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INVESTIGATION OF EDIBLE OILS OXIDATION STABILITY USING PHOTOOXIDATION AND SPME/GC METHOD FOR DETERMINATION OF VOLATILE COMPOUNDS – PRELIMINARY INVESTIGATION

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The resistance of edible oils to oxidative degradation, leading to rancidification, is an important parameter for assessing the quality of oil. This paper presents a new promising method to diagnose the volatile compounds which are products of oil oxidation reactions. Our experiment was carried out using a combined method of UV irradiation as an oxidation acceleration technique, headspace solid-phase microextraction (HS-SPME) as an extraction technique and capillary gas chromatography (GC) as an assay technique. Method precision and sensitivity expressed as RSD (<19%) and LOD (between 18 and173 ng/mL) are satisfactory. The induction period set on hexanal / *t*-2-nonenal ratio (IP=6.67 h) is comparable with those obtained with the Rancimat method (4.64–6.73 h).

INTRODUCTION

In recent years rapeseed/canola oil has becoming increasingly popular again. The oil available nowadays comes from rapeseed, twice improved with lower glucosinolates and erucic acid content, and is referred to as double zero oil ("00"). It competes with olive oil, on account of its low price and nutritional advantages. Rapeseed oil is a rich source of fat-soluble vitamins A, D_3, E, K and essential polyunsaturated fatty acids, like linoleic acid (LA) and alpha-linolenic acid (LNA) with their good proportion equal to 2:1 [Pijanowski *et al*., 2004; Drozdowski, 2002]. However, due to a high content of polyunsaturated fatty acids this oil impairs lipid changes in oxidation by atmospheric oxygen in the autooxidation process or during the lipooxygenase pathway [Cavalli *et al*., 2004]. The rate of oxidation reaction increases with the degree of unsaturation or temperature increase (*e.g.* frying process). This reaction badly effects the natural aroma of the oil. In vegetable oils, like in other food ingredients, natural aroma is created by a characteristic composition of volatile compounds. In the creation of aroma bouquet both natural volatile compounds and derivative substances have a part, which came from extraction or pressing of oil and which are dissolved in oil [Gromadzka & Wardencki, 2007]. Short-chain hydrocarbons, ketones, aldehydes, alcohols, epoxides, esters and lactones may be formed, giving a smell and taste of rancidity [Drozdowski, 2002].

Among commonly used methods for determination of oil oxidation stability there are the Rancimat test and peroxide value determination. Peroxide value allows to determine only the total amount of oxidation products. It strongly depends on the analytical experience and measurement cannot be automated. In contrast, the Rancimat test is fully automated, but is usually performed at higher temperatures (100-120°C). Furthermore, the Rancimat test is an indirect method based on measurement of water conductivity in which volatile oil oxidation compounds are absorbed.

The promising method to diagnose the volatile compounds can be the solid-phase microextraction technique connected with gas chromatography coupled to mass spectrometry or flame ionization detector (SPME/GC/MS or SPME/GC/FID) [Gromadzka & Wardencki, 2007]. There are many different substances which can be indicators of the oxidation process but the level of hexanal in the sample or even the hexanal/nonanal ratio [Jimenez *et al*., 2004] seem to be the most suitable. Hexanal is generated both in the autooxidation process and during lipooxygenase pathway. In turn, nonanal is formed only in the autooxidation process of oleic acid and shows the highest rate of increase during the oxidation process and therefore may be the appropriate oxidation marker [Vichi *et al.*, 2003].

The aim of the present work was to develop a simple and reproducible method for the determination of the degree of rancidity of edible oil by analysing volatile compounds, using headspace solid-phase microextraction (HS-SPME) and capillary gas chromatographic (GC) techniques. In comparison to the Rancimat test, the developing method should allow

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to identify and trace amounts of oxidation products of edible oils.

MATERIALS AND METHODS

Samples and reagents

Rapeseed oil used in this research was Kujawski Oil from Fat Factory Kruszwica S.A., purchased from a local supermarket. Solvents used in sample preparation: acetone – GC grade, methanol – gradient grade and hexane – GC grade, were from Merck and standard reagents: hexanal, purity: >97% (GC) (Fluka), *t*-2-nonenal, purity: 97% (Aldrich), *t*-2-octenal, purity: tech. 94% (Aldrich), *t*-2-heptenal, purity: ~98% (GC) (Fluka), *t,t*-2,4-heptadienal, purity: >97% (GC) (Fluka), *t,t*-2,4-nonadienal, purity: 85% (Aldrich), and *t,t*-2,4--decadienal, purity: >85% (GC) (Fluka).

