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Slovak Journal of Food Sciences

Potravinarstvo Slovak Journal of Food Sciences
vol. 11, 2017, no. 1, p. 664-672
doi: https://dx.doi.org/10.5219/823
Received: 31 July 2017. Accepted: 02 November 2017.
Available online: 18 December 2017 at www.potravinarstvo.com

ISSN 1337-0960 (online)

EVALUATION OF SELECTED PHYSICOCHEMICAL PARAMETERS OF EXTRA VIRGIN OLIVE OIL COMMERCIALIZED IN THE CZECH MARKET AND STORED DURING A PERIOD OF 5 MONTHS

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ABSTRACT

The scope of this work was to evaluate the development of selected physicochemical parameters (free acidity, peroxide value and specific extinction coefficients in ultraviolet) of extra virgin olive oil, commercialized in the Czech market and stored for a time period of 5 months (at 20 ±5 °C). The tested extra virgin olive oil samples were stored under conditions simulating domestic and commercial storage environment, in which the impact of light and headspace volume were also examined. Moreover, all the analyzed samples fell within the established "extra virgin olive oil category", thus proving their legitimacy, authentication and excellent quality. Furthermore, all the monitored physicochemical parameters were affected by the progress of the storage period, the rising volume of headspace (due to more available oxygen in the container) and exposition to light, resulting in decreasing quality of the examined extra virgin olive oil samples. In addition, the storage of extra virgin olive oil samples in dark containers reported sufficient resistance to oxidation processes up to a period of 3 months, however, after this period signs of oil quality deterioration were reported. Nevertheless, if exposition to light occurred, accelerated decrease in the quality of the extra virgin olive oil samples was observed.

Keywords: extra virgin olive oil; oxidation; quality; storage period; storage conditions

INTRODUCTION

Extra virgin olive oil (EVOO) is a "natural juice" obtained only from fresh and mature fruit of the olive tree (*Olea europaea* L.) through solely mechanical or other physical operations (in particular thermal means) under conditions not leading to product alterations. Moreover, EVOO shall not undergo any treatment other than washing, decanting, centrifuging and filtering. High quality EVOO is an ingredient of great importance of the Mediterranean diet, due to its nutritional, health benefits and its organoleptic properties **(Kanavouras et al., 2005; Dabbou et al., 2011; Condelli et al., 2015; Jabeur et al., 2015; Gomez-Caravaca et al., 2016; Kotsiou and Tasioula-Margari, 2016)**. In addition, the health-promoting characteristics of EVOO concern of the ability preventing diseases (cardiovascular, cancer, and neurodegenerative) **(Ben-Hssine et al., 2013; Condelli et al., 2015)**. Furthermore, the high proportion of monounsaturated fatty acids, namely oleic acid and a balanced presence of polyunsaturated fatty acids and minor components (tocopherol, phenolic compounds – hydroxytyrosol, oleuropein) result in the protective effect of EVOO **(Kiritsakis, 1998; Dais and Hatzakis, 2013; Condelli et al., 2015, Frančáková et al., 2015)**.

The hard and time-consuming tasks involved in the cultivation of olive trees, the harvesting of the fruits and the extraction process are the main factors that cause the increase in the price of EVOO. The price of EVOO can be 6 to 7 times higher than that of other edible vegetable oils. In general, adulteration of food products involves the replacement of high cost ingredients with lower grade and cheaper substitutes. Nevertheless, for the above-mentioned reasons, adulterations of EVOO with lower quality/price olive oils and/or with oils of different botanical origin are monitored. Olive oil adulteration is one of the leading food frauds and is a serious problem for regulatory agencies, oil suppliers and could also threat health of consumers **(Tay et al., 2002; Vlachos et al., 2006; Gurdeniz and Ozen, 2009; Poulli et al., 2009)**. The International Olive Council defines four commercial graded groups of olive oils based on their free acidity (FA) value (expressed as percentage w/w of oleic acid) – extra virgin (≤0.8% w/w), virgin (≤2.0% w/w), ordinary virgin (≤3.3% w/w), and lampante (>2.0% w/w). The value of olive oil FA can be affected by factors such as variety, method of harvesting, extraction process and storage parameters **(Tsimidou et al., 2005; Hirri et al., 2016)**.

