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EFFECT OF PROCESSING AND STORAGE ON SAGE (*SALVIA OFFICINALIS* L.) HONEY QUALITY

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ABSTRACT

The aim of this study was to evaluate the effect of processing (45 °C/48 h and 65 °C/6 h) and two years' storage on sage (*Salvia officinalis* L.) honey and to determine the period in which the compliance to the regulations is fulfilled and degradation of nutritive components are minimal. The results showed that processing at higher temperature for shorter period had a greater impact on enzyme activity decrease and increase of color than heating at lower temperature for longer time. Processing had no significant effect on HMF increase, while during storage HMF content increased above maximum prescribed value. Although processing and storage increased phenolic content and antioxidant capacity, at the same time, quality was significantly degraded regarding enzyme activity and HMF content. Therefore, it is recommended to store sage honey for less than two years and if processing is necessary to heat it at as low temperature as possible.

Keywords: sage honey, processing, storage, quality, shelf life

INTRODUCTION

Chemical composition and nutritional value of honey, as well as consumer's preferences, depends mainly on its botanical origin. Many honey components responsible for specific nutritive and biological properties are known to be unstable during storage and sensitive to heat (**Escriche *et al.*, 2009; Fauzi *et al.*, 2014; Karabagias *et al.*, 2017; Önür *et al.*, 2018**). Before placing to the market, honey is often processed with the aim of viscosity decrease, delaying and/or preventing crystallization and fermentation control in order to facilitate manipulation and ensure storage stability (**Subramanian *et al.*, 2007**). Although, several innovative techniques have been used for honey processing (**Kowalski, 2013; Wilczyńska, 2014; Fauzi *et al.*, 2014; Önür *et al.*, 2018**), the beekeepers and traders usually still heat honey to achieve desirable processing effect. It is known that heating causes honey quality degradation, like enzyme activity decrease, increase of 5-(hydroxymethyl)furan-2-carbaldehyde (HMF) content, darkening of honey and formation of Maillard reaction products (MRPs). The intensity of changes is strongly dependent on applied temperature and heating duration (**Turhan *et al.*, 2008; Escriche *et al.*, 2014; Karabagias *et al.*, 2017; Radtke and Lichtenberg-Kraag, 2018; Merve Turkut *et al.*, 2018**). Heat treatments lasting 1-2 days at 40-50 °C will not cause significant changes, while the application of temperatures of 50-60 °C increases the intensity of changes and impairs the quality of honey. Application of pasteurization temperatures (63-68 °C/30-35 minutes or 77 °C/several seconds) will completely inactivate the yeasts responsible for fermentation and delay crystallization, but at these temperatures a decrease in enzyme activity is visible (**Dimiņš *et al.*, 2006; Merve Turkut *et al.*, 2018; Radtke and Lichtenberg-Kraag, 2018**). It is recommended to heat the honey at the lowest possible temperatures and for as short a time as possible in order to achieve the desired processing effect.

Honey is packed in glass or plastic containers that are intended for consummation over a longer period and therefore it is important to maintain quality as much as possible during storage. Critical factors that affect honey quality during storage are relative humidity, temperature and the presence of light in storage rooms. Improper storage conditions can lead to the changes of sensory characteristics (mainly color and aroma), the loss of biologically active components, like enzymes and other antimicrobial components, and honey fermentation (**Brudzynski and Kim, 2011**). Furthermore, storage affects carbohydrate composition, increase of honey acidity and HMF formation (**Monggudal *et al.*, 2018; Radtke and Lichtenberg-Kraag, 2018**). Due to the natural variability of honey components and the number of factors that affect its' quality, it is very difficult to estimate the processing and storage conditions that minimize degradation of honey. Namely, available studies showed that the intensity of changes during processing and storage is strongly dependent on honey botanical origin (**Wang *et al.*, 2004; Dimiņš *et al.*, 2006; Brudzyinski and Miotto, 2011a; Kowalski, 2013**).

Sage (*Salvia officinalis* L.) honey is one of the most important Croatian unifloral honey type and one of the most appreciated among consumers. Characterization of this specific honey type from different aspects is documented in our previous papers (**Kenjerić *et al.*, 2006; Kenjerić *et al.*, 2008; Čačić Kenjerić *et al.*, 2009; Primorac *et al.*, 2011; Flanjak *et al.*, 2016a; Flanjak *et al.*, 2016b; Strelec *et al.*, 2018**).

