

## Stability of olive leaf drink upon storage at different temperatures

### Abstract

In the olive oil production, olive leaves mostly often remain insufficiently used and represent waste, although many studies showed its anticancer, antiinflammatory and antimicrobial properties due to abundance of polyphenols, which makes olive leaves potential ingredient for functional food production. Also, olive leaf drink due to its valuable composition represents functional drink itself. But, functional properties can reduce due to polyphenols degradation, especially during storage. Hence, this research aimed to investigate the stability of olive leaf drink during nine weeks storage at different temperatures (8, 22 and 32 °C). Influence of storage time and storage temperature was monitored by analysis of total phenols, antioxidant capacity by DPPH, color parameters ( $L^*$ ,  $a^*$ ,  $b^*$ ,  $\Delta E_{ab}^*$ , BI) and sensory properties of olive leaf drink samples. Obtained results showed that total phenols slightly decreased during storage, being most stable on 8 °C. Antioxidant capacity showed approximately similar trend. Color of all samples darkened by the storage with increase rate of  $L^*$ ,  $\Delta E_{ab}^*$  and BI related to temperature increase. Major color changes were observed by the storage ending at 32 °C. Sensory analysis confirmed change of olive leaf drink color, where yellowness decreased as brownness increased by the storage time and temperature. Odor and flavor decreased with time and at higher storage temperatures. Intensity of drink's main taste attributes (astringent and bitter taste) decreased by storage time, especially on higher temperatures, resulting with higher scored harmony taste. Obtained results showed good stability of olive leaf drink, suggesting its potential as a functional product or semi-product.

**Keywords:** olive leaf, storage, phenols, color, sensory

### Introduction

The olive tree (*Olea europaea* L.) is a traditional plant widely grown in Mediterranean area, where it provides major economic and nutrition benefits to the population (Japón-Luján et al., 2006). The largest olive tree utilization refers to the processing its fruit into olive oil, anciently called „liquid gold“ and „the great healer“ (Clodoveo et al., 2014) as it possesses many nutritionally prosperous features (Galanakis et al., 2010).

In the production of olive oil other parts of plant, e.g., olive leaves (OL), are an agricultural by-product, often insufficiently used and remaining as a waste. Although it has been traditionally used as a folk remedy (Altiok et al., 2008) and commonly consumed as a „tea“ (El and Karakaya, 2009), health benefits from the OL itself have only recently been investigated (Brahmi et al., 2012).

El and Karakaya (2009) and Rahmanian et al. (2015) described that various studies reported antioxidant, antimicrobial, hypoglycemic, antihypertensive, antiatherosclerotic, antiproliferative and anticancerous properties of OL, which are attributed to the presence of high quantities of phenolic compounds (Erbay and Icier, 2010) such as oleuropeosides, flavones, flavonols, flavan-3-ols and substituted phenols, with oleuropein being the most abundant compound (El and Karakaya, 2009). Phenolic compounds are known as strong antioxidants, which scaven-

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ging power of free radicals can delay or prevent oxidation of cellular oxidative substrates (Pourmorad et al., 2006) and are considered very important additives in food processing (Rahmanian et al., 2015). Due to its functional properties, OL extracts have a great potential as a functional food ingredient and could employ for the fortification and increased stability of various food products, e.g., enrichment of edible oils (Erbay and Icier, 2010) and beverages (Kranz et al., 2010) or animal feed (Molina-Alcaide and Yáñez-Ruiz, 2008). Also, OL "tea" could represent a functional drink by itself, as herbal decoctions and infusions are natural sources of bioactive compounds, which promote well-being benefits.

Applied storage conditions, such as storage time and temperature, have a great impact on the stability of initial plant antioxidants (Wahid et al., 2007) and are attributed to the development of undesirable chemical reactions, which can cause severe degradation of functional properties and color of plant extracts (Jiménez-Zamora et al., 2016). Such physical and chemical changes can reflect on sensory properties of plant extracts, consequently affecting consumer acceptability.

Therefore, the aim of this research was to investigate the influence of storage time and different storage temperatures on the total phenols, antioxidant capacity, color and sensory properties of olive leaf drink (OLD).

