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EFFECT OF FREEZING ON PHYSICO-CHEMICAL, ANTIOXIDANT, AND SENSORY PROPERTIES OF HONEYDEW HONEY

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ABSTRACT

Freezing food slows down the physico-chemical and biochemical reactions in food. In honey, freezing is primarily used to delay or halt crystallization, reduce viscosity, and preserve nutritional components. However, low temperatures may influence the physico-chemical, antioxidant, and sensory properties of honey.

This study compares the properties of honeydew honey before and after freezing treatment. The analysis included the evaluation of physico-chemical parameters such as moisture content, water activity, pH value, acidity, hydroxymethylfurfural (HMF) content, diastase activity, sugar content, ash content, and electrical conductivity. Antioxidant properties were assessed by determining total phenolic and flavonoid content, as well as FRAP, ABTS, and DPPH assays, along with HPLC analysis. Sensory evaluation encompassed assessments of color, consistency and texture, odor, taste, and aroma.

The obtained results indicated a decrease in water activity and pH value after freezing, accompanied by an increase in acidity. HMF content and diastase activity exhibited only minor changes compared to the control sample. Additionally, an increase in total flavonoid content and higher FRAP and DPPH values were observed. HPLC analysis revealed elevated levels of catechin and malvidin in the treated sample. Sensory evaluation showed no significant differences in the taste and aroma of honeydew honey following the freezing treatment.

Keywords: honeydew honey, freezing treatment, antioxidant activity, sensory properties.

INTRODUCTION

Freezing food slows down, but does not stop, the physicochemical and biochemical reactions that lead to food spoilage (Rahman, & Velez-Ruiz, 1999). Hermetically sealed honey can be stored at freezing temperatures, as it is considered that this method allows for longer preservation, while having minimal impact on its physicochemical, antioxidant, and antimicrobial properties (Kędzierska-Matysek, et al., 2016).

Lower temperatures slow down chemical reactions in honey, which is particularly noticeable at temperatures below 0 °C (Kędzierska-Matysek, et al., 2016). In addition to halting microbiological growth, freezing temperatures aim to preserve all nutrients present in the food matrix (James, & James, 2014). However, frozen food undergoes changes in its physicochemical properties, most notably in pH value, viscosity, water activity, and redox potential (Rahman, & Velez-Ruiz, 1999).

Low temperatures slow down or completely inhibit the crystallization process in honey, reduce viscosity, and prevent the onset of fermentation (Kędzierska-Matysek, et al., 2016). Most types of honey contain a higher proportion of glucose compared to fructose, which may lead to spontaneous crystallization at room temperature, resulting in the formation of glucose monohydrate. In addition to the glucose-to-fructose ratio, the water content also influences honey

crystallization. Conforti, et al. (2006) found that honey types with higher water content crystallized more at −20 °C, which is the opposite of the results obtained at room temperature.

The aim of this study was to investigate the effect of low temperature on the physicochemical, antioxidant, and sensory properties of honeydew honey.

MATERIAL AND METHODS OF WORK

As the sample for the analysis was used honeydew honey from the territory of the Republic of Srpska, Bosnia and Herzegovina. The untreated honeydew honey was used as the control sample (C), as also reported in the study by Stojković, et al. (2021). The honeydew honey sample was subjected to freezing at temperature -18 °C (sample T). The freezing process involved transferring the honey into plastic screw-cap tubes, followed by storage in a freezer. The honey remained frozen under these conditions until subsequent analysis. The experiment was carried out in triplicates.

The characteristics and satisfactory quality of all samples were assessed through an analysis of the following parameters: water content, diastase activity, HMF content, acidity, reducing sugars, saccharose, electrical conductivity as described by Ordinance on methods for control of honey and other bee products (Official Gazette of BiH no 37/2009, 2009). The pH was measured with a pH meter (Hanna Instruments HI-2211). All the chemicals and reagents used were of analytical grade.

The water activities of samples were analyzed at 25 ± 0.2 C by using the instrument LabMaster-aw (Novasina, Switzerland). The detection limit was ± 0.003 a_w. Calibration was performed with saturated salt solutions in the a_w range of 0.2–0.6.

