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Evaluation of Photooxidation of Olive Oil by Determining the Concentration of Hexanal as an Oxidative Marker Using an Electronic Nose

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To date, for the evaluation of olive oil lipid oxidation, certain oxidative parameters, including free fatty acids (FFAs), peroxide value (PV), and the specific extinction coefficient at 270 nm (K270), have been used, and the use of one parameter alone is insufficient to evaluate oil quality. Volatile compounds such as hexanal, which is directly related to oxidative off-flavour, have the potential to be alternative markers for oil oxidation. However, static headspace gas chromatograph analysis is generally not sensitive enough to quantify oil oxidation markers at the levels required. The HERACLES e-nose (Alpha MOS, France) is based on dual fast gas chromatography technology, which is a dynamic headspace analysis that enables the sensitive and rapid determination of volatile compounds in oils. In this study, as a new approach for evaluating photooxidation of olive oil, the e-nose was employed to determine the concentration of hexanal in olive oil, as an oxidative marker during storage under light. Comparative studies revealed that changes in olive oil hexanal content were similar to those of conventional oxidative indicators during storage under light. The FFAs, PV, and K270 showed good correlations with hexanal concentration. In addition, determining the hexanal content by the e-nose analysis also enabled the evaluation of the antioxidant effect of α-tocopherol added to olive oil on oil quality. These results improve our understanding of the shelf-life of olive oil and provide strategies for maintaining the quality of olive oil through determining the concentration of hexanal using e-nose, in addition to conventional oxidative indicators.

1. Introduction

Olive oil is the central component of the Mediterranean diet. In particular, extra virgin olive oil (EVOO) is the juice obtained from olive fruits by mechanical procedures with no chemical additives. Therefore, EVOO is the highest grade of olive oil and is recognised as a premium edible oil with beneficial health effects. Its consumption is increasing around the world, with the European Union as the leading producer, consumer, and exporter. Since EVOOs produced in one crop season are usually consumed before the next crop season (Morello et al., 2004), it is necessary to minimize oil deterioration during the storage period.Olive oil producers, suppliers, and consumers need to prevent olive oil exposure to diffuse light and direct sunlight during processing, transportation, handling, and storage. Fluorescent light has been reported to cause lipid oxidation (Sattar et al., 1975). Unfortunately, oil products are often exposed to fluorescent lighting in supermarkets, which may result in light-induced deterioration of the oil and the production of off-flavours and colour defects. Eventually, these oil products lose their commercial value. The assurance of the shelf-life stability of EVOOs is a matter of great concern for the olive industry.The quality and stability of edible oils are affected by light (Sohail et al., 2010). Exposing vegetable oils to light induces photooxidation through the action of natural photosensitizers, such as chlorophylls (Lee, 1955; Usuki et al., 1984). The main chlorophyll pigment present in EVOOs is pheophytin a (phy-a) (Psomiadou and Tsimidou, 2001; Giuliani et al., 2011). Phy-a is present at higher concentrations in EVOO that in other types of vegetable oil (Sena et al., 2017). The colour of EVOO is mainly related to the presence of chlorophyll and carotenoid pigments, which are responsible for producing

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green-yellowish colouration, respectively. These pigments affect consumer acceptance of oil, and the chlorophyll pigments in EVOO are extremely sensitive to sunlight (Kishimoto, 2019b).

Nowadays, for the evaluation of olive oil lipid oxidation, certain indicators are available, including free fatty acids (FFAs), peroxide value (PV), which determines the amount of primary oxidation products, and the specific extinction coefficient at 270 nm (K270), which measures the formation of secondary oxidation products (IOC, 2019; Malheiro et al., 2009). These methods supply only limited information regarding the level of olive oil oxidation. Recently, gas chromatography/mass spectrometry (GC/MS) was applied to detect changes in the chemical composition of olive oil during storage. Previous studies have reported that certain aldehydes, ketones, and other compounds could also be markers for oil oxidation, of which hexanal could be an alternative marker for lipid oxidation (Jeleń et al., 2000; Ha et al., 2011; Sun et al., 2015). The concentration of hexanal is directly related to oxidative off-flavours. Its detectable odour threshold was reported to be very low (García-Llatas et al., 2007; Kalua et al., 2007). However, static headspace GC/MS analysis is generally not sensitive enough to quantify oil oxidation markers. The GC/MS technique is complex, expensive and time-consuming. Gas phase/solid phase microextraction (SPME) has provided better sensitivity and has been used widely for monitoring purposes (Bicchi et al., 2004). Kiritsakis (1998) and Vichi et al. (2003) suggest that the occurrence of particular volatile compounds, such as nonanal or hexanal, can be a good indicator of olive oil for lipid oxidation. The olive oil industry needs to be able to verify quickly the oxidative level of oil to estimate its shelf-life. Therefore, the development of analytical methods that can execute quick and reliable quality checks on olive oil is required.

