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VARIATIONS OF SOME NUTRITION VALUES OF OLIVE OIL BY HOUSEHOLD USING

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ABSTRACT

This study examines of virgin olive oil variations after microwave heating, boiling in vinegar or salt solution, as well as UV irradiation. The heating and UV irradiation were performed using both continuous and discontinuous profiles. Measurements of selected nutrition values involving chlorophylls, carotenoids and alpha-tocopherol contents were carried out applying visible and fluorescence spectrometry. Development of fatty acid oxidation products was verified from relation of intensity ratio of 445/520 nm peaks in fluorescence spectrum. The continual microwave heating or UV irradiation showed results different from those using the discontinuous exposure despite the same cumulative treatment time. Chlorophylls and carotenoids were less sensible to the treatment than alpha-tocopherol. The boiling with the vinegar solution affected each from the followed components more destructively than the salt medium.

Keywords: Olive oil, α-tocopherol, carotenoids, chlorophylls, microwave, UV irradiation

INTRODUCTION

Olive oil is very popular for its nutritive and health-promoting potential, especially against cardiovascular disorders due to the presence of high levels of monounsaturates and other valuable minor components such as phenolics, phytosterols, tocopherols, carotenoids, chlorophyll and squalene **(Ghanbari *et al.*, 2012)**.Olive oil resistance against oxidation process is an important indicator of performance and shelf-life. In general, oil is oxidized when subjected to oxygen, heating, exposure to light, catalyzers, etc. **(Vlachos *et al.*, 2006)**.High oxidative resistance is attributed to both triacylglycerol composition low in polyunsaturated fatty acids and a group of phenolic antioxidants composed mainly of polyphenols and tocopherols **(Velasco and Dobarganes, 2002)**. Also carotenoids have many important positive effects in human alimentation, mainly based on their antioxidative, antimutagenic and photoprotective properties. They influence immune system, participate in eyesight and act as precursors of vitamin A **(Lehninger*et al.*, 2008; Vodrážka, 1992)**. Chlorophylls are a rich source of magnesium, improve oxygen distribution into brain, they are necessary for heart activity and effective in anemia therapy and organism regeneration. These compounds are important for oxygen and electron transports in biological structures acting as "quenchers" of free radicals. However, they must not be oxidized to be exploited in the organism **(Kleňová, 2011)**.

Culinary treatment of olive oil includes the use as is in vegetable salads and in cooked, baked or fried meals. In households and restaurants, olive oil is stored for various periods of time and under different temperature and light conditions depending on the consumption rate. It is well known the above mentioned factors affectolive oil properties. Therefore before consumption of the oil, its initial components and properties can be changed significantly in many different ways. Some papers related to this subject have already been published **(Vieria and Retigano-d´Arce, 1998; Velasco and Dobarganes, 2001; Goméz-Alonso *et al.*, 2004; Vlachos *et al.*, 2006; Malheiro *et al.*, 2009)**. However, many aspects are still to be further investigated, due to the complexity and variable composition of olive oil.

Besides the classical meal preparations, microwave (MW) cooking and heating are used more and more. This study was conducted with the aim to verify impact of some model culinary treatments on changes of chlorophyll, carotenoid and α-tocopherol contents in virgin olive oil. The treatments involved the heating of oil in a microwave oven using different heating time and applying continuous or discontinuous heating profile, the boiling with vinegar and salt aqueous solutions, as well as UV light irradiation under continuous and discontinuous conditions. Fluorescence spectroscopy was used to measure α-tocopherol amount and determination of chlorophylls and carotenoids was carried out by means of UV-VIS spectroscopy.

MATERIAL AND METHODS

Materials

The sample of virgin olive oil (VOO) was obtained from retail outlet (Billa Nitra):100% EXTRA VIRGIN OLIVE OIL marked BORGES, Spanish provenance, 1 liter packaging in dark green bottle, expiration to 02.04.2014. The sample was stored in dark. The following experiments were carried out in triplicates.
Cyclohexane p.a., sodium chloride p.a. and anhydrous sodium sulphate p.a. were supplied by Centralchem, s.r.o., Banská Bystrica, Slovakia.
Consumer 8% vinegar marked Pagusa was bought in retail outlet (Lidl Nitra).