However, the quality of EVOO decreases during storage, and is attributable mainly to lipid oxidation mechanisms leading to rancidity and hydrolytic reactions which cause the partial loss of minor compounds having health-promoting benefits. Several factors can affect the oxidative processes that influence the shelf-life of olive oil. Concretely, temperature, light, oxygen availability, packaging and storage conditions are the main parameters influencing the rate of the phenomena mentioned above **(Brenes et al., 2001 Vacca et al., 2006; Kotsiou and Tasioula-Margari, 2016)**. Therefore, the packaging material applied for EVOO must adequately protect it against light, oxygen and the autooxidation process that can cause rancidity. Autooxidation is a chemical reaction occurring at ambient temperatures between atmospheric oxygen and an organic compound. Plethora of types of packaging materials (plastic films, metal containers) can be used, whereas glass containers of various shapes and colours are the most common. However, the main disadvantage of "clean" glass and transparent plastic containers is that the product might be subjected to photooxidation. Moreover, real time storage of EVOO in the marketplace or under domestic conditions may expose the product to light and/or elevated temperatures (typically 28 – 30 °C). The latter storage conditions are not optimum of preservation the high quality of EVOO, as are among the main factors affecting the rate of degradation reactions **(Gutiérrez et al., 2002; Kanavouras and Coutelieris, 2006; Cozzolino, 2015)**.

The per capita consumption of olive oil in the Czech Republic together with other Eastern European countries is very low (0.6 kg per capita per year). This phenomenon is probably related to the high price of EVOO and to the population's poor dietary habits of this region (high intake of saturated fats), which is also linked with increased cardiovascular diseases and other chronic disorders **(Ness and Powles, 1997; Boylan et al., 2009)**. However, these facts indicate that olive oil is not so significant commodity in the Czech Republic and some neighboring countries and thus, rising advertence should be provided to the legality standards of EVOO sold at these regions. To our knowledge, in the available scientific literature there has not been found yet a study focused on the physicochemical quality indices of EVOO sold exclusively in the Czech Republic or other countries with minimum olive oil consumption.

The present study was undertaken with the primary objective to evaluate the legitimacy and selected physicochemical quality parameters (free acidity, peroxide value and specific extinction coefficients in ultraviolet) of EVOO samples commercialized in the Czech market and stored in dark glass containers during a 5 month storage period (at 20 ±5 °C). The storage parameters were simulating mainly domestic and commercial conditions. Moreover, a supplementary aim of the current study was to investigate the phenomenon mentioned above on an EVOO sample which was exposed to light.

Scientific hypothesis

The storage conditions (storage period, light exposition) of EVOO can influence its physicochemical quality characteristics.

MATERIAL AND METHODOLOGY

Samples and storage conditions

EVOO monovarietal samples of five different commercial brands were considered in this work in total. Four of the evaluated samples originated from two distinctive producing countries, including Spain (samples A and E) and Greece (samples C and D). In addition, one sample was a mixture of EVOOs originating from areas of the European Union and outside the European Union (sample B; Spain and Morocco). All the samples were manufactured from olives of one crop harvesting season (2014/2015). The examined samples were purchased from Czech local retail. Furthermore, the samples were transferred in dark glass containers and were stored in a room without light exposition for a period of 5 months at 20 ±5 °C. Moreover, one of the samples (sample B) was transferred also in a transparent glass container and was stored on a laboratory shelf, where was exposed intermittently 12 h to artificial light for a period of 5 months at 20 ±5 °C. All glass containers (Sklárny Moravia, Úsobrno, Czech Republic) were of 500 mL in capacity and after the filling contained 450 mL of oil. Finally, the containers were tightly sealed with aluminum screw-type caps of negligible permeability to oxygen.

Solvents and reagents

For the determination of free acidity (free fatty acids), ethanol (≥99.9%) and diethyl ether (≥99.7%), potassium iodine (>99.0%), sodium thiosulphate and sodium hydroxide (>99%) were purchased from Sigma-Aldrich (Schnelldorf, Germany). For the determination of peroxide value, chloroform (>99.1%) and acetic acid (100.0%) were also purchased (Sigma-Aldrich, Schnelldorf, Germany). For the determination of absorption indices isooctane was purchased from Sigma-Aldrich (Schnelldorf, Germany).