HPLC analysis: The chemicals and reagents used were of analytical grade. All the standards: catechin hydrate, gallic acid, malvidin, rutin, hlorogenic acid, benzoic acid, quercetin, caffeic acid, ferulic acid and naringenin were of purity > 95%. Measurements were performed on HPLC agilent 1260 infinity (USA) equipped with DAD detector. The HPLC analysis of phenolics was conducted according to Hussein, et al. (2011) method with some modifications. Honey samples (10 g) were dissolved in 50 mL of deionized water and filtered through a membrane filter (Liofilchem, Italy) 0.20 µm. Chromatographic conditions: reversed phase column EC-C18, Poroshell-120 (4.6 x 50 mm, particle size 2.7 µm), the mobile phase 0.1% HAC (solvent A) and acetonitrile (solvent B), flow rate 0.5 mL/min, column temperature 25 °C, sample injection volume 2 µL. The chromatograms were evaluated at λ = 280 nm. The following gradient was used for separation: 9% acetonitrile (B) was flowed through the column isocratically with 91% solvent (A) for 0.8 min which was then increased to 55% acetonitrile (B) for 1.5 min, changed to 40% acetonitrile (B) for 2.3 min, to 30% acetonitrile (B) for 6.8 min, and finally 40% acetonitrile (B) for 8 min. The phenolic compounds were identified by comparing the chromatograms and retention times of the analytes with the reference standards.

Antioxidant activity determination: The total phenolic content was determined using the modified method of Folin-Ciocalteu (Wolfe, et al. 2003), and the content of flavonoids was determined using the method of Ordonez, et al. (2006). The testing of antioxidant activity using the Ferric reducing/Antioxidant power (FRAP) assay was carried out in accordance with Benzie, & Strain (1996); the 2,2-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) assay using the modified method of Re, et al. (1999) and the 2,2 diphenyl-1-picryl-hydrazyl (DPPH) assay using the method of Brand-Williams, et al. (1995) with some modification. A 0.1 mM solution of DPPH (1,1-diphenyl-2-picrylhydrazyl) in methanol was prepared. 1 mL of aqueous honey solution was mixed with 1 mL of DPPH solution. The mixture was left to stand for 30 min in the dark and absorbance was read spectrophotometrically at 517 nm. All the chemicals and reagents used were of analytical grade.

Sensory analysis of the initial and treated honey samples was conducted using a partially modified ,quantitative descriptive evaluation of sensory properties, based on the method described in Grujić (2015), applicable to honey of varying quality levels.

RESULTS AND DISCUSSION

Table 1 presents the results of the physicochemical analysis of honeydew honey before and after freezing treatment. The water content in the control sample was 18.50%, while a slight increase was observed after treatment, reaching 18.74%. Subramanian, et al. (2007) reported a slight increase in water content in samples stored at low temperatures for an extended period, whereas Kędzierska-Matysek, et al. (2016) stated that freezing honey can lead to a reduction in water content, which contrasts with the values obtained in this study. Their analysis showed that honey stored at room temperature had a water content of 18.53%, while freezing at −20 °C reduced it to 18.06%. The water activity (a_w) of the control sample was 0.597, which decreased to 0.587 after the treatment. Scripcă, & Amariei (2021) emphasized that water activity is directly related to the water content in the sample. A higher amount of free water facilitates the onset of fermentation and honey spoilage; therefore, reducing a_w through treatment is beneficial for extending honey's shelf life.

The pH value was 4.33, and after freezing, it decreased to 4.19. In the study by Kędzierska-Matysek, et al. (2016), a similar trend was observed, where the application of freezing led to a decrease in pH from 4.20 to 3.93. Chaikham, & Pranghtip (2015) attribute the reduction in pH to the release of organic acids from pollen during honey processing. The acidity of the control sample was 45.17 mmol/kg, and after freezing, it increased to 45.75 mmol/kg. Similar results were reported by Kędzierska-Matysek, et al. (2016), who measured a total acid content of 18.71 mmol/kg in untreated honey, while freezing at −20 °C led to an increase in acidity to 21.71 mmol/kg.

Based on the presented results, it can be observed that the sucrose content decreased from 2.01% to 1.96%. In contrast to the sugar content, the ash content increased significantly from 0.34 g/100 g to 0.62 g/100 g. These results correlate with the values of electrical conductivity, which measured 1.168 mS/cm in the control sample and increased to 1.235 mS/cm after treatment. Kędzierska-Matysek, et al. (2016) reported that the application of different storage temperatures does not significantly affect electrical conductivity. In their experiment, identical conductivity values were obtained before and after freezing, which was not confirmed in this study.