## Materials and methods

### Sample preparation

For the preparation of OLD, OL were collected in olive orchard located in Ravni Kotari (Croatia) as a mixture of Oblica, Leccino, Karbunčela and Marokanka cultivars. After harvesting, OL were transported to the laboratory and air dried at room temperature. Prior drink preparation, dry OL were pre-washed, dried and chopped into smaller pieces.

OLD was prepared by boiling 4 g of OL in 100 mL of distilled water for 3 minutes and then soaking at room temperature for additional 10 minutes. The OLD was decanted and then pasteurized at 85 °C for 10 minutes. Prepared OLD was transferred to prior washed and sterilized glass jars (200 mL), hermetically sealed and stored in thermostated containers at three temperatures (8, 22 and 32 °C) for 9 weeks. Analysis were conducted in freshly prepared OLD (day 0) and on the 1<sup>st</sup>, 21<sup>st</sup>, 42<sup>nd</sup> and 63<sup>rd</sup> day of storage.

### Determination of total phenols and antioxidant capacity

Total phenols (TP) in OLD were determined according to the method described by Shortle et al. (2014), with some modifications, i.e., 100 µL of sample, 200 µL of Folin Ciocalteu's reagent, 2 mL of distilled water and 1 mL of sodium carbonate solution (1:4, w/v) were mixed thoroughly and incubated at 50 °C for 25 minutes. The absorbance was measured against the blank at 765 nm. Calibration curve was obtained using different concentrations of gallic acid (5 mg mL<sup>-1</sup>) and TP were expressed as mg of gallic acid (GAE) equivalents per L of OLD.

The determination of antioxidant capacity (AC) was carried out using DPPH free radical scavenging method (Prior et al., 2005; Ansari et al., 2013), as follows: the mixture of 0.75 mL of sample and 1.5 mL of 0.2 mM 2,2-Diphenyl-1-picrylhydrazyl (DPPH) solution in methanol incubated for 20 minutes in dark at room temperature before the absorbance was measured at 517 nm with methanol set as blank. Different concentrations of 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) (1mM) were used for calibration curve and AC was expressed as µmol of Trolox equivalents (TE) per L of OLD.

All spectrophotometric measurements were performed by UV-VIS spectrophotometer UV-1600PC (VWR International, Radnor, USA) in three replicates (n=3) and data are presented as the mean value±SD.

### Color measurement

Color analysis of OLD was conducted by applied CIELab color space (CIE, 1976) and the transmittance color measurements were performed by Konica Minolta CM-5 spectrophotometer (Konica Minolta Sensing, Inc., Osaka, Japan). Before the measurement, instrument was calibrated with a standard black and white in accordance with the manufacturer's instructions. Values of  $L^*$  (lightness),  $a^*$  (redness/greenness) and  $b^*$  (yellowness/blueness) were recorded in triplicate ( $n=3$ ) for D65 illuminant with  $2^\circ$  standard angle, and the mean value was calculated. For monitoring the change of color during storage, total color difference ( $\Delta E_{ab}^*$ ) and browning index ( $BI$ ) were calculated using following equations:

$$\Delta E_{ab}^* = \sqrt{\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}} \quad (1)$$

$$BI = \frac{100 \cdot (x - 0.31)}{0.17} \quad (2)$$

$$x = \frac{(a^* + 1.75 \cdot L^*)}{(5.645 \cdot L^* + a^* - 3.012 b^*)} \quad (3)$$

where all  $\Delta L^{*2}$ ,  $\Delta a^{*2}$ ,  $\Delta b^{*2}$  were calculated in reference to the storage time = 0 day.

### Sensory analysis

Sensory properties of OLD were evaluated using Quantitative Descriptive Analysis (QDA). For that purpose, OLD samples were served in a sensory laboratory equipped according to ISO 8589 at room temperature in randomly ordered coded transparent plastic cups. A group of ten trained panelists ( $n=10$ ) evaluated twelve OLD sensory properties with 10-point interval scale (1 = none; 10 = extremely strong), including color intensity (yellow (YCI), brown (BCI)), odor (olive leaf odor (OLO), off-odor (OO)), taste (astringent (AT), bitter (BT), olive leaf taste (OLT), off-taste (OT), harmony taste (HT)) and flavor (olive leaf flavor (OLF), green flavor (GF), off-flavor (OF)) (Stone et al., 2012). Sensory scores are shown as the mean value.