Determination of moisture content

The mass assessment by heating in a drying oven (103 ±2 °C) standardized method was performed in order to determine the moisture content of the samples (EC No 1989/2003). In brief, in a capsule, previously dried at 103 ±2 °C and cooled, 20.00 ±0.05 g of the examined were weighted. Thereafter, the samples were transferred into a drying oven at 103 ±2 °C for a period of 24.0 ±0.5 h, after which the samples were removed and weighted again. The operation was repeated until constant weight was obtained. Finally, the moisture content was calculated as the difference in weights. The analysis was performed in triplicate for each sample on each day of analysis.

Determination of free acidity

The free acidity (FA) is indicative of the free fatty acid content of the examined oil and is expressed as percentage of oleic acid, since it is the major fatty acid found in olive oils (corresponding to 55 – 83% of the total fatty acid content) **(Rodrigues et al., 2016)**. The current determination was performed by titration with the aqueous solution of sodium hydroxide (0.1 $mol.L^{-1}$) of an oil solution in a previously neutralized solvent (ethanol/ethylether, 1 : 1 v/v) and using phenolphthalein as indicator. The applied method was according the standard analytical methods described by the Regulation 1989/2003

of the Commission of the European Union (EC No 1989/2003). For each sample at each day of analysis the determination was performed in triplicate.

Determination of peroxide value

The peroxide value (PV) is a measurement of the amount of hydroperoxides formed through oxidation during storage. Peroxide value is expressed as milliequivalents of active oxygen per kilogram of oil. Peroxide value was determined as follows: olive oil (2.5 g) was dissolved in a mixture of chloroform/acetic acid (2 : 3, v/v) and was left to react with a solution of potassium iodide in the darkness; the free iodine was then titrated with a sodium thiosulfate solution. The analysis was assessed following the analytical methods described by the Regulation EC/1989/2003 of the Commission of the European Union (EC No 1989/2003). For each sample the analysis was performed in triplicate.

Analysis of spectroscopic indices in ultraviolet

The ultraviolet characteristics, i.e. extinction coefficients determined from absorption at 232 nm (K232), at 270 nm (K270) and ΔK value [difference between absorbance at 270 nm and (266 nm +274 nm)/2], by an UVmini-1240 UV-VIS spectrophotometer (Shimadzu, Kyoto, Japan), using a 1.0% (v/v) solution of oil in isooctane and a path length cuvette of 1 cm. The ΔK value was calculated according to Eq. 1 as follows:

$$\Delta K = A - \frac{B+C}{2} \quad (1)$$

where; A, B and C are the absorbencies at wavelengths of 270 nm, 266 nm and 274 nm, respectively.

The determinations describing the samples characteristics in ultraviolet were performed according to the European Union Commission Regulations 1989/2003 (EC No 1989/2003). For each sample the determination of spectroscopic characteristics in ultraviolet was performed in triplicate.

Statistic analysis

The obtained data were subjected to analysis of variance using the Minitab 17 statistical software program (Minitab, Coventry, United Kingdom). Where statistical differences were noted, differences among data were determined, using the Tukey's test. Significance was defined at $p < 0.05$.

RESULTS AND DISCUSSION

Determination of moisture content

The moisture content of the analyzed EVOO samples ranged from 5.0 x 10^{-2} to 4.5 x 10^{-1} g·kg^{-1}, a range similar to that was reported in the study of **Ragni et al. (2016)**. The moisture content of the samples was in accordance to the established regulations and standards by the European Union (EC No 1989/2003). The quality of EVOO is known to be closely related to its composition and the moisture content is considered one of the basic criteria of evaluating its quality. Moreover, the amount of water content which is indirectly correlated with some organoleptic characteristics, such as pungency and bitterness, can also affect the stability and preservation of EVOO during storage **(Fregapane et al., 2006; Hatzakis and Dais, 2008)**. Moreover, the amount of water in commercial EVOO samples is highly affected by the applied technological processes, such as extraction and filtration operations. However, the high polar phase (water) may augment the alteration of EVOO during storage, by increasing the hydrolytic rate of the present triacylglycerols. However, the latter process increases FA, exposing EVOO to oxidation in the presence of oxygen, light or high temperature. This could be explained by the hydrolysis of the ester linkage of triacylglycerols, resulting in the production of free fatty acids. Hence, as these are less resistant to autooxidation, their presence could lead to the development of rancidity, off-flavours and decrease of the smoke point **(Ragni et al., 2012; Yun and Surh, 2012)**.

