic crystals into more stable forms. Two endothermic events were well distinguishable: a major event that peaked at −3.5 °C, and a minor, smaller and flatter peak at 8.0 °C. It might be possible that an additional endothermic event, peaking at ∼−12 °C was present. The major endothermic peak may be ascribed to the melting of the most unsaturated fraction of TAG (TUTAG), and the minor event to less unsaturated TAG (MSTAG), as previously reported (Jiménez Márquez & Beltrán Maza, 2003).

HaO heating thermograms showed a well defined endothermic event peaking at −7.0 °C, with two less distinct shoulder peaks, at lower (∼−15 °C) and higher temperatures (∼−2 °C). An exothermic event at lower temperatures may be present in HaO but, even if present, it is not as well defined as the exothermic event observed in EvoO. Previous DSC reports on HaO indicated the presence of either a single melting peak (Tan & Che Man, 2000), or a wide endothermic event consisting of four overlapped peaks (Tan & Che Man, 2002) that were ascribed to the melting of TAG. Different experimental conditions and/or DSC instrumentation and/or nature of the sample may explain the differences observed in HaO melting profiles.

Heating thermograms of EvoO and HaO admixtures showed some evident differences from EvoO. The exothermic event, originating at −27 °C and characteristic of EvoO, decreased at 5% of HaO and almost disappeared at 30% of HaO added, also causing the shift of the overall transition toward higher temperatures. The change in chemical composition of EvoO upon HaO addition may have allowed TAG crystallisation during cooling. The shoulder endothermic peak at −12 °C, present in pure EvoO thermograms, was no longer evident even at the lowest percentage of HaO added (5%). The major melting peak gradually changed lineshape by shifting toward lower temperatures and becoming more asymmetric with increasing HaO, presenting a very different shape at 40% HaO (Fig. 2A). The increase of the higher unsaturated and lower melting lipid fraction (TUTAG, oleic and linoleic acid) upon HaO addition to EvoO, may have induced the lineshape change of the major endothermic peak profile and the lower transition temperature.

The smaller event peaking at higher temperature, characteristic of EvoO but not present in pure HaO, gradually shifted toward lower temperature and slowly disappeared

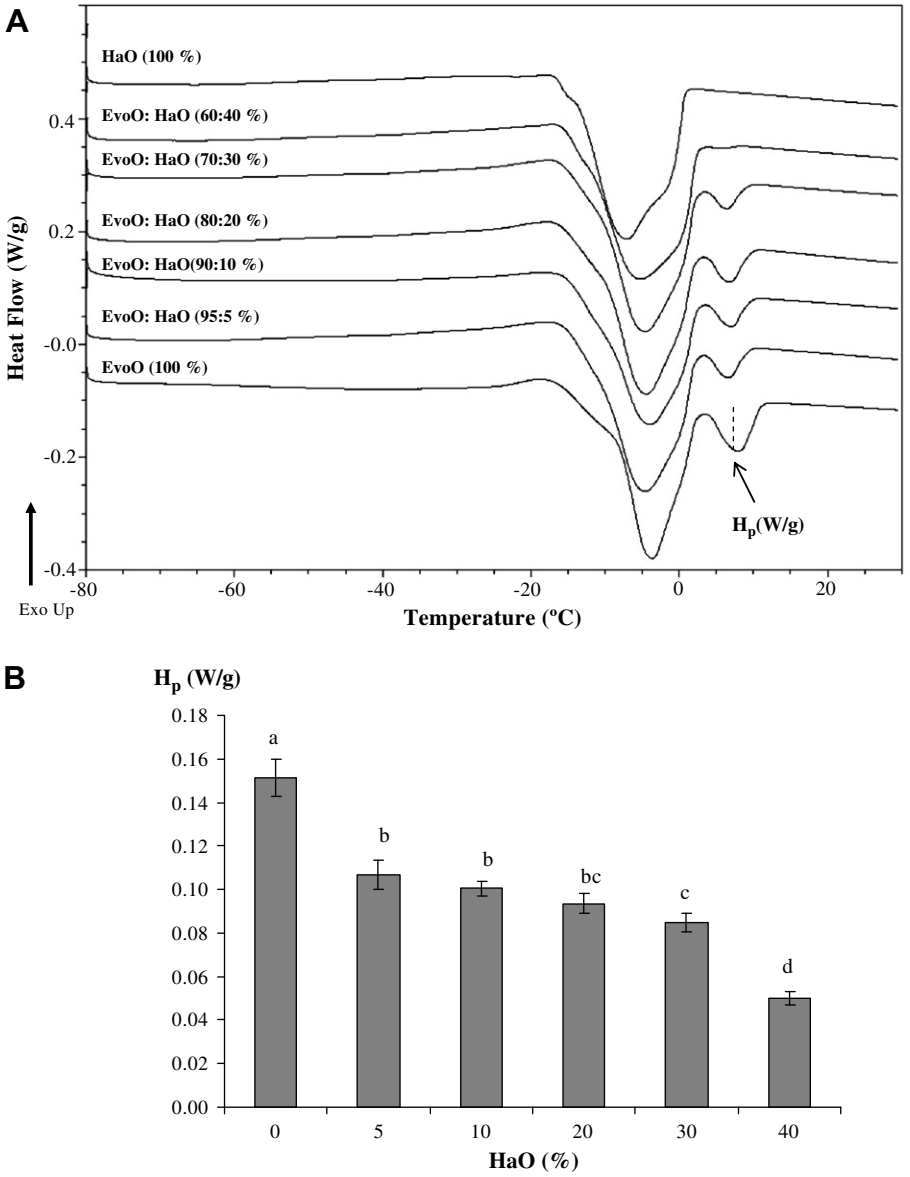

Fig. 2. (A) Representative DSC heating thermograms of EvoO and HaO and their admixtures. (B) Peak height (W/g) of the minor endothermic event (dotted line in A) of the heating thermograms at different HaO percentages added. Error bars represent ±1SD ($n = 3$). Bars with the same letters are not significantly different ($p < 0.05$). *Abbreviations*: EvoO, extra virgin olive oil; HaO, hazelnut oil.