The HERACLES electronic nose (e-nose) (Alpha MOS, Toulouse, France) is based on ultra-fast gas chromatography technology and holds the promise of being much smaller, cheaper, and easier to use and maintain than the GC/MS. The e-nose with built-in pre-concentration trap allows to reach very low detection thresholds on odorous molecules. Besides, the e-nose coupled with an autosampler allows rapidity and high-throughput analysis. Therefore, with respect to the human nose the e-nose does not fatigue, is capable of performing repeated discriminations with high precision. It also features two metal capillary columns of different polarities mounted in parallel and coupled to two flame ionization detectors. Therefore, two chromatograms are obtained simultaneously, which allows the sharper identification of chemical compounds. The system also provides a sensory feature by directly clicking on the chromatogram peaks generated by the e-nose. Especially, the e-nose technique is used to detect volatile compounds originated from hydroperoxide degradation and to identify the products of triglyceride oxidation, allowing to characterise aspects of the oxidation process since it is characterized by simplicity of sample preparation. Notably, the odour profiles obtained by the HERACLES e-nose system have been shown to explain the sensory attributes of olive oil (Melucci et al., 2016; Kishimoto et al., 2017; Kishimoto, 2018 and 2019a, b; Kishimoto and Kashiwagi, 2019). Thus, the e-nose approach could be a faster recognition method for monitoring oil oxidation and characteristics of olive oil. In this study, as a new approach for the evaluation of olive oil lipid oxidation, the HERACLES e-nose was employed to determine the concentration of hexanal in olive oil as an oxidative marker during storage under light conditions and was then validated using the e-nose technique for the analysis of hexanal in olive oil.

2. Experimental

2.1 Materials

EVOO was obtained by dual-phase decanter centrifugation using industrial processors. Refined olive oil (ROO) (Toyo Olive Co., Ltd., Kagawa, Japan) and medium-chain triglyceride (MCT) oil (The Nisshin Oillio Group, Ltd., Tokyo, Japan) were purchased from a market. The amount of α-tocopherol in the oil was 20.0 mg/100 g oil. Phy-a (purity, >95 %) and hexanal (purity, >95 %) were purchased from FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan). α-Tocopherol (purity, >97 %) was purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Phy-a was added to the ROO at the desired treatment concentrations of 2, 5, and 10 mg/kg, with no phy-a added to the control sample. α-Tocopherol was added to enriched ROO with phy-a (5 mg/kg) at the desired treatment concentrations of 100, 300, and 500 mg/kg, and the control sample lacked α-tocopherol.

2.2 Storage under light and dark conditions

Three samples of ROO (40 g) were weighed into 50-mL clear glass bottles with the headspace occupied by air. To shield the oil from fluorescent light, these bottles were covered completely with aluminum foil (Kishimoto, 2019b) and then placed in a dark room. To simulate possible fluorescent light exposure at the consumer level, the oil samples were then placed on a benchtop in our laboratory at room temperature (24 ± 2 °C) for five weeks, with exposure to fluorescent light at a lux intensity of 1,000 (room luminance) for 10 hours

per day. For each set of experiments, an equal amount of oil (3.0 g) was taken from the same bottle and analysed weekly.

2.3 Analytical procedures

FFAs, PV, and K270 of the oil samples were measured using an OxiTester (CDR; Ginestra Fiorentina, Italy) (Kamvissis et al., 2008). Preliminary confirmation of the FFAs determined using the OxiTester method was conducted by comparing the results for oil samples over a wide range of values with those from the official analysis method (Gucci et al., 2012; Kishimoto, 2019b). Oil samples were added to prefilled cuvettes for analysis. The volume of oil used was 2.5 µL for measuring FFAs, 0.5–2.5 µL for PV, and 10 µL for K270.