Table 1. Results of physico-chemical analysis of control and treated sample

Parameter	Control sample	Treated sample
Water content (%)	18.50 ± 0.19	18.74 ± 0.02
Water activity	0.597 ± 0.000	0.587 ± 0.001
pH value	4.33 ± 0.02	4.19 ± 0.00
Acidity (mmol/kg)	45.17 ± 0.29	45.75 ± 0.25
Sucrose content (%)	2.01 ± 0.00	1.96 ± 0.00
Ash content (g/100 g)	0.34 ± 0.01	0.62 ± 0.02
Electrical conductivity (mS/cm)	1.168 ± 0.00	1.235 ± 0.000
HMF content (mg/kg)	5.32 ± 0.25	24.78 ± 0.11
Diastase activity	47.67 ± 0.32	39.51 ± 1.63

Diastase activity and HMF content are indicators of honey freshness and exposure to heat (Fallico, et al., 2008; Hasan, 2013). The HMF content before treatment was 5.32 mg/kg, increasing to 24.78 mg/kg after freezing, which is in accordance with the current Regulation. Piekut, & Baranowska (2001) stated that minimal changes in HMF occur at freezing temperatures. Similarly, Kędzierska-Matysek, et al. (2016) emphasized that honey freshness is preserved under freezing conditions. Basmaci (2010) and Chua, et al. (2013) explained that HMF is formed as a result of the Maillard reaction, through the action of acids on hexoses, and that this process is accelerated at elevated temperatures. In addition to HMF content, honey freshness is also indicated by diastase activity, which decreased from 47.67 before treatment to 39.51 after freezing. Kędzierska-Matysek, et al. (2016) reported that freezing temperatures do not cause significant changes in diastase activity.

Table 2 presents the results of the antioxidant properties of honeydew honey before and after freezing treatment, as well as the results of HPLC analysis. The total phenolic content in the control sample was 1.485 mg GAE/g. After freezing, a slight decrease was observed, with the value reaching 1.415 mg GAE/g. The flavonoid content in the control sample was 0.846 mg GAE/g, and after treatment, a slight reduction was recorded, amounting to 0.714 mg GAE/g. The effect of low temperatures on phenolic compounds present in the product matrix has been examined in a limited number of studies.

Antioxidant activity was evaluated using FRAP, DPPH, and ABTS assays. The FRAP value of the control sample was 8.040 mmol Fe^{2+}/g, and a slight increase was observed after the treatment, reaching 8.916 mmol Fe^{2+}/g. A significant increase was observed in the DPPH value following freezing: the control sample showed 0.186 µg GAE/mg, while after treatment, the value rose to 0.368 µg GAE/mg. An improvement in antioxidant activity was also confirmed through the ABTS assay, expressed as IC_{50} value, which represents the concentration of the sample required to inhibit 50% of free radicals. A greater ability to neutralize free radicals corresponds to lower ABTS values, i.e., lower IC_{50} values indicate higher antioxidant activity (Pontis, et al., 2014). In the control sample, the IC_{50} value was 0.0044 mg/g, which decreased to 0.0038 mg/g after freezing.

Neri, et al. (2020) reported that freezing has a negative impact on the antioxidant properties of fruit purees and juices, while Çubukçu, et al. (2019) analyzed the antioxidant activity of various types of vegetables before and after freezing and found that antioxidant activity increased in some types and decreased in others.

Table 2. Results of antioxidant properties analysis and HPLC analysis

Parameter	Control sample	Treated sample
Total phenolic content (mg GAE/g)	1.485 ± 0.0238	1.415 ± 0.002
Total flavonoid content (mg GAE/g)	0.848 ± 0.005	0.714 ± 0.003
FRAP (mmol Fe2+/g)	8.040 ± 0.081	8.916 ± 0.162
DPPH (µg GAE/mg)	0.186 ± 0.001	0.368 ± 0.002
ABTS (g/mg)	0.0044 ± 0.0006	0.0038 ± 0.0003
Catehin (mg/100 g)	0.804	1.636
Malvidin (mg/100 g)	0.264	0.499
Galic acid (mg/100 g)	0.014	0.077

Table 2 also includes the results of the HPLC analysis. Numerous studies have shown that honey contains a wide variety of phenolic and flavonoid compounds. The composition of these compounds depends on the botanical origin, as well as ecological, seasonal, and processing conditions (Lachman, et al., 2010; Oliveira, et al., 2017). HPLC-DAD analysis of the examined samples confirmed the presence of catechin, malvidin, and gallic acid, with catechin being the most abundant and gallic acid the least abundant compound.