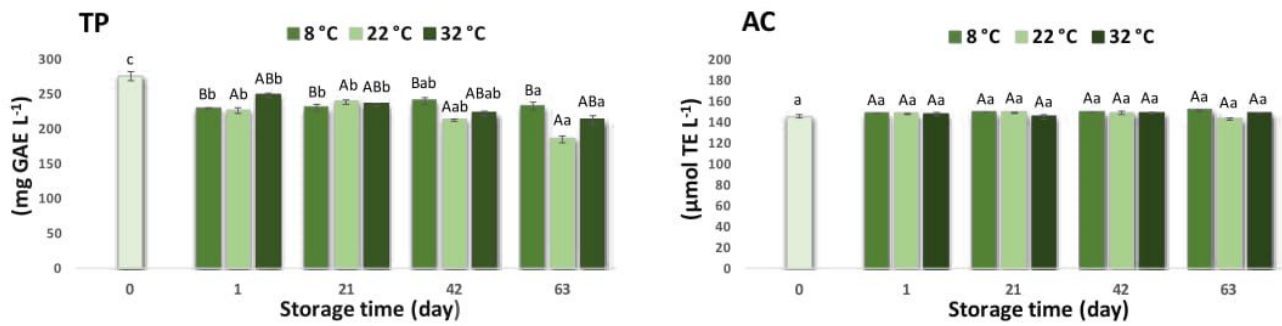
### Statistical analysis

Two-way analysis of variance (ANOVA) was used to evaluate the effects of storage time and storage temperature on TP, AC, color parameters and sensory properties of OLD, and marginal means were compared by Tukey's HSD test ( $\alpha \leq 0.05$ ). Correlation analysis was performed to examine the relationships among determined parameters. All tests were carried out using a statistical software Statistica ver. 8.0 (Statsoft Inc., Tulsa, USA).

## Results and discussion

Decoctions are generally consumed as fresh and have 72-hour maximum storage if stored in a very cool conditions (Green, 2011), but their functional properties and color can diminish during storage and have a severe impact on sensory properties, resulting with subsided consumer acceptability. Therefore, we evaluated the effect of storage conditions, such as time and temperature, over the TP, AC, color and sensory properties of OLD.

Obtained results for TP are presented in Graph 1. Statistical analysis showed significant influence ( $p \leq 0.05$ ) of storage temperature and storage time on OLD TP. The highest TP amounts were determined in fresh OLD ( $275.71 \text{ mg L}^{-1}$ ). Compared to our results, Jiménez-Zamora et al. (2016) reported almost threefold lower TP in OL infusion and Büyükbacı and El (2008) determined almost fourfold lower TP amounts in OL decoction, what is explainable by the differences in sample preparation, as the amount of compounds transferred to the beverage may be affected by the preparation procedure (Komes et al., 2010).



**Graph 1** Content of total phenols (TP) (mg GAE L<sup>-1</sup>) and antioxidant capacity (AC) (μmol TE L<sup>-1</sup>) of olive leaf drink during storage at different temperatures

**Grafikon 1.** Sadržaj ukupnih fenola (TP) (mg GAE L<sup>-1</sup>) i antioksidacijski kapacitet (AC) (μmol TE L<sup>-1</sup>) napitka od maslinovog lista tijekom skladištenja na različitim temperaturama

*Different uppercase letters indicate significant difference in storage temperature and different lowercase letters indicate significant difference in storage time between samples ( $p \leq 0.05$ ). Results are expressed as mean  $\pm$  SD.*

As for OLD storage, it can be observed that TP generally decreased by the storage time at all temperatures, remaining most stable at 8 °C, with 16 % of decrease at the 63rd day in regards to fresh OLD. Observed TP decrease over time could be due to the oxidation of antioxidant compounds (Guimarães et al., 2011), while low decrease is expected at lower temperatures, since the cool storage slows chemical reactions rate. Considering the variation of the applied conditions, TP decrease at 22 °C and 32 °C (33 %, 23 %) are in accordance with those in study of Jiménez-Zamora et al. (2016), where TP in OL infusion decreased for 39 % during three months storage at room temperature, while at 50 °C the decrease was 29 %.

