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Characterisation of traditional Macedonian edible oils by their fatty acid composition and their volatile compounds

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ABSTRACT

The fatty acid composition and volatile compounds of selected traditional Macedonian edible oils of several varieties, including sunflower seeds, pumpkin seeds, flax seed, rapeseed and sesame seeds, were analysed. The fatty acid (FA) composition was determined by GC-FID analysis after transesterification into the corresponding methyl esters. α-Linolenic acid (C18:3) was the main unsaturated fatty acid in flax seed oil (56.2% of total FA), oleic acid (C18:1) dominated in rapeseed and sesame seed oils (65.3 and 43% of total FA, respectively), and linoleic acid (C18:2) was the dominant compound in sunflower and pumpkin seed oils (59.2 and 59.5% of total FA, respectively). The volatile flavour compounds were determined using headspace solid phase microextraction (HS-SPME) using a DVB/Carboxen/PDMS fibre, coupled with gas chromatography–mass spectrometry (GC–MS). In total 97 volatile compounds were detected revealing a very complex aroma profile of the oils, composed of acids, alcohols, aldehydes, alkanes, alkenes, esters, furans, pyrazines, sulphur compounds and terpenes. Among them, aldehydes presented the highest proportion of the overall volatiles in rapeseed oil (76.8% of the total volatiles), followed by sesame seed oil (25% of the total volatiles), pumpkin seed (5.45% of the total volatiles), flax seed oil (2.5% of the total volatiles) and sunflower seed oil (0.95% of the total volatiles). Terpenes (41 detected) were the dominant compounds in sunflower seed oil and pumpkin seed oil (93.9 and 87.8% of the total terpenes, respectively), followed by flax seed oil (47.6% of the total terpenes), sesame seed oil (21.5% of the total terpenes) and rapeseed oil (10% of total the terpenes). Sunflower seed and pumpkin seed oil showed the highest number of volatile compounds identified, with the highest number of terpenes and esters within the investigated products.

1. Introduction

Edible oils are recognized as essential nutrients in both human and animal diets. From a nutrition point of view, they are concentrated sources of energy providing essential fatty acids which are considered as building blocks for hormones, as well as a carrier medium for the oil soluble vitamins A, D, E, and K (Tannenbaum, 1979). Different classes of compounds are present in edible oils, such as fatty acids, tocopherols, phenolic compounds, phytosterols, carotenoids and thioglycosides (Gromadzka & Wardencki, 2011).

The fatty acid composition varies significantly within oils from different plant sources, predominantly depending on the variety, but also on the state of ripeness, the area in which the plants are grown, climate conditions etc. (Murkovic, Hillebrand, Winkler, Leitner & Pfannhauser, 1996; Schuster, Zipse & Marquard, 1983). In general, fatty acids are classified according to their degree of saturation: saturated or unsaturated with one double bond (mono-unsaturated) or more than one double bond (poly-unsaturated). The major unsaturated fatty acids are oleic acid (OA), linoleic acid (LA) and α-linolenic acid (ALA). Fatty acids with even numbers of carbon atoms, from 16 to 18, with a single carboxyl group, are the most common fatty acids present in vegetable oils (Ballesteros, Gallego & Valcárcel, 1993; De Koning, van der Meer, Alkema, Janssen & Brinkman, 2001).

Unsaturated fatty acids tend to oxidize in the presence of radicals, oxygen, metal catalysts or lipoxygenase enzymes, producing volatile organic compounds (VOCs). VOCs have low molecular weights (usually less than 300 Da), which are easily vaporized at room temperature and which may produce an odour sensation (Temime, Campeol, Cioni, Daoud & Zarrouk, 2006). These compounds may have positive or negative (off-flavour) impact on the flavour of the oil. The presence/absence of VOCs in different proportions can be taken as a marker for identifying adulteration. Important flavour compounds of oils are mainly produced by the endogenous plant

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enzymes as a result of the lipoxygenase pathway. Further volatile and odour-active compounds are formed during the chemical oxidation (autoxidation) of lipids (Wilkes et al., 2000). These compounds contribute significantly to the flavour of the freshly pressed oils. During storage, autoxidation of edible oils continues whereas at some point — depending on the concentration of the volatile and odour-active autoxidation products — the desired flavour turns into off-flavour in terms of rancidity. How quickly the flavour of edible oils turns into off-flavour depends on various factors, like for example the fatty acid composition, storage conditions, influence of UV light or the presence of metal ions (Choe & Min, 2006; Jansen, 2015). Off-flavour of edible oils may also be caused by the presence of exogenous enzymes. Moreover, the flavour compounds in edible oils can be formed from amino acids such as valine and leucine, which can be converted into volatile compounds, such as esters and alcohols which may, as a consequence, also influence the sensory perception of edible oils such as olive oils (Kalua et al., 2007).

Since the chemical composition of edible oils is very complex, different analytical methods have been proposed to isolate, identify and quantify different compounds that characterise oils. High-performance liquid chromatography (HPLC) connected to different detection systems was successfully used for the identification and quantification of fatty acids, triacylglycerols, sterols, tocopherols and hydrocarbons (Cao et al., 2014; Gliszczyńska-Świgło, Sikorska, Khmelinskii & Sikorski, 2007; Murkovic, Piironen, Lampi, Kraushofer & Sontag, 2004; Zeb & Murkovic, 2010). Furthermore, gas chromatography (GC) is the technique of choice for the analysis of fatty acids, usually coupled with a flame-ionization detector (FID) or for the analysis of volatile compounds (Haiyan, Bedgood, Bishop, Prenzler & Robards, 2007; Murkovic et al., 1996). GC or HPLC in combination with mass spectrometry, as sophisticated techniques allowing structural identification and quantification by single-ion monitoring (SIM) or multiple-ion monitoring (MIM) of different classes of compounds, are used for the analysis of different classes of compounds present in the oils (Haiyan et al., 2007; Ma et al., 2014; Zhang et al., 2014). Recently, a headspace comprehensive two-dimensional gas chromatography time-of-flight mass spectrometry (Headspace GC × GC–TOF/MS) was used for the classification of volatiles from vegetable oils in order to build a statistical model that should help to identify adulteration of oils (Hu et al., 2014).