2.4 Flash gas chromatography electronic nose analysis of hexanal

To analyse hexanal concentration in the oil samples, the headspace (gas mixture) prepared in a temperature-controlled vial was analysed using a HERACLES II electronic nose (Alpha MOS). This instrument was equipped with a nonpolar column (MXT-5; 10-m length × 180 µm diameter) and a polar column (MXT-WAX; 10-m length × 180 µm diameter) in parallel to produce two chromatograms simultaneously. An HS100 auto-sampler (CTC Analysis AG; Zwingen, Switzerland) was used to automate sample incubation and injection. An alkane mixture (from *n*-hexane to *n*-hexadecane) was used to convert the retention times into Kovats indices for calibration. For analysis, an aliquot of oil (2.0 g) was placed in a 20-mL vial and then sealed with a magnetic cap. The vial was placed in the auto-sampler, which was then placed in the HERACLES II shaker oven and incubated for 15 min at 60 °C with shaking at 500 rpm. A syringe was used to sample 5 mL of the headspace, which was injected into the gas chromatographer. The oven temperature was initially 40 °C (held for 10 s), then increased to 250 °C at 1.5 °C/s and held at this temperature for 60 s. The total separation time was 120 s. Data were acquired and processed using AlphaSoft software v2020 (Alpha MOS).

2.5 Quantification of hexanal in olive oil

To determine the concentration of hexanal in each oil sample, a standard curve was established. Samples of MCT oil containing different hexanal concentrations were prepared and subjected to flash gas chromatography e-nose analysis using the HERACLES II e-nose. The concentrations of hexanal in the oil samples after storage were determined using a standard curve. The limits of detection (LOD) and the limit of quantification (LOQ) of the proposed method were calculated respectively, as three times and ten times of the standard deviation (SD) on analysing six blanks.

2.6 Statistical analysis

Data are presented as the mean ± SD of three replicates. The data were analysed using one-way analysis of variance followed by the Tukey-Kramer test in Microsoft Excel. Differences from mean values with $p < 0.05$ were considered statistically significant.

3. Results and Discussion

3.1 Flash gas chromatography electronic nose analysis of hexanal in oil

Figure 1 shows a representative chromatogram of the headspace gases obtained from hexanal in MCT oil and EVOO when the MXT-5 column was used. The hexanal peak was eluted at a retention time of approximately 45 s. At the same retention time, peaks of the major volatile compounds of the oil were also detected.

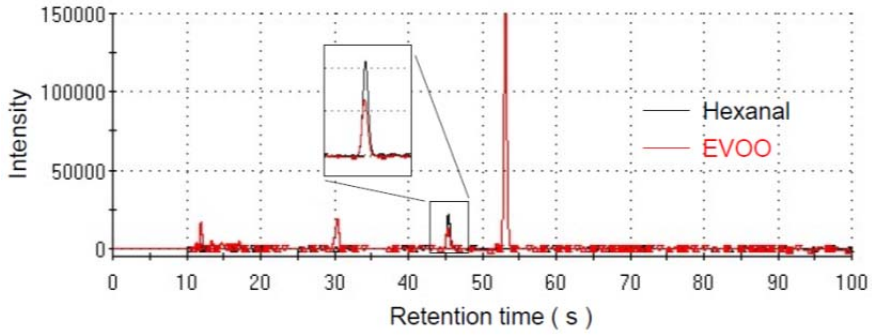

Figure 1: A representative chromatograms of headspace gases obtained from hexanal in MCT oil and EVOO.

The standard curve (y = 2.1942x + 400.18) for hexanal was used to determine its concentration in the oil samples. The concentration of hexanal correlated with the peak area over the range of 10–5,000 ppb. The

linear correlation coefficient (R^2 = 0.999) indicated that this standard curve allowed the quantification of hexanal in oils with high accuracy. The results of the calculations of LOD and LOQ for hexanal in oil were 5.6 and 18.6 ppb, respectively.