**Table 1** Color parameters of olive leaf drink during storage at different temperatures

**Tablica 1.** Parametri boje napitka od maslinovog lista tijekom skladištenja na različitim temperaturama

Temperature (°C)	Time (day)	$L^*$	$a^*$	$b^*$	$\Delta E_{ab}^*$	$BI$
8	0	91.14 $\pm$ 0.49 <sup>bc</sup>	-1.98 $\pm$ 0.12 <sup>a</sup>	19.18 $\pm$ 0.14 <sup>a</sup>	0.00 $\pm$ 0.00 <sup>a</sup>	21.41 $\pm$ 0.09 <sup>a</sup>
	1	94.22 $\pm$ 0.03 <sup>cd</sup>	-1.99 $\pm$ 0.01 <sup>Ab</sup>	18.51 $\pm$ 0.07 <sup>Aa</sup>	3.16 $\pm$ 0.04 <sup>Ab</sup>	19.74 $\pm$ 0.10 <sup>Aa</sup>
	21	93.09 $\pm$ 0.05 <sup>Cc</sup>	-0.88 $\pm$ 0.01 <sup>Ac</sup>	20.43 $\pm$ 0.05 <sup>Ab</sup>	2.57 $\pm$ 0.02 <sup>Ac</sup>	23.47 $\pm$ 0.07 <sup>Ab</sup>
	42	91.77 $\pm$ 0.02 <sup>Cb</sup>	-0.39 $\pm$ 0.02 <sup>Ad</sup>	22.16 $\pm$ 0.01 <sup>Ac</sup>	3.43 $\pm$ 0.01 <sup>Accd</sup>	26.65 $\pm$ 0.01 <sup>Ac</sup>
	63	90.97 $\pm$ 0.04 <sup>Ca</sup>	-0.02 $\pm$ 0.02 <sup>Ae</sup>	23.78 $\pm$ 0.01 <sup>Ad</sup>	5.00 $\pm$ 0.02 <sup>Ae</sup>	29.54 $\pm$ 0.04 <sup>Ad</sup>
22	1	92.85 $\pm$ 0.06 <sup>Bd</sup>	-1.24 $\pm$ 0.03 <sup>Bb</sup>	20.43 $\pm$ 0.08 <sup>Ba</sup>	2.25 $\pm$ 0.03 <sup>Bb</sup>	23.24 $\pm$ 0.14 <sup>Ba</sup>
	21	91.93 $\pm$ 0.11 <sup>Bc</sup>	-0.07 $\pm$ 0.01 <sup>Bc</sup>	23.28 $\pm$ 0.19 <sup>Bb</sup>	4.59 $\pm$ 0.15 <sup>Bc</sup>	28.44 $\pm$ 0.30 <sup>Bb</sup>
	42	90.52 $\pm$ 0.10 <sup>Bb</sup>	0.34 $\pm$ 0.02 <sup>Bd</sup>	25.73 $\pm$ 0.21 <sup>Bc</sup>	6.97 $\pm$ 0.21 <sup>Bcd</sup>	32.89 $\pm$ 0.37 <sup>Bc</sup>
	63	88.90 $\pm$ 0.06 <sup>Ba</sup>	0.89 $\pm$ 0.01 <sup>Be</sup>	28.64 $\pm$ 0.09 <sup>Bd</sup>	10.13 $\pm$ 0.08 <sup>Be</sup>	38.65 $\pm$ 0.14 <sup>Bd</sup>
32	1	92.71 $\pm$ 0.04 <sup>Ad</sup>	-1.07 $\pm$ 0.01 <sup>Cb</sup>	20.48 $\pm$ 0.08 <sup>Ca</sup>	2.23 $\pm$ 0.02 <sup>Cb</sup>	23.48 $\pm$ 0.12 <sup>Ca</sup>
	21	90.58 $\pm$ 0.02 <sup>Ac</sup>	0.56 $\pm$ 0.01 <sup>Cc</sup>	27.16 $\pm$ 0.07 <sup>Cb</sup>	8.39 $\pm$ 0.07 <sup>Cc</sup>	35.22 $\pm$ 0.12 <sup>Cb</sup>
	42	88.79 $\pm$ 0.21 <sup>Ab</sup>	1.01 $\pm$ 0.09 <sup>Cd</sup>	29.47 $\pm$ 0.54 <sup>Cc</sup>	10.97 $\pm$ 0.57 <sup>Ccd</sup>	40.15 $\pm$ 1.06 <sup>Cc</sup>
	63	87.49 $\pm$ 0.08 <sup>Aa</sup>	1.41 $\pm$ 0.03 <sup>Ce</sup>	31.54 $\pm$ 0.19 <sup>Cd</sup>	13.33 $\pm$ 0.20 <sup>Ce</sup>	44.72 $\pm$ 0.39 <sup>Cd</sup>