Having in mind that botanical origin and post extraction manipulation have a great impact on honey quality alterations, the aim of this work was to estimate dynamic of changes after different processing treatments and two years' storage on sage honey. The results of this study can be useful for estimation of "best before" date for sage honey in which the compliance to the regulations is fulfilled and degradation of nutritive components are minimal.

MATERIAL AND METHODS

Honey samples

Five sage (*S. officinalis* L.) honey samples (10 kg of each sample) were purchased from the beekeepers from different parts of Adriatic region of Croatia. Each sample was first homogenized in a large container and divided into 3 series (unprocessed, UP; processed, P1 and P2). The glass containers were filled to the top and sealed, and were not opened until analysis. Unprocessed samples (UP) were analyzed without any processing/heating while other two series of samples (P1 and P2) were heated under different temperature-time conditions. P1 processing treatment included heating of samples at 45 °C for 48 hours while the P2 processing treatment was performed by heating samples at 65 °C for 6 hours. Thermal processing was performed in the temperature controlled heating chamber with forced convection (BINDER FED 53, USA). After cooling at the room temperature, following physicochemical parameters were determined: HMF content, activities of diastase, invertase, glucose oxidase (GOX) and acid phosphatase (AP), phenolic content, antioxidant capacity with DPPH and FRAP assays, color determinations (color intensity and color grading) and fluorescence measurement.

Both unprocessed (UP) and processed samples (P1 and P2) were stored at the room temperature in the dark place. Determination of HMF content, activity of diastase, invertase, GOX and AP, phenolic content, antioxidant capacity (DPPH and FRAP assays), color determinations and fluorescence measurement were conducted after 12 and 24 months of storage, respectively.

Methods

Melissopalynological analysis of collected honey samples was performed as according to the harmonized methods of melissopalynology (**von der Ohe *et al.*, 2004**) while preparation of slides without acetolysis for microscopical examination was done according to the method of **Louveaux *et al.* (1978)**. Identification and counting of pollen grains in prepared slides was performed under a microscope at 400-1000x magnification. Pollen grains identification was made by reference to the literature data (**Von der Ohe and Von der Ohe, 2003; Kenjerić *et al.*, 2006**). Qualitative melissopalynological analysis, identification and counting of pollen grains of the predominant plant species, was performed on all samples in order to confirm uniflorality of colleted samples. Final confirmation of uniflorality was done based on the results of mellisopalinological analysis (**Ministry of Agriculture, Fisheries and Rural Development, 2009**), sensory characteristics (**Piana *et al.*, 2004; Kenjerić *et al.*, 2006**) and selected physicochemical parameters that are usually correlated to honey botanical origin.

Basic physicochemical parameters determination was conducted according to the international methods for honey analyses (**International Honey Commission, 2009; AOAC International, 2002**). Moisture content was determined using refractometric method, electrical conductivity was measured in a solution of 20 g honey dry matter in a low conductivity distilled water, HMF content was determined using method after White, diastase and invertase activities using spectrophotometric methods after Shade and Siegenthaler, respectively. Specific rotation was determined by means of polarimeter while carbohydrate content was determined by chromatographic (HPLC) method (**International Honey Commission, 2009**). Initial pH of honey, and afterwards free acidity, lactones and total acidity were determined by titrimetric method according to the AOAC Official Methods (**AOAC International, 2002**).

Glucose oxidase (GOX) activity was determined according to the **Schepartz & Subers (1964)**. The quantification was performed using H_2O_2 as standard (Fluka, Germany) with peroxidase and *o*-dianisidine and the results were expressed as µg H_2O_2/h g honey.

Acid phosphatase (AP) activity determination was performed as described **Bergmeyer *et al.* (1974)**. The results of acid phosphatase activity were expressed as mg P/100 g honey/24 h.

Phenolic content was determined by modified Folin-Ciocalteu method described by **Beretta *et al.* (2005)**. The quantification was performed using gallic acid (Sigma, USA) as standard and the results were expressed as mg gallic acid/kg honey.

Antioxidant capacity was determined using two spectrophotometric assays. The antiradical/antioxidant capacity by DPPH assay was performed as described **Brand-Williams *et al.* (1995)** and **Berreta *et al.* (2005)**. The antiradical capacity of honey samples was expressed as IC_{50}, a honey concentration (mg/mL) that causes a decrease of the initial DPPH radical concentration by 50 %. For the determination of total antioxidant capacity, the assay reported by **Benzie and Strain (1996)** was used. Aqueous standard solutions of $FeSO_4 \cdot 7H_2O$ (Kemika, Croatia) were used for calibration curve and the results were expressed as the FRAP value (µM Fe(II) of the 10 % honey solution).