3.2 Changes in FFAs, PV, K270, and hexanal in ROO enriched with phy-a during storage under light

Although the total phy-a content in EVOOs obtained from different olive cultivars ranges from 1.4 to 64.1 mg/kg, most varieties had values within a range of 10 mg/kg (Giuliani et al., 2011). To demonstrate the photooxidation effect on oil quality of olive oil, ROO, which is a photosensitizer-free olive oil enriched with phy-a at different concentrations (0, 2, 5, and 10 mg/kg) were stored under light and dark conditions. The changes in FFAs, PV, and K270 as quality parameters of the oils during storage under light and dark conditions are shown in Figure 2. All parameters of ROOs enriched with different phy-a concentrations increased with increasing light exposure time. The rate of increase was much higher in the enriched ROOs, compared to those with no phy-a added, and depended on the initial phy-a concentration in the enriched ROO. This is because auto-oxidation, once initiated, does not stop until all free radicals are inactive (Kiritsakis, 1992). The PVs of ROOs enriched with 2, 5, and 10 mg/kg phy-a stored under light were over the limit of 20 meqO_2/kg set by the International Olive Council (IOC, 2019) after storage for one week (Figure 2b). In contrast, the parameters of ROOs enriched with 10 mg/kg of phy-a stored in the dark slightly increased with increasing storage time. These results suggest that phy-a addition accelerates the primary oxidation of olive oil.

The changes in hexanal concentration during storage under light and dark conditions are shown in Figure 2d. The quality parameters and hexanal concentration did not significantly change in the dark, regardless of the initial phy-a content (0, 2, and 5 mg/kg) (data not shown). The concentration of hexanal in the oils with phy-a also increased with storage time under light. The change patterns were similar to those of FFAs and K270, and the changes in hexanal depended on the initial phy-a content (Figure 2a, 2c, and 2d). These results demonstrated that phy-a addition accelerates the photooxidation of olive oil and the olive oil photooxidation resulted in a similar trend of hexanal concentration to those obtained from other quality parameters.

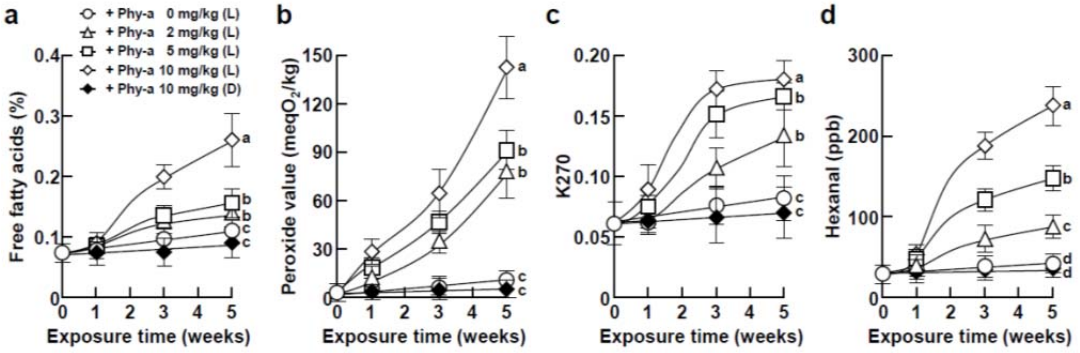

Figure 2: Changes in FFAs (a), PV (b), K270 (c), and hexanal content (d) in ROO enriched with different concentrations of phy-a during storage under light (L) and dark (D) conditions. [a-d]For the five-week values, mean values with different letters are significantly different ($p < 0.05$).

Table 1: Correlation coefficient, R, between hexanal and other oxidative indicators

Added phy-a (mg/kg)	FFAs–Hexanal	PV–Hexanal	K270–Hexanal
0	0.9470	0.9360	0.9855
2	0.9746	0.9423	0.9912
5	0.9962	0.9519	0.9961
10	0.9920	0.9298	0.9947

3.3 Correlation coefficient *R* between hexanal and other oxidative indicators

Regarding the possibility of hexanal concentration determination by the HERACLES II e-nose analysis as an oxidative indicator, the correlation coefficient, *R*, between hexanal and other oxidative indicators was calculated, as shown in Table 1. The results showed that the concentration of hexanal had good correlation coefficients with FFAs, PV, and K270 during storage of ROO enriched with phy-a at different concentrations under light. The *R* value between hexanal and other oxidative indicators, such as FFAs, PV, and K270, was good for the evaluation of olive oil photooxidation and revealed that hexanal can be used as an oxidative marker of olive oil.