*Different uppercase letters indicate significant difference in storage temperature and different lowercase letters indicate significant difference in storage time between samples ( $p \leq 0.05$ ). Results are expressed as mean  $\pm$  SD.*

AC of the samples didn't significantly differed by the investigated sources of variation (Graph 1) ranging from 143.58 – 151.64  $\mu\text{mol L}^{-1}$ , what is lower compared to literature data (Jiménez-Zamora et al., 2016). AC of the starting OLD sample (day 0) was 145.83  $\mu\text{mol L}^{-1}$ , while values of other samples slightly varied during storage period without major alterations. We believe that AC results obtained with selected method were preliminary insight for AC changes in stored OLD and that other more specific methods, e.g., ORAC, should be performed for better comprehension of OLD AC variations influenced by storage. Generally, it can be concluded that OLD demonstrated good TP and AC stability during storage and therefore the retention of its functional properties.

Color is major quality criterion for consumers acceptance of specific product, so color variations during storage should be monitored. Applied storage conditions significantly affected ( $p \leq 0.05$ ) OLD color parameters (Table 1). Fresh OLD was instrumentally defined as luminous and yellow/greenness colored ( $L^* = 91.14$ ,  $a^* = -1.98$ ,  $b^* = 19.18$ ). At the 1st day of storage, lightness ( $L^*$  values) slightly increased at all temperatures, probably due to the sedimentation during first 24 hours, where slight precipitate was noticed. Later, in the following storage period it continued to decrease, indicating darkening. The lowest  $L^*$  value amounted on the 63rd day at the 32 °C ( $L^* = 87.49$ ). It can be noticed that with temperature increase OLD started to darken faster, where  $L^*$  values below starting point (day 0) occurred on the 21st day at 32 °C and on the 42nd day at 22 °C, while at 8 °C color darkening was determined at the end of storage. Similar trend was observed among OLD  $a^*$  parameters, where transit from green (negative  $a^*$  values) to red (positive  $a^*$  values) area at 22 °C and 32 °C was recorded in the same storage period as for  $L^*$  values, confirmed by good correlations ( $-0.74$ ,  $p \leq 0.05$ ) (Table 2). Despite its increase, only  $a^*$  parameter of 8 °C samples remained in green area until the end of storage. Values of parameter  $b^*$  continuously increased during storage with higher increase at higher temperature, pointing to a higher yellow pigmentation with temperature increase. Development of yellowness highly correlated with storage darkening ( $-0.85$ ,  $p \leq 0.05$ ). Furthermore,  $\Delta E_{ab}^*$  was calculated to evaluate the total color difference between fresh OLD and stored ones and their browning was estimated by  $BI$ . It can be noticed that the last day of storage was marked with the maximum color difference followed by the highest  $BI$ , likewise.

Also, lowest  $\Delta E_{ab}^*$  and  $BI$  values were observed at 8 °C, but with the increase of storage temperature color changes were more prominent. All color parameters, except  $L^*$ , showed good correlation with TP (Table 2), indicating that determined color change during storage could be associated with TP degradation. It could be possible because phenolic compounds enter various chemical reactions through which colored products are developed, for example Maillard reaction (Jiménez-Zamora et al., 2016).