Photoreaction conditions

A photoreactor (homemade at the University of Santiago de Compostela, Spain) with two ultraviolet low-pressure mercury lamps (10 W and 8 W) with maximum emission at 254 nm was used in this research. The photoreactor has three possible modes of work (8 W, 10 W, 18 W). In this case all experiments were carried out with a power of 18 W, so both the lamps were turned on. A sample containing 6 mL of oil was poured to two quartz closed cells (3 mL in each). Later the cells were placed into the photoreactor for ultraviolet irradiation at different irradiation times (15 min – 12 h).

Headspace solid-phase microextraction (HS-SPME) conditions

Oil samples in volume of 4 mL were placed in 10 mL vials and closed by an aluminum cap with a PTFE-faced septum. Before extraction, stabilization of the headspace in the vial was conducted by equilibration for 3 min at 40°C. On the basis of literature studies a combined SPME fiber, coated with divinylbenzen/carboxen/polydimethylosiloxane (DVB/CAR/PDMS, 2 cm long, with 50/30 μm coating thickness, Supelco, Bellefonte, PA, USA) stationary phase, was chosen as most appropriate for the extraction of volatile compounds from the oil matrix. The extraction was carried out by inserting the fiber into the headspace of the oil sample for 30 min at 40°C with a magnetic stirring. After exposure, the fiber was thermally desorbed for 5 min into the GC injector at 250°C.

Gas chromatography conditions

Analyses were carried out on a CP-3800 (Varian, Palo Alto, CA, USA) gas chromatograph equipped with Varian Saturn 2000 Ion Trap Mass Spectrometer and Flame Ionization Detector. The injection was made for 2 min using the splitless mode. The temperature of the injector was 250°C and that of detector 240°C. The separation was carried out on two columns with different polarity: on a HP-5MS column with a stationary phase of 5% poly(diphenyl) and 95% poly(dimethylsiloxane) (30 m x 0.25 mm x 0.25 μm, Hewlette--Packard) and on a DB-WAX column with 20% poly(diphenyl) and 80% poly(dimethylsiloxane) (30 m x 0.25 mm x 0.5 μm, Supelco, Bellefonte, PA, USA) with an oven temperature program as follows: initial temperature was 32°C held for 3 min, then ramped at 5°C/min to 80°C, again ramped at 10°C/min to 200°C and then once again ramped at 10°C/min to a final temperature of 280°C. The total time of analysis was 29 min. The carrier gas was helium with a flow rate of 1.2 mL/min.

To identify oil oxidation volatiles a Varian Saturn 2000 Ion Trap Mass Spectrometer was used. The detector operated in electron impact mode (70 eV) at 250°C. The temperatures of the ion source and of the transfer line were 250°C and 300°C, respectively, and the carrier gas flow rate was 1 mL/min. Detection was carried out in the scan mode between 25 and 300 amu. Detected components were tentatively identified by matching EI^+ spectra against the NIST Mass Spectral Database containing about 100 thousand compounds and by comparing their retention times with those of standards.

RESULTS AND DISCUSSION

At the first stage of the experiment detection possibilities of the method were investigated. In order to calculate the repeatability and limits of detection and quantification (RSD, LODs and LOQs, respectively), three HS-SPME experiments were carried out using as a sample of oil (4 mL) spiked with a mixture of selected aldehyde standards. The obtained values of RSD, LOD and LOQ are shown in Table 1.

The method's precision expressed as the relative standard deviation (RSD) ranged form 6.6 to 19%. Data in Table 1 shows that repeatability achieved was satisfactory. The average error in SPME has been described by RSD=2.5–37% [Keszler & Heberger, 1998]. The precision of the proposed method seems to be better than the average observed for most headspace analyses. The LOD and LOQ (Table 1) were calculated on the basis of the signal to noise ratio obtained for the studied aldehydes in SPME experiments. The obtained detection limits (18-173 ng/mL) are generally acceptable for headspace analysis [Keszler & Heberger, 1998].

In this research UV irradiation was used to accelerate the oxidation process in real edible oil samples [Szukalska, 2003; Hęś *et al*., 2001]. Photodegradation experiments of rapeseed oil with different irradiation times (Figure 1) were carried out in order to assess the photoformation – photodegradation kinetics of volatile oil oxidation products.

In general, the concentration of detected compounds increases during the photo-induced process (Figure 2). Similar behaviour was observed during the investigation of the influence of temperature on oil volatiles [Jeleń *et al.,* 2000, 2007; Mildner-Szkudlarz *et al.,* 2003].