Determination of free acidity

The analysis of FA reports the level of hydrolysis in the examined oil. In the same token, could provide information correlated with how the olives were manipulated prior processing and the length of time from harvest to milling **(Borges et al., 2017)**. Furthermore, EVOO is composed by approximately 98% of neutral lipids, mainly triglycerides (96 – 97%), followed by a small quantity of diglycerides (1 – 2%) and a variable amount of free fatty acids which are used as a marker in quality evaluation **(Jabeur et al., 2015)**. The results of FA values development during storage in dark glass bottles are depicted in Figure 1 (part A). From the obtained results it can be assessed that the examined samples fell within the ranges established for EVOO category, as required by Regulation EC/1989/2003 (EC No 1989/2003). Concretely, the FA values at the initial stage of the experiment (month 0) were below 0.8% (0.2 – 0.4%), indicating that all the examined samples presented efficient resistance to oxidation, since FA could be used as a quality parameter contributing also to oil oxidative stability **(Dabbou et al., 2011)**. Additionally, it should be mentioned that the lower the values of FA, the higher of the oil obtained from fresh and healthy olives, harvested at the optimal maturity degree, followed by immediate extraction without proceeding to olive storage. In general, it is accepted that olives of higher maturity level result in products with elevated levels of FA, since they can undergo an increase in enzymatic activity (especially lipolytic enzymes) and are more sensitive to pathogenic infections and mechanical damage **(Manai-Djebali et al., 2012)**. Moreover, the results of FA values development during storage in transparent and dark glass containers are shown in Figure 2 (part A). From the results it could be depicted that the sample stored in dark container showed a minimum increase ($p > 0.05$) in FA during storage. Hence, the protective effect on light of the dark container was obvious during the storage of EVOO. Moreover, similar findings have been previously reported in the studies of **Dabbou et al. (2011)** and **Pristouri et al. (2010)**. On the other hand, the values of FA significantly rose ($p < 0.05$) in the samples stored in transparent glass containers and thus exposed to light. Concretely, after 3 months of storage the oil sample exceeded the established limit for EVOO category. A possible explanation of this phenomenon

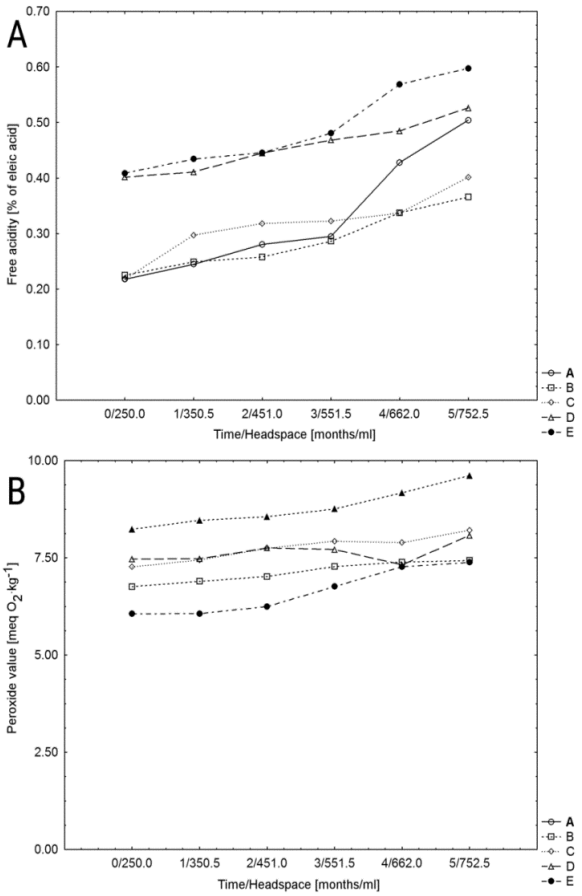

Figure 1 Parameters development in EVOO samples. Development of free acidity (Part A). Development of peroxide value (Part B). The samples (A, B, C, D, E) were stored in dark glass containers during a storage period of 5 months (at 20 ± 5 °C). The results are expressed as means (n = 3); standard deviations were in range of 0.01 – 0.03 and are not displayed.