with increasing HaO concentration. Peak heights of the minor endothermic event (dotted line in Fig. 2A) were measured on pure oils and their admixtures and are shown (absolute values) in Fig. 1B as a function of HaO content. A significant decrease of peak height was observed at the lowest percentage of HaO added (5%). Such height decrease became even more significant when HaO was present in percentage equal to or higher than 30%. The decrease of the lipid fraction with higher saturation degree (MSTAG, DSTAG, palmitic and stearic acid) that is expected to melt in the temperature range of the minor shoulder peak (Che Man, Haryati, Ghazali, & Asbi, 1999), may be partially responsible for the disappearance of this peak. It may also be hypothesised that the change of EvoO chemical composition induced by increasing HaO percentages may have influenced the formation of different and less stable polymorphic crystals than those of pure oil, which also may melt at lower temperature. No literature data were found to support this hypothesis, as scarce information is available on TAG polymorphism in olive oil.

The characterising overall thermal properties (enthalpy, T_{on} and temperature range) obtained from the heating thermograms of pure oils and their admixtures, from the beginning of the exothermic to the end of the endothermic events, are shown in Table 4. Enthalpy value of the total heating transition was calculated by subtracting the exothermic enthalpy from the endothermic peak enthalpies.

Table 4
DSC data obtained from the heating thermograms of EvoO, HaO and their admixtures[A]

	ΔH (J/g)	T_{on} (°C)	Range (°C)[B]
EvoO	73.2 (1.7)[c]	−26.7 (0.6)[c]	38.6 (0.6)[a]
5% HaO	77.1 (0.5)[b]	−26.5 (0.8)[c]	36.5 (0.9)[b]
10% HaO	78.1 (1.7)[b]	−26.7 (0.4)[c]	36.4 (0.8)[b]
20% HaO	77.9 (0.7)[b]	−25.9 (0.4)[c]	36.5 (0.7)[b]
30% HaO	76.5 (0.9)[b]	−23.9 (0.4)[b]	36.5 (0.7)[b]
40% HaO	84.1 (0.8)[a]	−20.2 (0.7)[a]	27.8 (0.6)[c]
100% HaO	85.7 (0.6)[a]	−20.6 (0.5)[a]	24.1 (0.3)[d]

Abbreviations: EvoO, extra virgin olive oil; HaO, hazelnut oil.
[A] Same letters within each column do not significantly differ; SD is given in parenthesis ($n = 3$, $p < 0.05$).
[B] Temperature difference between T_{on} and T_{off}.

EvoO displayed significantly lower heating enthalpy than did HaO and the admixtures. Enthalpy of the overall heating transition did not significantly change with 5–30% HaO addition. A significant increase was observed at 40% HaO, as the exothermic event disappeared. A more ordered crystal structure, having TAG chains more compactly associated that completely crystallised upon cooling, may be formed upon adding HaO, thus requiring higher energy to melt.

T_{on} of the total heating event significantly shifted toward higher temperature, starting from 30% of HaO added, as the first exothermic event shifted toward higher temperature and disappeared at 40% HaO. Range of transition also significantly narrowed as the minor endothermic peak shifted toward lower temperature. The increase of higher unsaturated lipid fractions, and the consistent decrease of the more saturated ones, may both be responsible for T_{on} shifting and narrowing of transition, making the major endotherm more similar to the HaO lineshape.

4. Conclusions

This preliminary work has shown that both cooling and heating DSC thermograms undergo significant changes as result of the addition of HaO to EvoO, and they may be a tool for the detection of adulteration of EvoO with refined HaO.

Thermal properties of cooling thermograms, such as enthalpy and T_{on} of transition, were affected by HaO addition at a concentration as low as 5%. However, it is not possible to evince the exact percentage of HaO added from the analysis of cooling thermograms, as thermal properties did not significantly differ in the range 5–20% and 30–40%, respectively. Heating profiles may offer a qualitative tool for investigating the presence of refined HaO as an adulterant by analysing the lineshape of the thermogram; a marked change of the major endothermic peak and a slight disappearance of the minor endothermic peak result from HaO addition. Moreover, the evaluations of the height of the major exothermic peak of crystallisation and the minor endothermic peak of the heating curve may both be attractive parameters for detecting HaO, even at low level (5%). In addition, the lowest percentage of HaO that could be detected by DSC was not completely established by these preliminary results. However, HaO addition to extra virgin olive oil at economically remunerative levels (20–40%) was clearly detectable by DSC analysis.

In conclusion, the results of this first investigation suggest that DSC may be used to detect EvoO adulteration with HaO. These preliminary findings must be confirmed by the analysis of a larger number of samples that take into consideration the variation of chemical composition of both EvoO and HaO, which may arise from different cultivars, agronomical practices, geographical origins, harvesting periods and processing technologies.

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