TABLE 1. RSD, LODs, LOQs for HS-SPME extraction in oil sample with spike standards (n=3).

Compound	RSD (%)	LOD (ng/mL)	LOQ (ng/mL)
hexanal	6.6	25	82
t-2-heptenal	10.8	21	70
t,t-2,4-heptadienal	19.0	18	61
t-2-octenal	11.1	22	72
t-2-nonenal	13.9	47	158
t,t-2,4-nonadienal	11.9	129	429
t,t-2,4-decadienal	18.5	173	577

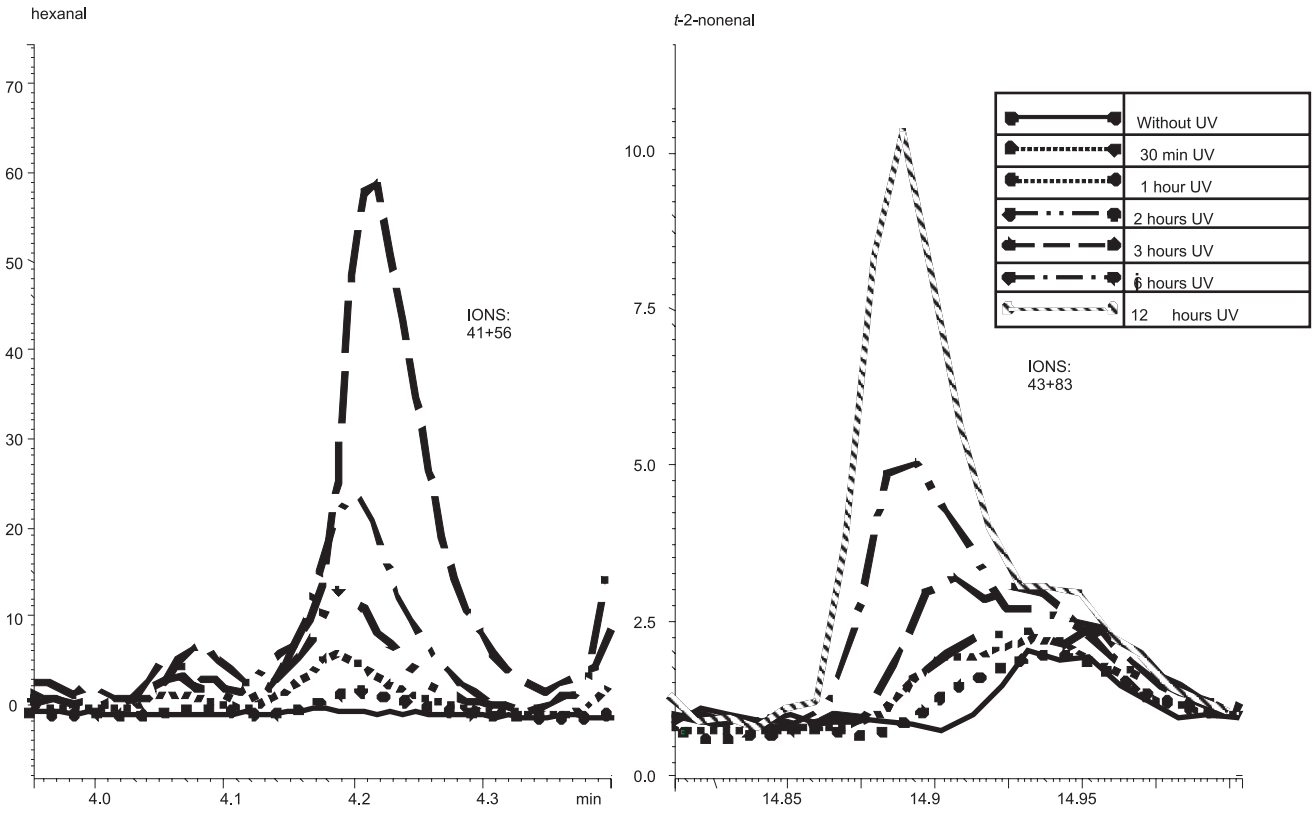

FIGURE 1. Chromatograms of hexanal and *t*-2-nonenal at different irradiation times.