MW heating

The heating was carried out in the MW oven (Orava MW 1707A, Slovakia; 17 l in volume with 24.4 cm rotating plate in diameter, 5 heating levels, total power 1150 W, ultimate MW power 700 W). Each heating experiment started with the oven cooled down. The oil samples in volume of 10 ml were heated in Pyrex Petri dishes of 9 cm diameter put on the centre of the rotating plate at maximum MW power for 1, 2, 3, 5, 10 min and 5x2 min.

Boiling with vinegar or salt water simulators

The heating 50 ml of the VOO with 500 ml of 1.6 % vinegar or 1% salt aqueous solutions, respectively, was performed in saucepan of 21 cm diameter on a hotplate for 1 h. Upon cooling down, the oil layers were removed into a test tube using a syringe, dried with anhydrous sodium sulphate and filtered through cotton-wool.

UV irradiation

The exposure was performed in the chamber with UV lamp (Electronic Ultraviolet Crosslinker λ = 254 nm, UltraLum CEX-1500 UV, USA; Intensity 8.2-8.5 mW/cm^2, energy 999.9 mJ). The sample of 10 ml volume in Pyrex Petri dishes was irradiated intermittently in 5 min intervals following 5 min pauses opening the door, number of the irradiating 5 min cycles 6 as well as continually 30 min.

In all cases, the exposed samples were either measured immediately or kept in refrigerator until analysis.

Analytical methods

Chlorophylls and carotenoids concentration was determined using procedures described by **Mosquera *et al.* (1991)** with no modification. VIS spectra of VOO in cyclohexane were taken using Cary 50 Scan UV-VIS Spectrophotometer (Varian) with 1 cm cell. Calculation of chlorophyll and carotenoids contents was carried out using relations (1) and (2) as follows:

$$\text{Chlorophylls} = \frac{A_{670} \times 10^6}{613 \times 100 \times \text{oil density}} \text{ mg/kg} \quad (1)$$

$$\text{Carotenoids} = \frac{A_{470} \times 10^6}{2000 \times 100 \times \text{oil density}} \text{ mg/kg} \quad (2)$$

where A_{670} and A_{470} are absorbances at 670 nm and 470 nm, respectively, read from related VIS spectra. The relative deviation was within 5% for chlorophylls and 10% for carotenoids, respectively.

Alpha-tocopherol was estimated according to **Escuderos *et al.* (2009)** from fluorescence spectra scanned by Cary Eclipse Fluorescence Spectrophotometer with 1 cm cell.

The fluorescent emission spectra (λ_{ex} = 350 nm) were taken from oil directly. α-Tocopherol amount (mg/kg) was calculated using the following regression equation (3):

$$\propto -\text{Tocopherol} = 209.6 - 106.4\, xI_{370} + 119.6\, xI_{371} - 13.997\, xI_{378} + 6.89\, xI_{414} - 6.41\, xI_{417} \quad (3)$$

where I is the peak intensity at related λ. The relative deviation did not exceed 20%.

RESULTS AND DISCUSSION

MW heating

The heating of VOO sample having the layer thickness of 1.6 mm showed that after 10 min, the content of chlorophylls was reduced to 57% and carotenoids to19% of the initial values, while the α-tocopherol content was 77% as can be seen in Figure 1. Such high carotenoids loss when compared with the chlorophylls supports opinion that in plants, the main role of carotenoids is protection of chlorophylls **(Jursík *et al.*, 2010)**.Consequently the lower carotenoids concentration means a lower protection of chlorophylls.

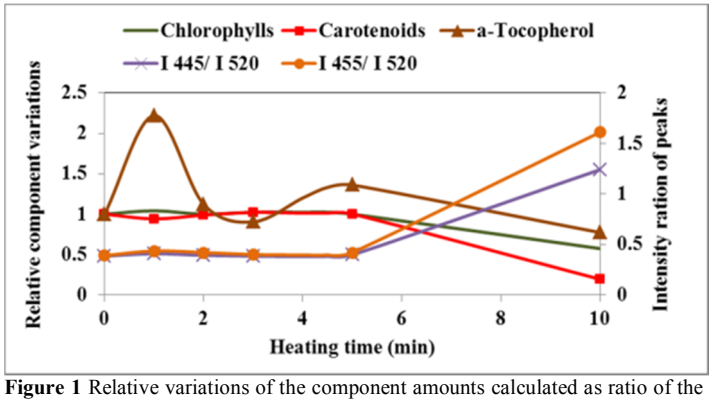

Figure 1 Relative variations of the component amounts calculated as ratio of the initial and measured amounts for chlorophylls, carotenoids and α-tocopherol amounts and selected intensity ratios in fluorescence spectrum (λ_{ex}=360 nm) of VOO with continual MW heating time. The initial amounts (mg/kg) are as follows: chlorophylls 3.0, carotenoids 2.3, α-tocopherol 158.6