could be the impact of light on triacylglicerides hydrolysis leading to free acid release, which in turn can cause rancidity. Moreover, another explanation could be the photosensitized oxidation mechanism, occurring via the action of light, present natural photosensitizers (such as chlorophyll) and atmospheric oxygen. Photosensitizers can react with atmospheric triplet oxygen (3O2) producing the excited state singlet oxygen (1O2). Therefore, 1O2 can form a free radical from unsaturated fatty acids, resulting in the development of lipid hydroperoxides (first lipid oxidation products). Nevertheless, the latter in EVOO can decompose immediately into aldehydes, ketones, alcohols and short-chain hydrocarbons, which are secondary lipid oxidation products and are responsible for the production of off-flavours **(Pristouri et al., 2010; Dabbou et al., 2011; Kim and Choe, 2013)**.

Determination of peroxide value

The PV could be characterized as quality parameter of mandatory importance, reflecting the onset of the oxidation process. The PV analysis determines mainly the content of hydroperoxides, which are unstable and can decompose producing aldehydes and ketones **(Borges et al., 2017, Šulcerová et al., 2017)**. The results of PV development during storage in dark containers are presented in Figure 1 (part B). The measured PV in all samples exhibited initial values within the range of EVOO category (≤20 meq·kg^{-1}), regardless of the storage time, signalizing EVOO of excellent quality. Moreover, another interesting observation was that the PV slightly rose ($p < 0.05$) during the 5-month storage, with a maximum at the last month of storage. Hence, this certain trend could be explained by the primary oxidation occurring in the presence of oxygen in the container headspace leading to the production of hydroperoxides. Additionally, the oxygen content in oil is dependent on the oxygen partial pressure in the headspace. Hence, higher oxygen partial pressure in the headspace results in higher levels of dissolved oxygen in the oil, which in turn enhances the lipid oxidation process **(Pristouri et al., 2010; Kim and Choe, 2013; Rodrigues et al., 2016)**. Furthermore, similar results were reported in the work of **Kotsiou and Tasioula-Margari (2016)** and **Vacca et al. (2006)**. The above-mentioned phenomenon was dramatically more intensive (p <0.05) in the sample which was exposed to light during storage (Figure 2; part B). From the results it could be depicted that the examined sample exceeded the permitted limit after 3 months of storage. However, after the completion of this period the combined effect of light and oxygen availability resulted in rapid EVOO oxidation acceleration during the remainder storage time. According to scientific literal sources photosensitized oxidation does not occur in olive oil stored in the dark at relatively low temperatures (13 – 20 °C). Thus, due to the present chlorophyll this can act as natural antioxidant along with

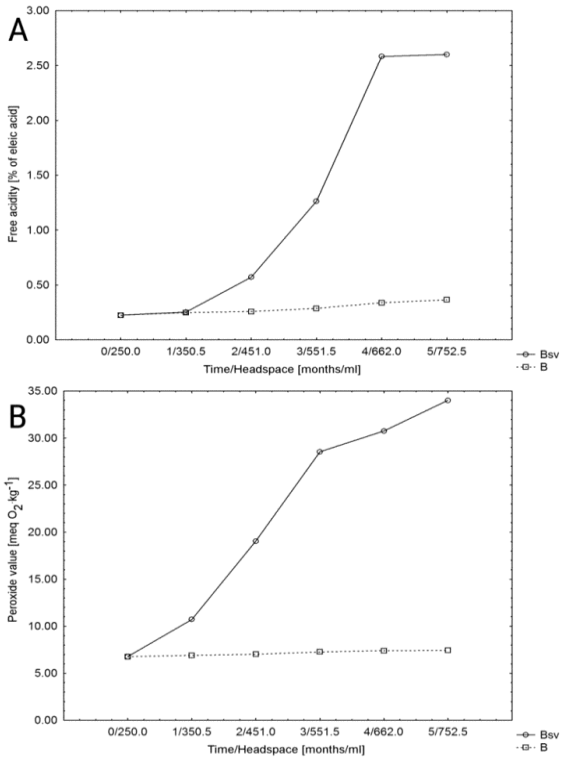

Figure 2 Parameters development in EVOO samples. Development of free acidity (Part A). Development of peroxide value (Part B). The samples were stored in dark glass (B) and transparent glass (Bsv) containers during a storage period of 5 months (at 20 ±5 °C). The results are expressed as means (n = 3); standard deviations were in range of 0.01 – 0.04 and are not displayed.