**Table 2** Correlations between total phenols content, color parameters and sensory properties of olive leaf drink**Tablica 2.** Korelacije između sadržaja ukupnih fenola, parametara boje i senzorskih svojstava napitka od maslinovog lista

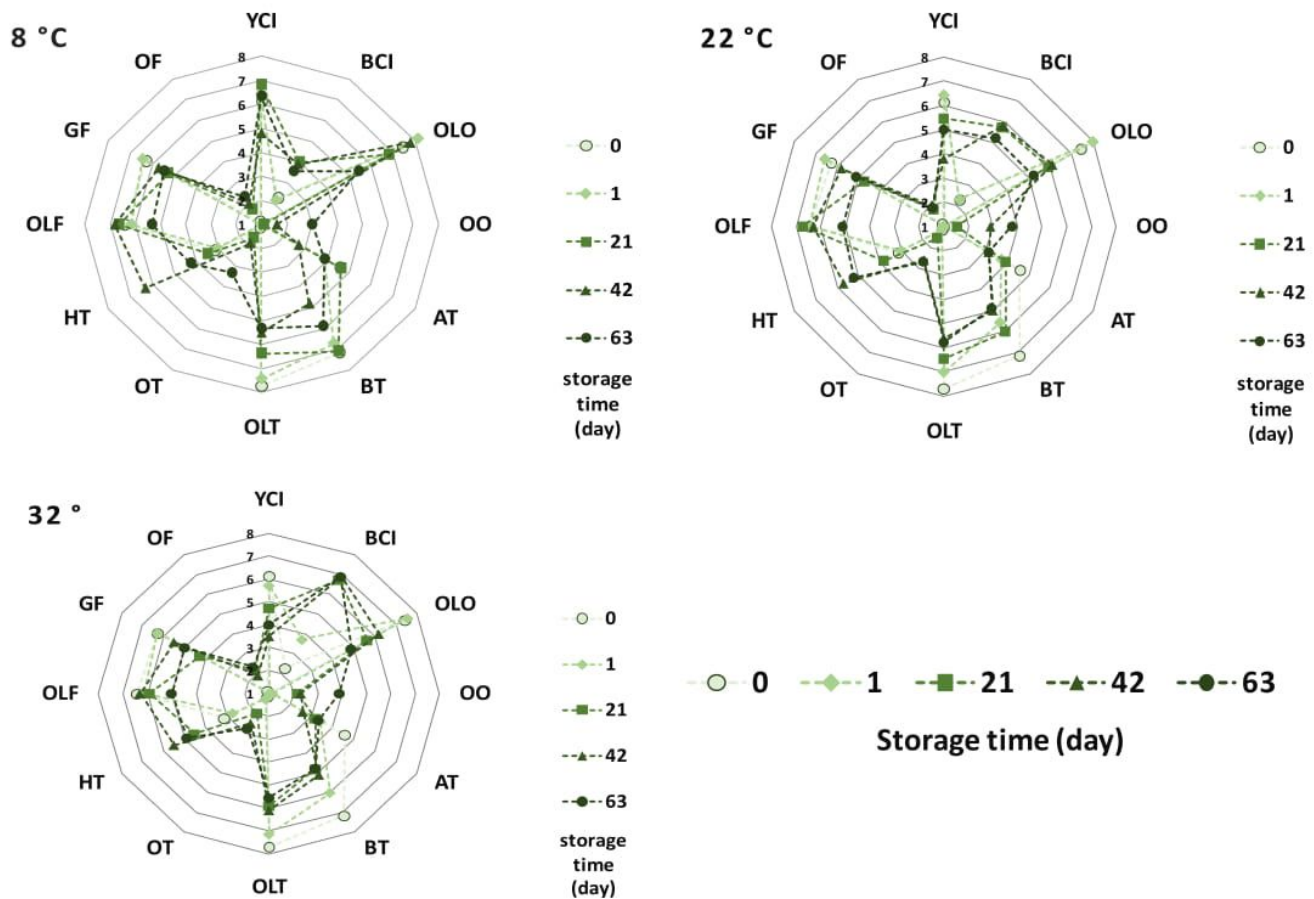
Correlation	Correlation coefficient	Correlation	Correlation coefficient	Correlation	Correlation coefficient
$L^* \times a^*$	-0.74*	$\Delta E_{ab}^* \times TP$	-0.78*	$BT \times \Delta E_{ab}^*$	-0.80*
$L^* \times b^*$	-0.85*	$BI \times TP$	-0.69*	$BT \times BI$	-0.80*
$L^* \times \Delta E_{ab}^*$	-0.76*	$YCI \times L^*$	0.74*	$YCI \times TP$	0.47ns
$L^* \times BI$	-0.87*	$YCI \times a^*$	-0.79*	$BCI \times TP$	-0.60*
$a^* \times b^*$	0.97*	$YCI \times b^*$	-0.83*	$AT \times TP$	0.65*
$a^* \times \Delta E_{ab}^*$	0.94*	$YCI \times \Delta E_{ab}^*$	-0.79*	$BT \times TP$	0.72*
$a^* \times BI$	0.96*	$YCI \times BI$	-0.83*	$HT \times TP$	-0.57*
$b^* \times \Delta E_{ab}^*$	0.98*	$BCI \times L^*$	-0.67*	$OLT \times TP$	0.72*
$b^* \times BI$	1.00*	$BCI \times a^*$	0.93*	$OLF \times TP$	0.54*
$\Delta E_{ab}^* \times BI$	0.97*	$BCI \times b^*$	0.91*	$AT \times HT$	-0.79*
$L^* \times TP$	0.35ns	$BCI \times \Delta E_{ab}^*$	0.88*	$BT \times HT$	-0.80*
$a^* \times TP$	-0.76*	$AT \times \Delta E_{ab}^*$	-0.72*	$OLT \times HT$	-0.83*
$b^* \times TP$	-0.70*	$AT \times BI$	-0.68*	$OLF \times HT$	-0.21ns