Even though vegetable oils from several plant species have traditionally been produced in Macedonia, there are only very few studies dealing with the properties of these products. Few studies focusing on the chemical composition of essential oils from various plant and herb species grown in Republic of Macedonia, such as *Salvia officinalis* populations, have been performed (Kostadinova et al., 2007; Najdoska, Bogdanov & Zdravkovski, 2010; Stefkov, Cvetkovikj, Karapandzova & Kulevanova, 2011). Only a preliminary study about fatty acid composition of vegetable oils and fats (Kostik, Memeti & Bauer, 2013) as well as one study concerning on antioxidative capacity determined by a voltammetric method (Gulaboski, Mirceski & Mitrev, 2013) are available in literature. However, detailed knowledge about the properties of these traditional oils is required in order to further improve their quality.

To the best of our knowledge, there has been no report on the identification and quantification of individual flavour compounds of Macedonian edible oils from different cultivars. Concerning this, the objectives of the present work were twofold: (1) characterisation of the fatty acids and the volatile compounds of the local edible oils and (2) correlation of the data in order to receive a useful data set for the oil producing industry in the country. Automated headspace solid-phase microextraction coupled with gas chromatography–mass spectrometry (HS-SPME GC–MS) was used for the analysis of the volatile compounds, and gas chromatography coupled with a flame ionization detector (GC-FID) was used for the analysis of the fatty acid after transesterification of the glycerides into the corresponding fatty acid methyl esters.

2. Materials and methods

2.1. Chemicals and reagents

The reference materials (fatty acid methyl esters) were purchased from Nu-Chek Prep, (Elysian, Minnesota, USA). The boron trifluoride–methanol complex (BF_3 solution 20% in methanol), which was used for derivatisation of the fatty acids, was purchased from Merck (Darmstadt, Germany). All chemicals and solvents used (e.g. hexane, heptane) were of analytical grade and were purchased from Merck (Darmstadt, Germany).

2.2. Oil samples

Five oil samples from different seed varieties (sunflower seeds, pumpkin seeds, flax seeds, rapeseeds and sesame seeds) were produced in 2014, in an oil factory located in the eastern part of the Republic of Macedonia. Prior to the production of the oils, the seeds from sunflower, flax, rape and sesame, were preconditioned by heating to temperatures between 40–50 °C before pressing. Only pumpkin seeds were heated to temperatures of 90–100 °C. Pressing of the seeds was performed by using a single press of a capacity of 20–25 kg h^{-1}. The press was equipped with an electric motor of an electric capacity of 1.5 kW. After pressing, the oils were stabilized for a period of 1 to 2 months, afterwards filtered and bottled. All oils were produced in three lots (in triplicates). To obtain the final oil, equal portions of each lot were blended. For the purpose of this study, samples were collected out of the final blends in 15 mL plastic tubes and purged with nitrogen in order to avoid oxidation. In order to protect the oils from UV light, the tubes were covered with aluminum foil, packed in a box with dried ice and transported to the laboratory for analysis. To keep oxidation as low as possible, the oil samples were stored in a refrigerator (4 °C) until use.

2.3. Fatty acid derivatisation and GC-FID analysis

The previously described transesterification method was used to transform the fatty acid glycerides into the corresponding methyl esters (Murkovic et al., 1996) Transesterification was performed by the use of boron trifluoride methanol complex (20% BF_3 in methanol). BF_3-methanol is one of the fastest and most convenient ways to convert fatty acids to their methyl ester derivatives. Thus, approximately 20 mg of an oil sample was transferred into a screw-cap test tube (30 mL) and 1 mL of a solution containing 1 g L^{-1} of pentadecanoic acid (C15:0 in methanol) was added as internal standard (IS). The mixture was reduced to dryness under nitrogen flow (N_2) and the residue was redissolved in 6 mL of 0.5 M methanolic NaOH solution. The tube was capped and stirred on a magnetic stirrer while heating to 80 °C for 30 min. After that, the tube was cooled to room temperature and 6 mL BF_3-methanol solution was added to the sample. The sample was stirred again on a magnetic stirrer at 80 °C for 15 min. After cooling down to room temperature, 10 mL H_2O and 10 mL heptane were added to the samples, followed by stirring on a magnetic stirrer for 10 min and subsequent vortexing for 1–2 min. Aliquots of the methylated samples were placed into an autosampler and analysed with GC-FID. Each oil sample was transesterified and analysed in four replicates. GC-FID analyses were performed on a gas chromatograph (Hewlett Packard 5890 series II, Wilmington, DE, USA) equipped with a split/splitless injector (split ratio of 1:30) and a flame ionization detector (FID), using an HP Innowax (30 m × 0.32 mm I.D.) capillary column with a film thickness of 0.25 μm. The temperature programme for the separation of the fatty acid methyl esters was as follows: 1 min isothermal at 50 °C, 8 °C min^{-1} to 140 °C followed by a temperature ramp of