Following the freezing treatment, an increase in the content of all three components was observed. The catechin content increased from 0.804 mg/100 g to 1.636 mg/100 g. The malvidin content rose from 0.264 mg/100 g to 0.499 mg/100 g, while gallic acid content increased from 0.014 mg/100 g to 0.077 mg/100 g. Based on these results, it can be concluded that most analyses indicate an improvement in the antioxidant properties of this honeydew honey as a result of the freezing treatment.

Table 3 presents the results of the sensory analysis of the treated honeydew honey and the differences in individual sensory attributes between the control and treated samples. Honeydew honey is characterized as a dark-colored type of honey (Ngoi, 2016). The honeydew honey used in this study had a dark, homogeneous color. The application of freezing treatment did not significantly affect the color, indicating good preservation of product quality under low-temperature conditions.

The consistency of the control sample was thick and viscous, with fine granules perceptible under the tongue. After treatment, small lumps formed in the structure of the honeydew honey.

This consistency led to uneven dripping from a spoon, as the treated sample was significantly thicker than the control. The aroma of the control sample was described as clean, intense, and highly pleasant. Freezing largely preserved the aroma. The taste of the control sample was described as pleasant and clean, specific to honeydew honey, with no presence of off-flavors or odors. After treatment, the taste and aroma did not change significantly.

The overall impression indicates that freezing may represent an alternative method for preserving the quality and sensory characteristics of honeydew honey.

Table 3. Sensory analysis results of honeydew honey, including the evaluation of differences between the treated and control samples.

Parameter		Control sample	Treated sample
Color	evaluation	5 ± 0.00	4.10 ± 0.22
	difference	-	2.00 ± 0.71
Consistency and texture	evaluation	5 ± 0.00	4.60 ± 0.22
	difference	-	0.80 ± 0.45
Aroma	evaluation	5 ± 0.00	4.40 ± 0.42
	difference	-	1.20 ± 1.10
Taste and odor	evaluation	5 ± 0.00	4.50 ± 0.35
	difference	-	1.20 ± 1.10
Overall impression	evaluation	5 ± 0.00	4.40 ± 0.22
	difference	-	1.20 ± 0.45

CONCLUSIONS

The application of low temperatures represents a suitable alternative method for preserving the quality of honeydew honey, particularly in terms of its sensory and antioxidant properties. Moreover, low-temperature treatment did not negatively affect the majority of physicochemical parameters, with the exception of hydroxymethylfurfural (HMF) content and diastase activity, where an increase in these values was observed. These parameters are commonly associated with prolonged storage or thermal treatment of honeydew honey.

DECLARATIONS OF INTEREST STATEMENT

The authors affirm that there are no conflicts of interest to declare in relation to the research presented in this paper.

LITERATURE

Basmaci, İ. (2010). *Effect of Ultrasound and High Hydrostatic Pressure (HHP) on liquefaction and quality parameters of selected honey varieties.* Doctoral thesis. The graduate school of natural and applied sciences of Middle East Technical University.

Benzie, I. F., & Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Analytical biochemistry*, *239*(1), 70-76.

Brand-Williams, W., Cuvelier, M. E., Barset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT – Food Science and Tehnologie*, *30*(7),748-753.

Chaikham, P., & Prangthip, P. (2015). Alteration of antioxidative properties of longan flower honey after high pressure, ultra-sonic and thermal processing. *Food Bioscience*, 10,1-7.

Chua, L. S., Lee, J. Y., & Chan, G. F. (2013). Honey protein extraction and determination by mass spectrometry. *Analytical and bioanalytical chemistry*, *405*(10), 3063-3074.

Conforti, P. A., Lupano, C. E., Malacalza, N. H., Arias, V., Castells, C. B. (2006). Crystallization of Honey at -20 ˚C. *International Journal of Food Properties, 9*, 99-107.

Çubukçu, H. C., Kılıçaslan, N. S. D., & Durak, İ. (2019). Different effects of heating and freezing treatments on the antioxidant properties of broccoli, cauliflower, garlic and onion. An experimental in vitro study. *Sao Paulo Medical Journal*, *137*(05), 407-413.