*YCI = yellow color intensity, BCI = brown color intensity, AT = astringent taste, BT = bitter taste, OLT = olive leaf taste, HT = harmony taste, OLF = olive leaf flavor, TP = total phenols (mg GAE L<sup>-1</sup>); \*p ≤ 0.05, ns = not significant*

**Table 3** F-distribution and the probability level in the ANOVA analysis of olive leaf drink sensory properties**Tablica 3.** F-distribucija i stupanj vjerojatnosti u ANOVA analizi senzorskih svojstava napitka od maslinovog lista

Sensory attribute	Storage time	Storage temperature	Sensory attribute	Storage time	Storage temperature
YCI	2.28ns	4.01*	OLT	11.42*	0.07ns
BCI	15.74*	11.11*	OT	6.96*	0.11ns
OLO	6.50*	1.73ns	HT	10.35*	0.02ns
OO	10.42*	1.81ns	OLF	3.30*	0.78ns
AT	1.22ns	0.29ns	GF	1.59ns	0.26ns
BT	3.35*	0.92ns	OF	6.29*	0.14ns

*YCI = yellow color intensity, BCI = brown color intensity, OLO = olive leaf odor, OO = off-odor, AT = astringent taste, BT = bitter taste, OLT = olive leaf taste, OT = off-taste, HT = harmony taste, OLF = olive leaf flavor, GF = green flavor, OF = off-flavor; \*p ≤ 0.05, ns = not significant*



**Graph 2** Sensory properties of olive leaf drink during storage at different temperatures

**Grafikon 2.** Senzorska svojstva napitka od maslinovog lista tijekom skladištenja na različitim temperaturama

YCI = yellow color intensity, BCI = brown color intensity, OLO = olive leaf odor, OO = off-odor, AT = astringent taste, BT = bitter taste, OLT = olive leaf taste, OT = off-taste, HT = harmony taste, OLF = olive leaf flavor, GF = green flavor, OF = off-flavor. Results are expressed as mean value.

Sensory analysis conducted by the QDA evaluated several OLD sensory attributes of color, odor, taste and flavor (Graph 2). Statistically, storage temperature didn't have significant impact on tested sensory properties, except on the yellowness and brownness ( $p \leq 0.05$ ), but storage time significantly affected ( $p \leq 0.05$ ) the majority of evaluated attributes (Table 3).

In fresh OLD YCI scored 6.1, and by the storage time it was slightly lower graded. Panelists observed larger differences in samples yellowness with respect to temperature, where samples stored at 8 °C were more yellowish opposite to samples stored at 22 °C and 32 °C, especially at the end of storage. Greater distinctions in sensory scores reached BCI, as it was significantly influenced ( $p \leq 0.05$ ) by both storage conditions. With initial score of 2.3, at 63rd day scores reached up to 3.6 at 8 °C, 5.2 at 22 °C and peaked at 32 °C with 6.9. It can be stated that instrumentally recorded color changes were sensory noticed, as both of evaluated color attributes highly correlated with instrumentally determined color parameters (Table 2). Additionally, BCI showed good correlation with TP ( $-0.60$ ,  $p \leq 0.05$ ).