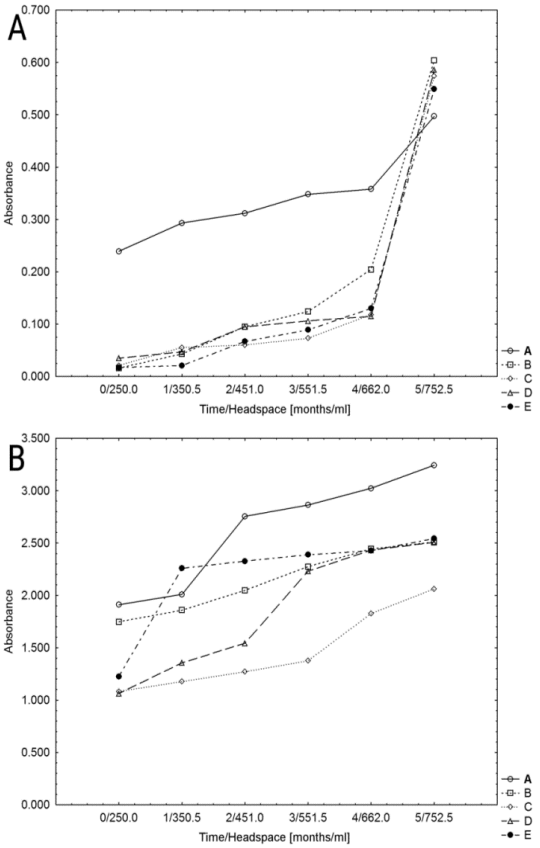

Figure 3 Absorption coefficients development of the EVOO samples. Development of K_{232} values (Part A). Development of K_{270} values (Part B). The samples (A, B, C, D, E) were stored in dark glass containers during a storage period of 5 months (at 20 ±5 °C). The results are expressed as means (n = 3); standard deviations were in range of 0.02 – 0.05 and are not displayed.

polyphenols protecting the EVOO from oxidation. In general, higher PV is an indicator of low quality EVOO with weak oxidative stability **(Pristouri et al., 2010; Pizarro et al., 2013)**.

Analysis of spectroscopic indices in ultraviolet

The absorption coefficients in ultraviolet are used as indicators of olive oil oxidation, allowing the measurement of certain oxidized compounds resonating at wavelengths of 232 nm and 270 nm. Moreover, the K232 index corresponds to the absorbancy of conjugated dienes and their oxidation products. In addition, the K270 index corresponds to the absorbancy of the conjugated trienes and secondary products of oxidation (carbonyl compounds) **(Manai-Djebali et al., 2012; Bachari-Saleh et al., 2013)**. The ΔK value can provide information about detection of oil treatments with colour removing substances and the presence of refined or pomace oil. The results of spectroscopic characteristics in ultraviolet for the examined EVOO samples stored in dark containers (at 20 ±5 °C) as a function of storage period are depicted in Fig. 3 (parts A and B). From the results it could be reported that all samples presented values of K232, K270, and ΔK below the established critical limits (K232 ≤2.50, K270 ≤0.22, ΔK ≤0.01). Hence, all the samples fell within the established for EVOO category. Moreover, the obtained results indicate that the analyzed samples were of superior quality, obtained from fresh and health raw material, harvested at the ideal ripening level, followed by immediate extraction without proceeding to olive storage. However, if high initial values of K232 and K270 were detected this could indicate the presence of conjugated dienes and trienes, which are formed in oils that have been heat-treated during the refining, process **(Bachari-Saleh et al., 2013)**. According to **Ben-Hassine et al. (2013)** fresh EVOO are characterized by low values of K232 and K270 in contrast to samples of extended storage. The K232 value development rose as the storage period progressed. Therefore, the K232 of most of the samples did not exceed the limit of 2.5 during the 5 month storage period. However, exception was sample E, whose measured values exceeded the established limit after 2 months of storage. The development of K270 value rose (p <0.05) with the increase of the storage period. All the samples at the initial stage (month 0) of the experiment reported values below 0.22 (which is the permitted limit); however, sample A from the beginning was slightly above the permitted limit. Moreover, the monitored values (with exception sample A) were below the limit up to the 4th month of storage, after this period the values rose significantly (p <0.05). The increase in the values of K232 and K270 during the evolution of storage was due to the formation of conjugated dienes and trienes, respectively. Their formation is proportional to the oxygen uptake and formation of hydroperoxides in the early stages of oxidation **(Kim and Choe, 2013)**. Moreover, this phenomenon was more intensive when the examined sample was exposed to light (Figure 4; parts A and B).