Milijaš, M., Žabić, M., & Cvetković, D. (2025). Effect of freezing on physico-chemical, antioxidant, and sensory properties of honeydew honey. *STED Conference 14*(2), 253-258.

Fallico, B., Arena, E., & Zappala, M. (2008). Degradation of 5-hydroxymethylfurfural in honey. *Journal of Food Science*, *73*(9), C625-C631.

Grujić, S. (2015). *Senzorna ocjena kvaliteta i prihvatljivosti prehrambenih proizvoda*. Banja Luka: Tehnološki fakultet, Univerzitet u Banjoj Luci.

Hasan, S. H. (2013). Effect of Storage and Processing Temperatures on Honey Quality *J. Babylon Univ. Pure Appl. Sci, 21*(6), 2244-2253.

Hussein, S. Z., Yusoff, K. M., Makpol, S., & Yusof, Y. A. M. (2011). Antioxidant capacities and total phenolic contents increase with gamma irradiation in two types of Malaysian honey. *Molecules*, *16*(8), 6378-6395.

James, S. J., James, C. (2014). *Chilling and Freezing of Foods*. Food Processing: Principles and Applications, Second Edition. Wiley Library.

Kędzierska-Matysek, M., Florek, M., Wolanciuk, A., & Skałecki, P. (2016). Effect of freezing and room temperatures storage for 18 months on quality of raw rapeseed honey (Brassica napus). *Journal of Food Science and Technology*, *53*(8), 3349-3355.

Lachman, J., Orsák, M., Hejtmánková, A., Kovárová, E. (2010). Evaluation of antioxidant activity and total phenolics of selected Czech honeys. *LWT – Food Science and Technology, 43*(1) 52-58.

Neri, L., Faieta, M., Di Mattia, C., Sacchetti, G., Mastrocola, D., & Pittia, P. (2020). Antioxidant activity in frozen plant foods: Effect of cryoprotectants, freezing process and frozen storage. *Foods*, *9*(12), 1886.

Ngoi, V. (2016): *Effect of processing treatment on antioxidant, physicochemical and enzymatic properties of honey (Trigona spp.).* Universiti Tunku Abdul Rahman, Faculty of Science.

Oliveira, R. G., Jain, S., Luna, A. C., Freitas, L. S., Araujo, L. D. (2017). Screening for quality indicators and phenolic compounds of biotechnological interest in honey samples from six species of stingless bees (Hymenoptera: Apidae). *Food Science and Technology, 37*(4) 552-557.

Official Gazette of BiH no 37/2009. (2009). Ordinance on methods for control of honey and other bee products.

Ordonez, A. A. L., Gomez, J. D., & Vattuone, M. A. (2006). Antioxidant activities of Sechium edule (Jacq.) Swartz extracts. *Food chemistry*, *97*(3), 452-458.

Piekut, J., & Baranowska, E. (2001). Storage of natural bee honeys: descriptive sheets. *Apidologie*, *35*(1), 38-81.

Pontis, J. A., Costa, L. A. M. A., Silva, S. J. R., Flach, A. (2014). Color, phenolic and flavonoid content, and antioxidant activity of honey from Roraima, Brazil. *Food Science and Technology*, *34*(1), 69-73.

Rahman, M. S., & Velez-Ruiz, J. F. (1999). *Food Preservation by Freezing*. Handbook of Food Preservation, Second Edition. CRC Press.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., Rice-Evans, C. (1999). Antioxidant activity applying an improves ABTS radical cation decolorization assay. *Free Radical Biology and Medicine, 26*(9-10), 1231-1237.

Scripcă, L. A., & Amariei, S. (2021). The use of ultrasound for preventing honey crystallization. *Foods*, *10*(4), 773.

Stojković, M., Cvetković, D., Savić, A., Topalić-Trivunović, L., Velemir, A., Papuga, S., & Žabić, M. (2021). Changes in the physicochemical, antioxidant and antibacterial properties of honeydew honey subjected to heat and ultrasound pretreatments. *Journal of Food Science and Technology*, *58*(7), 2555-2566.

Subramanian, R., Umesh Hebbar, H., & Rastogi, N. K. (2007). Processing of honey: a review. *International Journal of Food Properties*, *10*(1), 127-143.

Wolfe, K., Liu, R. H. (2003). Apple peels as a value added food ingredient. *Journal of Agricultural Food Chemistry, 51*(6), 1676-1683.