So it did not surprise in our case, that the chlorophylls and carotenoids changes were parallel, while more remarkable extremes could be observed in the α-tocopherol curve (Figure 1). For the monitored components, **Malheiro *et al.* (2009)** obtained analogous courses of those dependencies including some initial increase in all component, what he explained by loss of moisture. After 10 min heating of several olive oil samples having the layer thickness of 5.3 mm, he found the residual amount of chlorophylls was between 65-93%, carotenoids 31-37% and α-tocopherol 0.03-0.18%. He also observed that after heating during 15 min α-tocopherol was not any longer detectable, while residual chlorophylls were 50-70% and carotenoids 11-23%. From this comparison one can assume that layer thickness or heated oil volume are relevant, since after 10 min heating, our colorant figures were close to the values obtained by **Malheiro *et al.* (2009)** after 15 min heating. Our experiments showed that the heating up to 5 min did not affect significantly either amount of chlorophylls or carotenoids. However, the heating time 1 min for α-tocopherol exhibited larger variations than **Malheiro´s *et al.* (2009)** ones. Our results supported his opinion that this variation could be due to transformation of other VOO components linked to α-tocopherol.

The oil sample subjected to fivefold 2-min re-heating simulates the re-heating of meals in household. Comparison of both interrupted and continuous heating for the same time in total is presented in Figure 2. It is obvious the heating 5x2 min did not provide the same results as continuous 10 min heating.

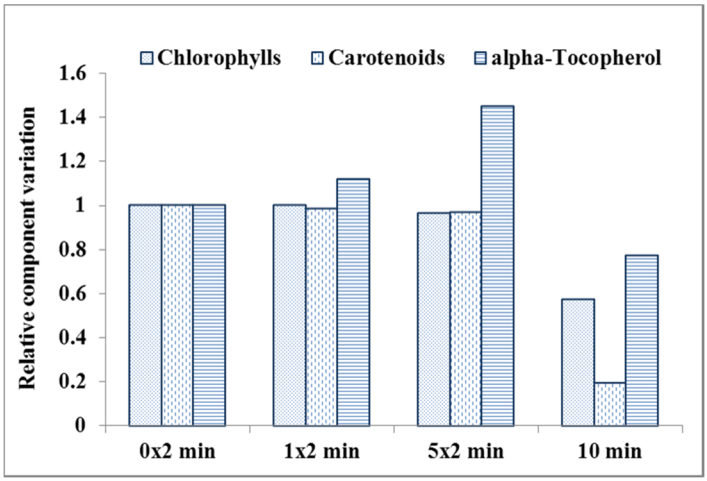

Figure 2 Effect of continuous and discontinuous MW heating of VOO on selected component variations

No significant differences were observed in chlorophylls and carotenoids concentrations between the re-heating when compared with continuous heating. In the case of the re-heating for 1x2 and 5x2 min, as shown in Figure 2, the increased concentration of α-tocopherol against the initial value was consistent with its growth for 1 min heating as in the previous case (Figure 1).This indicates a possible continuing transformation of some VOO components initiated by the short heating. The 10 min continual heating caused a destructive effect on all three components, the most of which were carotenoids. Reason for this could be a higher temperature reached under long time heating. These findings indicate that multiple short heating of meal containing VOO does not affect level of the monitored characteristics.

Estimation of oxidative level by fluorescence spectroscopy

European Commission Regulation (EEC) No 2568/91 defines specific extinction coefficients at 232 nm [conjugated dienes (K_{270})] and 270 nm [conjugated trienes (K_{232})] as the parameters related to the oxidation state of olive oil. **Kyriakidis and Skarkalis (2000)** found that in fluorescence spectrum of VOOs (Figure 3) with comparable intensity of chlorophyll peaks showed intensities of fluorescence peaks around 445 nm and 455 nm that, compared with 520 nm intensity as stable, may correlate with the values of K_{270} and K_{232} as well as hydrolysis products (acidity). Their related regression equations gave R^2 very close to 1. Thus the intensity rations can be used as indicators of oxidative changes in our sample, too. Figure 1 shows also the relationships between the monitored components and the intensity ratios obtained in our experiments. Course of the content variations of the components and the peak intensity ratios showed expected character and supports the findings of **Kyriakidis and Skarkalis (2000)**. It can be seen that the variations of chlorophylls, carotenoids and α-tocopherol are closely related with oxidative changes of the heated oil and thus confirming their antioxidant properties. Decreasing amounts of the chlorophylls and carotenoids caused increasing intensity of the selected characteristic bands in the fluorescence spectrum indicating a growth of oxidative products with antioxidant reduction. In some frame approximation, also the development of α-tocopherol amount can be involved here. However, the running changes of the included linked structures within early phases need to be still examined in more detail.