The color of honey was evaluated using two methods. Color grading was determined by Lovibond Honey ColorPod that measures transmittance at 430 and 530 nm of homogeneous, liquid honey, as compared with the transmittance of pure glycerin. The transmitted light was related to the Pfund color scale and the results were expressed as mm of Pfund (**AOAC International, 2002**). Spectrophotometric honey color determination (net absorbance), defined as the difference between absorbance measurements at 450 and 720 nm was performed as described **Beretta *et al.* (2005)** and the results were expressed mAU.

Fluorescence measurement was performed according to the procedure described in our previous study (**Strelec *et al.*, 2018**). Three dimensional fluorescence spectra recordings were performed by scanning with excitation wavelengths from 260 to 400 nm, and emission wavelengths from 300 to 600 nm while fluorescence intensity was measured at 280/350 nm (excitation/emission), and at 360/460 nm.

Statistical analysis

Average values, standard deviations and range for each parameter were given. In order to evaluate statistical difference between processing conditions and storage factorial analysis of variance (ANOVA) and post-hoc Tukey HSD test were performed using software STATISTICA® 13.3 (Dell Inc., Round Rock, TX, USA). p-values < 0.05 were considered significant.

RESULTS AND DISCUSSION

Botanical origin confirmation

With the aim of botanical origin confirmation of collected samples, melissopalynological analysis was performed. The results of melissopalynological analysis were presented in table 1. All analyzed samples fulfilled the requirement of minimum 15 % of *S. officinalis* L. pollen share in insoluble honey sediment, or 10 % together with characteristic sensory attributes for sage honey (**Ministry of Agriculture, Fisheries and Rural Development, 2009**). Sensory characteristics (colour, odour, and aroma) of collected samples correspond to those described in our previous paper where sage honey characteristics are described (**Kenjerić *et al.*, 2006**). Furthermore, determined physicochemical quality parameters (table 1) were in compliance to national (**Ministry of Agriculture, 2015**) and international regulations (**Codex Alimentarius Commission, 2001; Council of the European Union, 2002**). Based on the results of melissopalynological analysis, physicochemical parameters and sensory analysis, samples can be classified as unifloral sage (*S. officinalis* L.) honey.

Table 1 Average values, standard deviations (SD) and range for melissopalynological analysis and physicochemical parameters of analyzed sage (*S. officinalis* L.) honey samples

Parameter	Average	SD	Minimum	Maximum
S. officinalis L. pollen share (%)	19	6	11	26
Moisture (%)	16.8	0.8	15.8	17.8
Electrical conductivity (mS/cm)	0.299	0.062	0.221	0.386
pH	4.03	0.08	3.97	4.16
Free acidity (mmol/kg)	20.7	3.3	15.4	23.4
Lactones (mmol/kg)	6.5	1.5	4.6	8.0
Total acidity (mmol/kg)	27.2	4.7	20.0	31.2
Specific rotation $(\alpha)_D^{20}$	-16.8	1.4	-18.5	-14.9
F/G	1.26	0.05	1.20	1.32
F+G (g/100 g)	72.0	2.4	69.9	75.9

Effect of processing and storage on enzyme activities and HMF content

Honey is a food product that is often processed to ease further manipulation. Processing usually refers to heating honey at different temperatures for different time. The beekeepers and traders often heat honey at temperatures from 40 to 45 °C for few days in hot air chambers to recrystallize honey since crystallized honey is less attractive to consumers than liquid one but also to facilitate packaging (**Escriche *et al.*, 2009, Karabagias *et al.*, 2017**). Temperatures higher than 45 °C are necessary for inactivation of yeast responsible for fermentation of honey in inadequate conditions. Temperatures between 63 to 68 °C for few hours are used for honey pasteurization (**Babacan *et al.*, 2002; Subramanian *et al.*, 2007; Merve Turkut *et al.*, 2018**). Processing conditions applied in this study (P1 and P2) were selected with the aim of evaluation of decrystallization and pasteurization temperatures on sage honey quality. Honey is packed in glass or plastic containers, stored usually at room temperature and not consumed at once but for some time. "Best before" date for honey is stated two years but honey quality alters during storage (**Brudzynski and Kim, 2011; Monggudal *et al.*, 2018; Radtke and Lichtenberg-Kraag, 2018**). Additionally, the aim of this study was to evaluate the intensity of changes in unprocessed and processed sage honey for two years, and to investigate possible synergistic effect of processing and storage on sage honey quality.