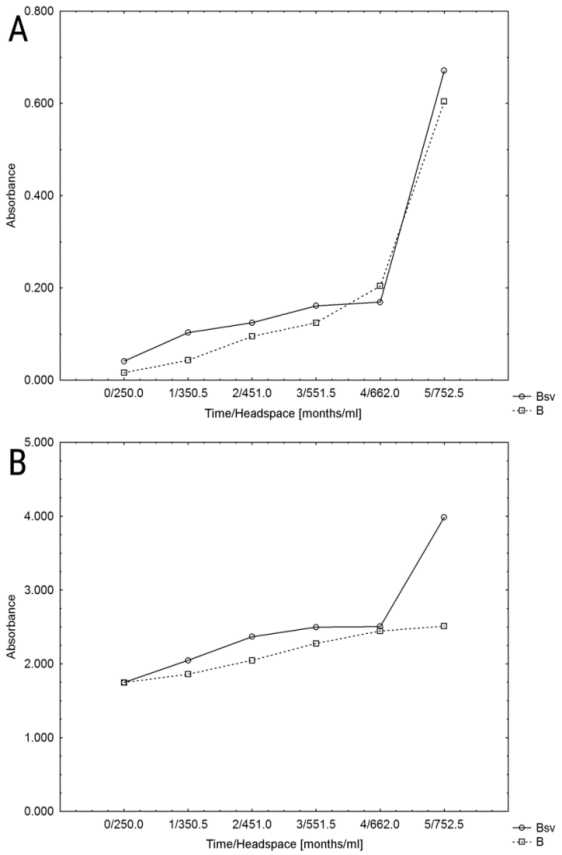

Figure 4 Absorption coefficients development in the extra virgin olive oil samples.Development of K_{232} values (Part A). Development of K_{270} values (Part B). The samples were stored in dark glass (B) and transparent glass (Bsv) containers during a storage period of 5 months (at 20 ±5 °C). The results are expressed as means (n = 3); standard deviations were in range of 0.01 – 0.03 and are not displayed.

Specifically, after 4 months of storage the EVOO sample exceeded the permitted limit. Similar results were found in the work of **Pristouri et al. (2010)** and **Stefanoudaki et al. (2010)**. The results of ΔK values ranged from 0.0005 to 0.0020, reporting that none of the analyzed samples were adulterated with refined or pomace oil and none of them were processed with colour removing substances.

CONCLUSION

The evolution of EVOO selected quality factors which simulated real-time household conditions was examined. The examined parameters of EVOO samples stored during 5 months at dark or transparent containers were affected by the storage time, lighting conditions and oxygen availability (headspace volume). Furthermore, all the samples merchandised in the Czech market fell within the established legal limits of EVOO category, thus proving not only their legitimacy and authentication whereas also their high quality. During the storage all the samples showed an increase in FA, PV and specific extinction coefficients at 232 nm (K232) and 270 nm (K270), respectively. Moreover, it could be concluded that the quality of the samples decreased with the increasing storage period, the rising volume of headspace (due to more available oxygen in the container) and exposition to light. Furthermore, the quality factors of the sample which was exposed to light decreased dramatically after 4 months of storage, depicting the significance of the packaging material on EVOO quality. The results indicated that transparent glass containers were unsuitable for long-term storage (≥4 months) of EVOO. In general, the EVOO samples when were stored in dark containers presented sufficient resistance to oxidation, reporting minimal quality changes. Finally, it could be assessed that even if EVOO stored under inadequate conditions presented minimal quality changes up to a period of 3 months; however, after this period signs of deterioration in olive quality were observed.

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Acknowledgments:

This study was kindly supported by a project of the internal grant agency of Tomas Bata University in Zlin, Czech Republic (IGA/FT/2017/004) funded from the resources of specific university research.

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