These results showed that more significant changes occur only after VOO was heated for 5 min. In addition, they confirm that the use of fluorescence spectrometry is an excellent tool for rapid evaluation of VOO quality.

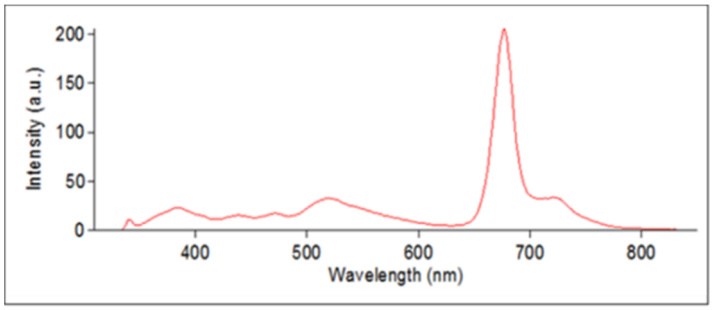

Figure 3 Fluorescence spectrum of VOO (λ_{ex}=360 nm, path 1.0 cm)

The boiling with vinegar or salt aqueous simulators

The following experiments should simulate impact of the 1 h boiling of vinegar and salt aqueous meals (e.g. soups) on change of the monitored VOO components. The results are presented in Figures 4, 5 and 6.

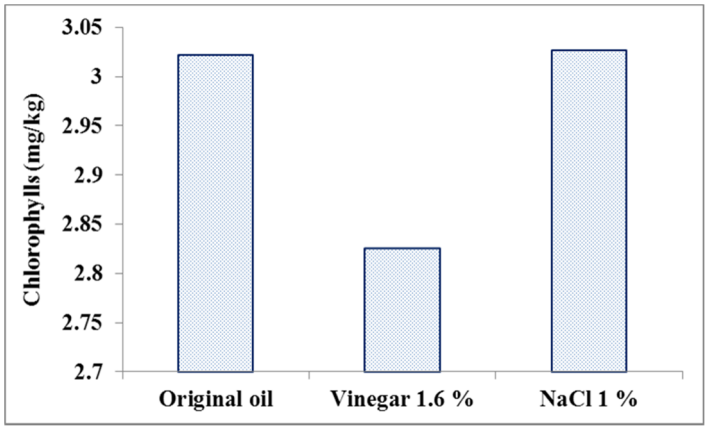

Figure 4 Variation of chlorophyll content in VOO after 1 h boiling with vinegar (1.6%) or salt solutions (1%)

After 1 h boiling the vinegar solution with VOO sample, the initial amounts of the chlorophylls fell to 93 % while the chlorophyll concentration was almost identical (102%) when boiled with the salt solution (Figure 4). Some hydrolysis of the chlorophylls by effect of H^+ ions can be explanation of the larger change in the vinegar medium, when acid removes the magnesium ion replacing it with two hydrogen atoms giving an olive-brown solids, phaeophytins (http://www.ch.ic.ac.uk/local/projects/steer/chloro.htm). The changes of the carotenoids (Figure 5) in the vinegar and salt solutions (reduction to 94% and 100% of the initial concentration) ran practically in parallel with the chlorophylls (Figure 4). The largest α-tocopherol reduction (Figure 6) was in the vinegar solution again (drop to 88%) while in the salt medium, the α-tocopherol content was almost identical with the original value (99%). The latter finding is in principled accordance with the microwave oven when already during 1 min heating, the α-tocopherol content grew up to double of the initial figure. Certainly, the variation course using the mentioned heating way cannot be considered to be identical.