Enzyme activity and HMF content are considered as indicators of honey processing and storage conditions. Average HMF content of collected samples was 2.0±0.04 mg/kg (table 2, figure 1) while average diastase activity, expressed as diastase number (DN), was 17.5±4.6 (table 2) which purports the fact that samples were fresh and unprocessed. Both processing treatments (P1 and P2) caused decrease of enzyme activity.

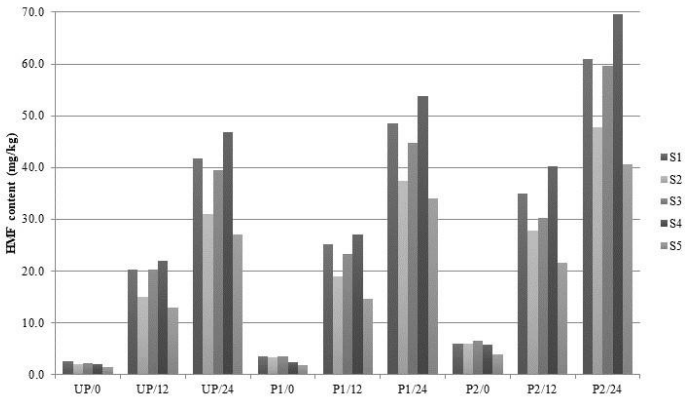

Figure 1 HMF content of analyzed sage honey samples (S1-S5) after processing and storage (UP-unprocessed, P1-45 °C/48 h, P2-65 °C/6 h; 0, 12 and 24 represent months of storage)

Table 2 Effect of different processing conditions (P1 and P2) and storage on selected physicochemical parameters (average ± SD)