Attributes of OLO, OLT and OLF significantly decreased by the storage time ( $p \leq 0.05$ ) with no larger differences regarding storage temperature, where their grades went down for around 2 points by the 63rd day. Attributes that most characterized the taste of OLD were AT and BT. At day 0 they were graded with 4.6 (AT) and 7.1 (BT) and the panelists observed the decrease of

its intensity by the storage duration. Also, the decrease was more noticeable with the increase of temperature. This decrease of astringency and bitterness provided more harmony in OLD taste (Graph 2), approved by high correlation (Table 2). As oleuropein, being the most abundant phenolic compound in OL and responsible for its predominant bitter taste, converses due its hydrolysis into bitterless hydroxytyrosol, (Ramírez et al., 2016), it can be observed that previously mentioned TP decrease reflected on AT and BT, as well as HT, respectively. Again, as shown in Table 2, correlation coefficients confirmed good relationship between TP and mentioned taste attributes. As for GF, panellists detected its decrease towards storage ending, from 6.4 (day 0) to the range of 5.0 – 5.4 (63rd day), depending on the applied storage temperature. The appearance of undesirable sensory properties (OO, OT and OF) was noticed only by the end of storage, but it wasn't remarkable.

## Conclusion

Results of this research showed that the most changes influenced by storage of olive leaf drink occurred by the end of storage, as well as on higher storage temperatures. The content of total phenols remained pretty stable (84 % at 8 °C, 67 % at 22 °C and 77 % at 32 °C), as well as the antioxidant capacity (by DPPH method). Also, color change during storage didn't respond negatively and olive leaf drink adopted more harmonious taste along the storage. It can be concluded that despite the effect of storage, olive leaf drink showed good stability in most of its desirable possessions and therefore it represents potential ingredient for functional food products, e.g., for beverage enrichment, whereby it retains the best quality at cool storage temperatures.

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## Stabilnost napitka od maslinovog lista ovisno o skladištenju na različitim temperaturama

### Sažetak

U proizvodnji maslinovog ulja, maslinovo lišće najčešće ostaje nedovoljno upotrebljeno i predstavlja otpad, iako su mnoge studije pokazale njegova antikancerogena, protuupalna i antimikrobna svojstva zbog obilja polifenola, što ga čini potencijalnim sastojkom za proizvodnju funkcionalne hrane. Također, napitak od maslinovog lista predstavlja samostalno funkcionalno piće zbog njegovog vrijednog sastava. No, funkcionalna svojstva se, osobito tijekom skladištenja, mogu smanjiti zbog degradacije polifenola. Stoga je ovo istraživanje imalo za cilj istražiti stabilnost napitka od maslinovog lista tijekom devet tjedana skladištenja na različitim temperaturama (8, 22 i 32 °C). Utjecaj vremena i temperature skladištenja ispitan je određivanjem ukupnih fenola, antioksidacijskog kapaciteta DPPH metodom, parametara boje ( $L^*$ ,  $a^*$ ,  $b^*$ ,  $\Delta E_{ab}^*$ ,  $BI$ ) i senzorskih svojstava napitka od maslinovog lista. Dobiveni rezultati pokazali su da se ukupni fenoli tijekom skladištenja neznatno smanjuju, pokazujući najveću stabilnost na 8 °C. Antioksidacijski kapacitet pokazao je približno isti trend. Boja svih uzoraka je skladištenjem potamnila uz porast  $L^*$  i  $\Delta E_{ab}^*$ ,  $BI$  povećanjem temperature. Najveće promjene boje zabilježene su na 32 °C krajem skladištenja. Senzorska analiza potvrdila je promjenu boje napitka od maslinovog lista smanjenjem žute te povećanjem smeđe boje s vremenom i temperaturom skladištenja. Miris i aroma su se s vremenom i na višoj temperaturi smanjili. Intenzitet glavnih svojstava okusa napitka (trpak i gorak okus) se za vrijeme skladištenja smanjio, osobito na višim temperaturama, što je rezultiralo više ocijenjenom harmoničnosti okusa. Dobiveni rezultati pokazali su dobru stabilnost napitka od maslinovog lista ukazujući na njegov potencijal kao funkcionalnog proizvoda ili poluproizvoda.

**Ključne riječi:** list masline, skladištenje, fenoli, boja, senzorika