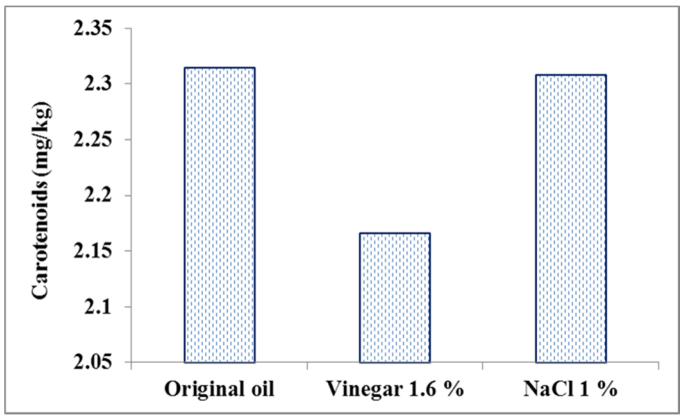

Figure 5 Variation of carotenoids content in VOO after 1 h boiling with vinegar (1.6%) or salt solutions (1%)

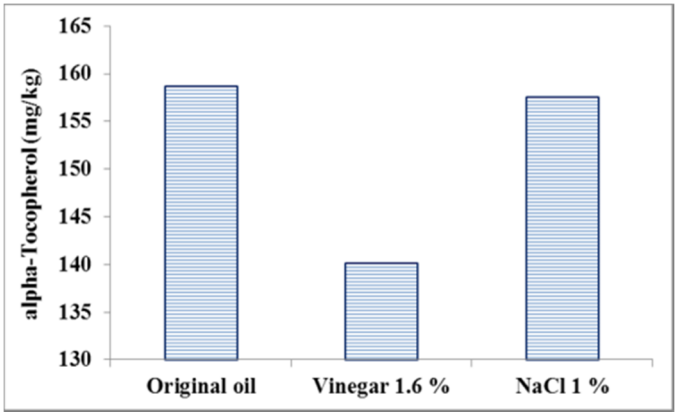

Figure 6 Variation of α-tocopherol content in VOO after 1 h boiling with vinegar (1.6%) or salt solutions (1%)

From the particular changes under boiling VOO with aqueous solutions it can be concluded that to save a maximum α-tocopherol content, it is recommended to acidify boiled meals just after are ready, if possible.

UV irradiation

UV irradiation of VOO in the chamber with defined wavelength and radiation energy was chosen as a model simulating oil storage in a place unprotected against sunlight. We are aware that it is impossible to establish any conversion factor between exposure under controlled conditions and very variable storage conditions in a real environment. However, this experiment provided at least some idea of UV irradiation impact on the selected VOO components. Figure 7 illustrates variations of the selected VOO components with UV exposure time.