Parameter	Storage period	Unprocessed (UP)	After P1 (45°C/48 h)	After P2 (65°C/6 h)
Diastase activity (DN)	0	17.5±4.6^{a}	16.0±4.3^{a,b}	12.5±3.3^{a,b}
	12 months	13.4±3.4^{a,b}	12.4±3.2^{a,b}	10.7±2.7^{a,b}
	24 months	11.9±3.0^{a,b}	11.5±2.9^{a,b}	9.8±2.5^{b}
Invertase activity (U/kg)	0	92.5±27.8^{a,b}	66.4±25.6^{a,b,c,d,e}	7.0±5.6^{d,e,f,g}
	12 months	58.3±20.2^{a,b,c,d,e}	44.9±20.6^{b,c,d,e,f}	3.9±2.7^{e,f,g}
	24 months	48.4±19.6^{b,c,d,e}	36.8±17.5^{b,c,d,e,f,g}	3.7±3.2^{e,f,g}
GOX activity (μg H_2O_2/h g)	0	223.6±104.9^{a}	211.9±94.0^{a}	34.9±46.3^{b}
	12 months	223.4±90.5^{a}	206.7±82.7^{a}	17.3±20.1^{b}
	24 months	220.0±85.5^{a}	212.0±86.4^{a}	27.6±30.0^{b}
AP activity (mg P/100 g honey/24 h)	0	47.3±18.4^{a}	37.2±11.6^{a}	29.6±6.7^{a}
	12 months	47.0±18.1^{a}	38.6±10.5^{a}	29.1±5.8^{a}
	24 months	33.4±14.0^{a}	34.3±14.9^{a}	26.4±15.0^{a}
HMF content (mg/kg)	0	2.0±0.4^{a,f}	2.9±0.8^{a,f}	5.6±1.0^{a,b,f}
	12 months	18.0±4.0^{b,d,f}	21.8±5.0^{b,d,g}	30.9±7.1^{b,c,d,e,g}
	24 months	37.2±8.1^{c,e}	43.7±8.1^{c,e,g,h}	55.7±11.5^{e,h}
Net absorbance (mAU)	0	228±45^{a,b}	248±50^{a,b,d}	275±57^{a,b,d}
	12 months	320±52^{a,b,c,d}	340±54^{a,b,c,d,e}	407±55^{b,c,d,e}
	24 months	406±52^{b,c,d,e}	441±62^{c,d,e}	528±69^{e,f}
Color (mm Pfund)	0	49±6^{a,d,e}	52±6^{a,b,d,e}	56±5^{a,b,c,d,e}
	12 months	59±2^{b,c,d,e,f}	60±2^{b,c,e,f}	66±3^{b,c,f}
	24 months	62±1^{b,c,e,f}	63±2^{b,c,e,f}	67±3^{b,c,f}
Phenolic content (mg gallic acid/kg honey)	0	90.9±16.4^{a,b}	102.7±18.6^{a,b,c}	112.5±21.6^{a,b,c,d}
	12 months	122.7±21.4^{a,b,c,d}	128.2±21.3^{a,b,c,d}	142.7±21.8^{b,c,d}
	24 months	132.5±20.5^{a,b,c,d}	141.9±20.4^{b,c,d}	151.6±22.9^{c,d}
DPPH-IC_{50} (mg/mL)	0	24.65±5.23^{a,b,d}	22.60±4.55^{a,b,d}	19.28±4.04^{a,b,c,d}
	12 months	17.02±2.75^{b,c,d}	15.64±2.12^{b,c}	14.74±1.74^{b,c}
	24 months	14.00±2.20^{b,c}	13.50±2.04^{b,c}	12.78±1.71^{b,c}
FRAP value (μM Fe(II))	0	149.6±40.4^{a}	164.0±41.8^{a,b}	194.7±51.2^{a,b}
	12 months	219.2±51.7^{a,b}	237.1±51.0^{a,b}	255.0±60.0^{a,b}
	24 months	237.2±56.8^{a,b}	249.6±53.8^{a,b}	267.0±60.8^{b}
Fluorescence intensity at 280/350 nm (a.u.)	0	45.00±6.94^{a}	41.96±6.39^{a}	37.04±4.36^{a}
	12 months	24.83±2.65^{b,c,d}	22.98±2.57^{b,c,d,e}	18.22±2.44^{b,c,d,e,f}
	24 months	17.66±1.75^{b,c,d,e,f}	15.48±2.04^{c,d,e,f}	12.72±1.96^{c,d,e,f}
Fluorescence intensity at 360/460 nm (a.u.)	0	152.90±14.56^{a,d}	149.14±17.43^{a,b,d}	149.04±15.88^{a,b,d}
	12 months	127.40±13.88^{b,c,d,e,g}	123.00±9.08^{b,c,e,f}	118.92±10.03^{b,c,e,f,g}
	24 months	106.48±8.12^{b,c,e,f,g}	101.38±8.46^{c,e,f,g}	94.71±6.69^{c,f,g}

Legend: GOX-glucose oxidase, AP-acid phosphatase, HMF-hydroxymethylfurfural, DPPH-IC_{50}-honey concentration that causes a decrease of the initial DPPH radical concentration by 50%. Values represented with same letters are not statistically different according to Tukey HSD test ($p>0.05$)

Having in mind that majority of enzymes are sensitive to elevated temperatures, causing enzyme denaturation, the obtained results are not surprising. The dynamics of change of selected physicochemical parameters was the same for all five analyzed samples. Statistically significant difference between unprocessed and processed samples, according to the Tukey HSD test, was determined for diastase, invertase and GOX activity while heating had no effect on acid phosphatase activity (table 2). Decrease of enzyme activity was more intense after heating at higher temperature, even 92 % for invertase and 84 % for GOX activity compared to unprocessed samples (table 2, figure 2). Obtained results confirm already reported information that invertase and GOX are more sensitive to heating than diastase (**Karabournioti and Zeralaki, 2001; Dimiņš *et al.*, 2006**). The intensity of changes depends greatly on honey botanical origin and therefore it is very difficult to compare the results for different honey types. The data about sage honey are very scarce while the data about the effect of processing and storage on sage honey characteristics are not available. **Karabournioti and Zeralaki (2001)** evaluated the impact of processing on thyme honey and they showed that invertase activity decreased from 70.64 U/kg (unprocessed honey) to 53.56 U/kg (after heating at 45°C) and 6.35 U/Kg (after heating at 65°C). The intensity of change is similar to those obtained in this study (28 % after P1, and 92 % after P2). **Dimiņš *et al.* (2006)** showed that invertase activity of heather honey decreased to 50 % of initial value after 2 years of storage that corresponds to the results in this study.