3.4 Evaluation of reduction effect of oil deterioration by α-tocopherol addition

Finally, as EVOO contains substantial amounts of antioxidants, such as α-tocopherol (Psomiadou et al., 2000), the antioxidant effect of α-tocopherol on the photooxidation of ROO enriched with phy-a was evaluated by determining hexanal and other oxidative indicators. To determine the effect of α-tocopherol on oil quality in the oils during storage under light, the results were compared for oil samples with different concentrations (0, 100, 300, and 500 mg/kg) of α-tocopherol in the presence of 5 mg/kg of phy-a, which is the average minimum content of total chlorophylls in 85 % of oils (Giulinani et al., 2011). Among these results, even the FFAs, PV, and K270 of the oils enriched with 100 mg/kg of α-tocopherol were within the IOC limit on these parameters, when the oils were stored under light for five weeks (Figure 3a, 3b, and 3c). In addition, adding at least 100 mg/kg of α-tocopherol effectively reduced hexanal formation in the ROOs enriched with phy-a stored under light, and the trend was similar to those obtained from other oxidative markers (Figure 3d). These results indicate that adding α-tocopherol to the oils enriched with phy-a resulted in appreciable resistance to oxidative deterioration after storage under light conditions for five weeks. Commercial EVOOs may exhibit resistance to fluorescent light irradiation, because the α-tocopherol contents in the oils range from 98 to 370 mg/kg (>200 mg/kg in 60 % of oils) (Psomiadou et al., 2000). Thus, hexanal could be used as an alternative oxidative marker to evaluate the effects of antioxidants on oil deterioration.

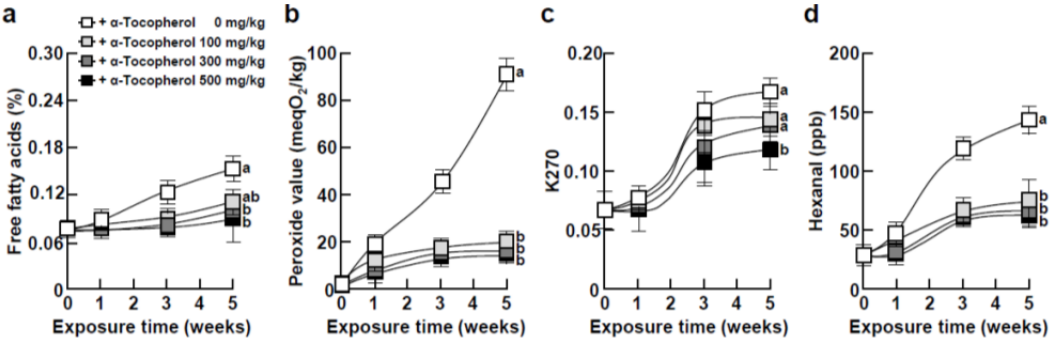

Figure 3: Changes in FFAs (a), PV (b), K270 (c), and hexanal content (d) in ROO enriched with different α-tocopherol concentrations in the presence of 5 mg/kg phy-a during storage under light. [a,b]For the five-week values, mean values with different letters are significantly different ($p < 0.05$).

4. Conclusions

The results of this study demonstrate that ROO photooxidation during storage with exposure to fluorescent light decreased upon adding phy-a, in a concentration-dependent manner, and that hexanal, produced by photooxidation of the oil, proved to be a reliable indicator for evaluating the degree of olive oil oxidation using an e-nose. The e-nose technique is a sensitive method that can be utilized to detect aldehyde compounds such as hexanal, which is formed by the oxidation of olive oil during storage under light. E-nose is a rapid method that can be utilized to measure olive oil oxidation and is also an effective method for evaluating the antioxidant effect of α-tocopherol on the oil quality of olive oil. The results showed that the effective content of α-tocopherol for anti-photooxidation to maintain oil quality for five weeks was at least 100 mg/kg, which is contained in commercial EVOOs. These EVOO shelf-life stability findings are important for manufacturers, and demonstrate that EVOO quality can be determined by measuring the concentration of hexanal using e-nose, in addition to conventional oxidative indicators. Thus, it is suggested that the artificial olfaction based on e-nose system is available to mimic the sensorial abilities of humans in detection of complex mixtures of chemical substances.

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