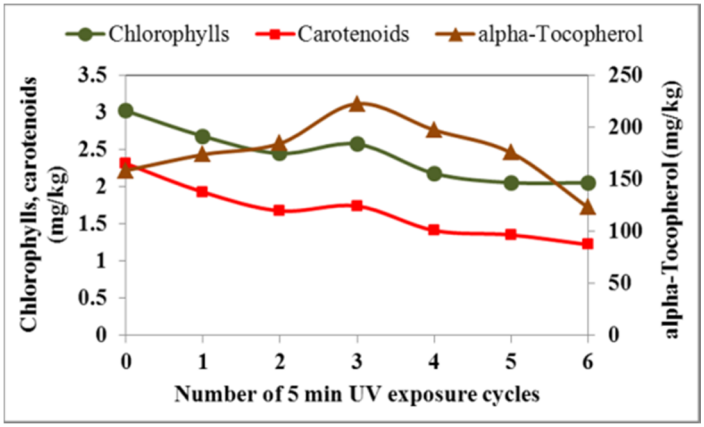

Figure 7 Variations of selected components in VOO with 5 min UV exposure cycles

Development of the chlorophylls and carotenoids contents was practically in parallel, but not consistent with that observed using MW heating, as shown in Figure 1. While under MW treatment more notable drop occurred after5 min, UV irradiation caused evident destruction of these two colorants from the start. It is obvious that the mechanism of UV degradation is different due to the presence of conjugated diene and triene systems which are very sensible towards UV light. After 2x5 min irradiation the residual amount of chlorophylls was 81% and carotenoids 72% from the initial values, and after 6x5 min it dropped down to 68% and 53%, respectively. The course of α-tocopherol amount was different from the other two monitored components in this case as well. It can be seen that UV radiation promoted transformation of some components into α-tocopherol what resulted in the α-tocopherol increasing within up to 3x5 min, while it readily decomposed during the next exposure. After 2x5 min the α-tocopherol content reached level 116% and, after 6x5 min dropped down to 78% of the initial value. A maximum observed on all experimental curves at 3x5 min suggests some connection between amounts of chlorophylls and carotenoids and a protective effect of α-tocopherol; the higher α-tocopherol content, the more moderate destruction of the other two colorants. Although chlorophylls and carotenoids are antioxidants, α-tocopherol is well above them due to photo-stabilizing effect and provides to them some protection, too. This observation confirms statement that α-tocopherol is the strongest antioxidant of lipids **(Larsen, 1991)**. Results of **Sabliov *et al.* (2009)** also suggest that free α-tocopherol is resistant to UV light relatively well. Degradation was due to UV light absorption by unsaturated fatty acids and phenolic compounds in VOO. In VOO due to oxygen fixation in linolenic and linoleic acids´double bond position, hydroperoxides arise producing later carbonyl species (aldehydes and ketones)

(Frankel, 2005; Goméz-Alonso *et al.*, 2004). These are subjected to Norrish I and II reactions creating free radical intermediates and next new products with changed spectral characteristics.
In the next experiments we compared impact of continuous and interrupted UV exposure (Figure 8). Contrary to the carotenoids and α-tocopherol, the interrupted irradiation 6x5 min was "friendlier" towards chlorophylls than the continuous one for 30 min.

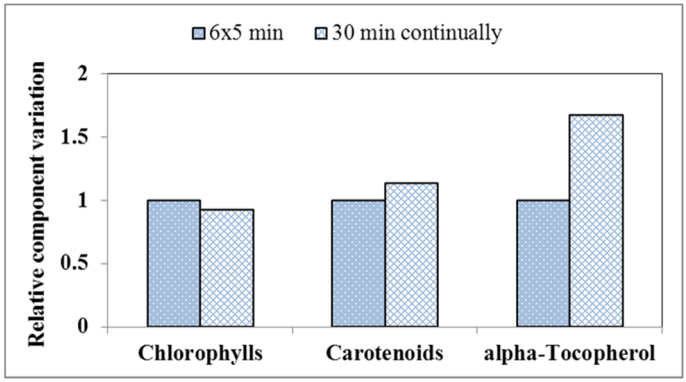

Figure 8 Effect of continuous and discontinuous UV irradiation of VOO on selected component variations

One can assume that destructive photoreactions of chlorophylls swept more deeply under the continuous irradiation because the relative loss was higher by 7% compared with the discontinuous exposure. On the other hand under the continuous irradiation, the relative carotenoids and α-tocopherol gains were by 14% and 67%, respectively, in comparison withthe discontinuous mode. The increased α-tocopherol level from the initial value of 159 mg/kg to 206 mg/kg can be explained by an effect of continuing transformation of related substances into the above mentioned forms. However, these results do not reveal UV radiation impact on other oil properties important for consumers.

CONCLUSION

MW heating of neat VOO caused significant reduction of the chlorophylls, carotenoids and α-tocopherol amounts just after 5 min heating. The impact of repeated short heating VOO was different from the heating conducted continuously for the cumulative time of the short cycles. The continuous heating resulted in greater losses of all monitored components. Therefore a short MW reheating meal involving VOO does not affect chlorophylls, carotenoids and α-tocopherol amounts.
The evaluation of relative peak intensities in range of 440-455 nm in fluorescence spectrum taken from the MW heated VOO was consistent with the component amount variations and confirmed applicability of this parameter to assess level of oil oxidation.
The 1 h boiling of the vinegar solution with VOO led to a moderate drop of the chlorophyll and carotenoid amounts and a higher loss of the α-tocopherol, while the same boiling of the salt solution with VOO had an irrelevant impact on the monitored components in VOO. This finding could be recommendation for meal cooking and means that vinegar should be added to meal just at the end of its preparation.
UV irradiation of VOO pointed out at different mechanism of changes when compared with the MW heating. Analysis revealed transformation of the components from the exposure start. Impact of the cyclic irradiation was different when compared with the continuous exposure way. Under the cyclic irradiation with six-multiple 5-min repetitions, all components showed drop of the initial content. The continuous exposure within 30 min led to significant α-tocopherol increase above the initial value but, the carotenoids went up softly and chlorophylls went down, respectively. Those results emphasize importance of VOO protection from sunlight in shelf generally.

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