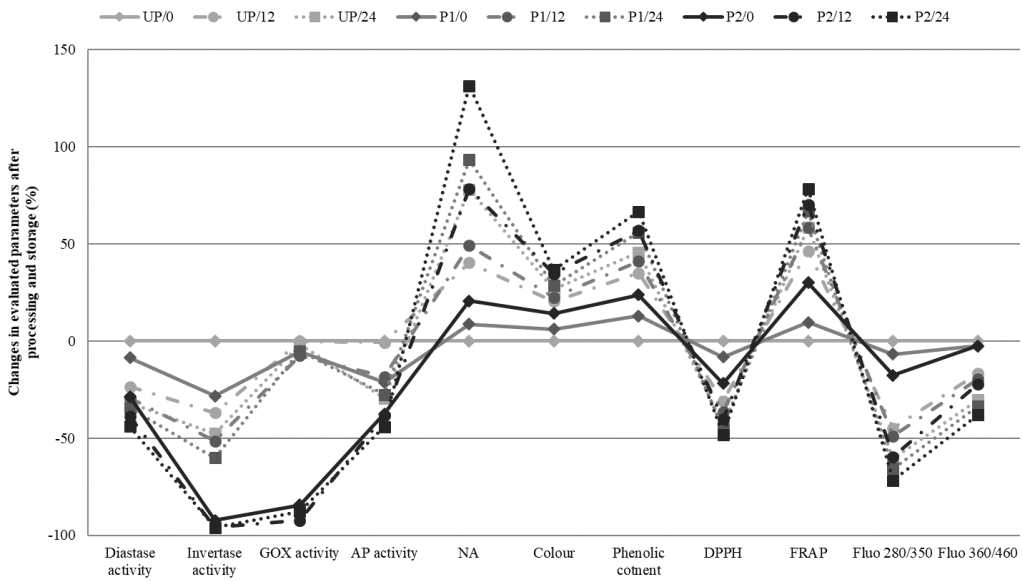

Figure 2 Intensity of changes (%) of selected physicochemical parameters after processing and storage (UP-unprocessed, P1-45°C/48 h, P2-65°C/6 h; 0, 12 and 24 represent months of storage)

Storage caused further decrease of enzyme activity while preheating had no effect on decrease dynamics (table 2). The effect of processing and storage on HMF content are well described in the literature, but is it emphasized that dynamic of HMF formation significantly depend on botanical origin and chemical composition, primarily pH value of honey. Heating at lower temperature (45 °C/48 h) had no effect on HMF content of sage honey, while higher temperature caused HMF increase by 1.5 times compared to unprocessed samples (table 2, figure 1). Nevertheless, all measured HMF values after processing/heating were lower than 10 mg/kg, which indicates that processing conditions are suitable and HMF content was within the limits for adequately processed honey. Limiting values for HMF as well as diastase activity are prescribed in national and international regulations (C**odex Alimentarius Commission, 2001; Council of the European Union, 2002; Ministry of Agriculture, 2015**). After two years of storage, average HMF content of preheated samples was higher than maximum value of 40 mg/kg (43.7±8.1 mg/kg after P1 and 55.7±11.5 mg/kg after P2). This indicates the combined effect of processing/heating and storage on HMF formation. Therefore, it is recommended to store and consume sage honey in a period of less than two years.

Effect of processing and storage on colour

Color of honey is one of the most important parameter for consumers' preference. Sage honey color (49±6 mm Pfund) is classified into extra light amber honey according to the USDA classification (**United States Department of Agriculture, 1985**). Processing and storage caused the darkening of sage honey, and average color values after two years of storage were 62±1, 63±2 and 67±3 mm Pfund (table 2), for unprocessed, and processed (P1 and P2) samples, respectively. According to the USDA classification, the color of analyzed samples after two years' storage changed into light amber (**U.S. Department of Agriculture, 1985**). Darkening of honey is attributed to the Maillard reaction products (MRPs) formation, fructose caramelization and phenolic components reactions (**Pereyra Gonzales *et al.*, 1999; Turkmen *et al.*, 2006; Brudzynski and Miotto; 2011a**). Increase of color was noted in both processed and unprocessed samples (table 2, figure 2) which indicated spontaneous formation of MRPs even in unprocessed samples during storage at room temperatures. Although color grading is standard method for honey color determination, spectrophotometric color determination (net absorbance) was proved to be better for monitoring of honey color change dynamics. Namely, melanoidins that are formed in final stage of Maillard reactions have characteristic absorption maximum between 420 and 450 nm (**Brudzynski and Kim, 2011**). Since, spectrophotometric color determination (net absorbance) is based on the absorbance measurement at 450 and 720 nm, the increase of absorbance at 450 nm indicates the formation of melanoidins during processing and storage of honey.