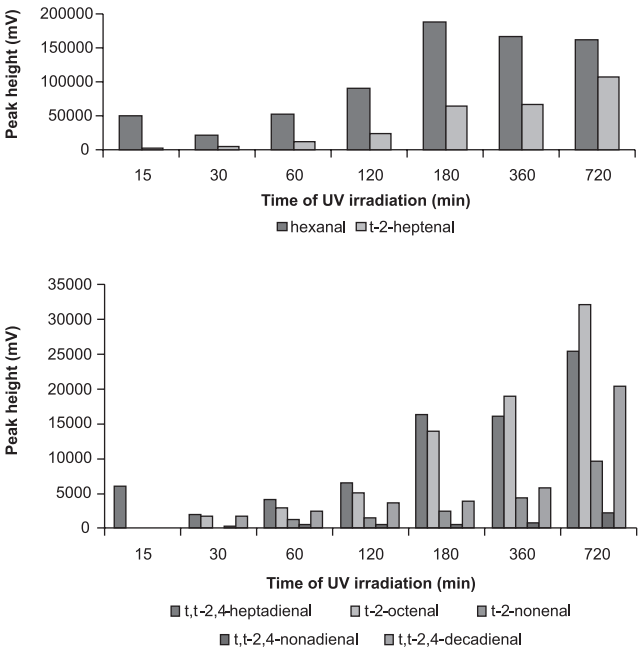

FIGURE 2. The increase of rapeseed oil oxidation products during UV irradiation time.

TABLE 2. Retention time of selected volatile photoproducts identified with high probability (HP-5MS column).

Compound	Retention time, tr (min)
1-pentanol	3.76
hexanal	4.27
2-hexenal	5.98
2-heptanone	7.14
t-2-heptenal	9.00
1-octen-3-ol	9.99
6-methyl-5-hepten-2-one	10.18
2-pentylfuran	10.27
hexanoic acid	10.55
octanal	10.66
t,t-2,4-heptadienal	10.77
t-2-octenal	12.17
t-2-nonenal	14.77
2-decanone	15.55
t,t-2,4-nonadienal	15.80
2-decenal	16.75
2-undecanone	17.26
t,t-2,4-decadienal	17.50
2-undecenal	18.29

Some identified oil oxidation volatiles with the highest probability factor are listed in Table 2. Most of these compounds were also mentioned by other authors who investigated oil degradation processes [Jeleń *et al*., 2000;, 2007; Keszler & Herberger, 1998; Mildner-Szkudlarz *et al*., 2003].

The best indicator of this process, as it was mentioned before, can be the hexanal/nonanal ratio. In our experiments, instead of nonanal *t*-2-nonenal – its derivative – was used.

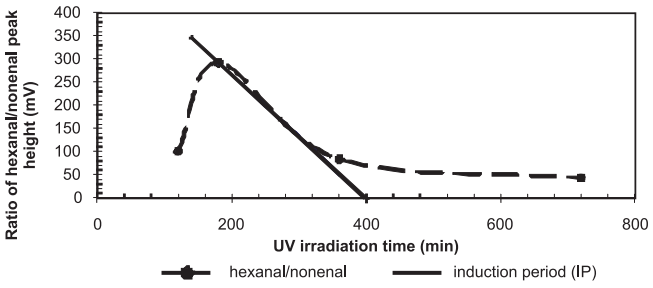

FIGURE 3. Dependence of hexanal on *t*-2-nonenal ratio in the UV irradiation process.

The induction period (IP), a period in which the testing oil remains oxidatively stable, was set graphically (Figure 3) on the basis of the hexanal/*t*-2-nonenal peak height ratio and its value is about 400 min, *i.e.* 6.67 h. This value corresponds to the IP determined by Maszewska & Krygier who used the Rancimat method [Płatek, 1995]. They applied the following conditions: mass of oil sample – 2.5 g, temperature – 120°C, air flow – 20 dm^3/h, and water volume in conductivity cell – 60 mL. They obtained the IP for rapeseed oil at a level of 4.64–6.73 h [Maszewska & Krygier, 2005]. According to Płatek [1995], who used the same conditions as mentioned above, average induction time for rapeseed oil in 120°C is 4.9 h. In our research, the investigated oil reached better stability – its induction period was nearly 2 h longer than that obtained by Płatek [1995].

CONCLUSIONS

The postulated method enables estimating the stage of oxidation of the analysed sample both qualitatively and quantitatively. Moreover, this method is very sensitive and affords the possibility of tracing the oxidation process, even during initial stage. It enables detecting classical oil oxidation products with satisfactory precision and repeatability expressed as the relative standard deviation (RSD) below 19% and with detection limits between 18-173 ng/mL, the values are generally acceptable for headspace analysis. This procedure can be applied for the classification and organoleptic quality assessment of vegetable oils of different quality or produced from various raw materials. It also allows the determination of the induction period using the hexanal/nonanal ratio, which corresponds to the Rancimat method. Furthermore the use of this method allows to detect adulteration of good quality oils by cheaper ones.

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