Effect of processing and storage on phenolic content and antioxidant capacity

Antioxidant capacity of honey depends primary on its botanical origin and mostly is correlated to phenolic content and color. It is known that darker honey types have higher phenolic content and antioxidant capacity than lighter types (**Bertoncelj *et al.*, 2007; Flanjak *et al.*, 2016a**). Phenolic content and antioxidant capacity of various honey types is well documented in the literature, but the effect of processing and storage conditions on antioxidant capacity are limited and the results are quite different (**Wang *et al.*, 2004; Turkmen *et al.*, 2006.; Brudzynski and Miotto, 2011a; Karabagias *et al.*, 2018; Monggudal *et al.*, 2018**). Differences in obtained results can be attributed to the differences in processing (temperature-time) and storage conditions (temperature, presence of light) and sensitivity and precision of used methods. Processing increased phenolic content (on average 13 % after P1 and 24 % after P2) of sage honey (Figure 2). Increase of antioxidant capacity was greater for FRAP assay than DPPH assay, and the intensity of increase was dependent of preheating. The highest increase of FRAP value was observed in unprocessed samples (average 59 %) followed by P1 processed samples (average 52 %) and P2 processed samples (average 37 %). Increase of antioxidant/antiradical capacity determined by DPPH assay was observed after one year of storage while further storage had no impact on DPPH values (table 2). The observed differences in antioxidant activity between these two assays, might be explained by differences in assays mechanisms and components that react with reagents. Nevertheless, the observed increase of antioxidant capacity during processing and storage of sage honey can be partially attributed to the MRPs formation because it is known that some of them possess antioxidant activity (**Brudzynski and Miotto, 2011a**). Besides, MRPs might be also partially responsible for sage honey darkening during processing and storage. Strong relationship between formation of MRPs and increase of antioxidant capacity and color of honey was reported by **Turkmen *et al.* (2006)** and **Brudzynski and Miotto (2011a,b)**.

Effect of processing and storage on fluorescence intensity

As reported in our previous study (**Strelec *et al*., 2018**) sage honey is characterized with two well defined fluorescence peaks, first dominant peak centered at 360/460 nm, and second peak of lower intensity at 280/350 nm. Fluorescence peak detected at 280/350 nm could be attributed to the fluorescence of aromatic amino acids Tyr, Phe and Trp and/or proteins, as well to some phenolic acids (**Strelec *et al.*, 2012; Strelec *et al.*, 2018**), while those at 360/460 nm to the fluorescence of flavonoids (**de Rijke *et al.*, 2002; Strelec *et al.*, 2018**). Heating had no significant effect on fluorescence intensity at both excitation/emission wavelengths (table 2). However, during the storage there was a slight decrease in honey fluorescence. This could be attributed to the increased level of melanoidins, whose higher level in honeys during storage was observed as increase of honey color (table 2). Since honey melanoidins are multi-component polymers consisting of protein-polyphenol-oligosaccharide complexes (**Brudzynski and Miotto, 2011a,b**), where additional interactions with honey flavonoids might not be neglected, multicomponent cross-linking of proteins, amino acids, sugars, polyphenols and flavonoids during honey storage probably caused decrease of fluorescence intensity. Namely, it is well known that interactions between flavonoids and proteins in solution might led to the shift and/or decrease of fluorescence intensity (**Liu *et al.*, 2010; Fu *et al.*, 2012**).

CONCLUSION

Processing and storage conditions used in this study were selected to simulate the real-life conditions that are mostly performed by the beekeepers and traders and afterwards to evaluate its effect on sage honey quality. The synergistic effect of processing and storage on honey quality was observed. Although the processing and storage increase phenolic content and antioxidant capacity, which is from nutritional aspect a desirable phenomenon, the general quality was considerably

degraded to such a degree that after two years of storage at room temperature, preheated samples were not in compliance to regulations. Therefore, "best before" date for sage honey in which the compliance to the regulations is fulfilled and degradation of nutritive components are minimal is less than two years and it is advisable to processed it at low temperature as possible to achieve the processing effect.

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Conflict of Interest Statement: Authors declare no conflict of interest.

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