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THE INFLUENCE OF PREVENTING CRYSTALLIZATION TREATMENT ON THE SELECTED QUALITY PARAMETERS OF HONEY

S u m m a r y

Background. Honey is a popular food product because of its taste, nutritional and health values. It is valued for its rich composition. During storage, honey changes its consistency from liquid to solid. The length of this process varies depending on the botanical origin of honey. The goal of the study was to determine the impact of thermal treatment on the selected qualitative features of honey and to extend the period during which honey remains liquid. The research material was non-purified, raw polyfloral honey. To prevent quick honey crystallization, hot air, microwave radiation and ultrasonic treatment was carried out. In order to identify changes in the structure of honey, its moisture content, hydroxymethylfurfural (HMF) content, the degree of honey crystallization during storage using the measurement of the amount of backscattered light (BS) and thermal properties using differential scanning calorimetry (DSC) were determined.

Results and conclusions. Each of these treatments was accompanied by an increase in the hydroxymethylfurfural content (HMF). However, the HMF content in heat-treated honey still meets the Council Directive 2001/110/EC criteria (< 40 mg/kg). To measure the progress of honey granulation during storage, the amount of backscattered light (BS) was checked. The microwave-heated honey remained in the liquid state for a long time, which was confirmed by a low amount of backscattered light. On the other hand, a common treatment preventing honey granulation, i.e. hot air, was not the most efficient method to prolong the storage time of liquid honey. Moreover, the honey heated with hot air had the highest HMF content and BS amount.

Keywords: honey quality, honey crystallization, thermal treatment, HMF, preventing honey crystallization

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Introduction

Honey is a popular food product because of its taste, nutritional and health values [22, 27]. Its properties result from its complex chemical composition. Honey contains more than 300 components and their number is determined, most of all, by the origin of honey and the type of raw material used by bees for its production [28, 8, 29]. The most important components of honey are: saccharides (glucose and fructose), organic acids (gluconic acid, acetic acid, butyric acid, citric acid, formic acid, lactic acid), nitrogen compounds (amino acids and enzymes), vitamins (C, H, and PP), as well as minerals (calcium, potassium, iron) [10]. Some of the substances contained in honey are sensitive to high temperatures and susceptible to degradation. That is one of the reasons why the prolonged thermal treatment of honey, where the heat transfer medium has a temperature higher than 40 ÷ 45 °C, is not recommended [40].

The quality of honey is affected by numerous factors, depending both on bees and beekeepers collecting it from beehives and processing it. In accordance with the Council Directive 2001/110/EC of 20 December 2001 [13] relating to honey, honey must meet specific criteria to be fit for human consumption. Honey must be free from foreign substances, including food additives, as well as must not exhibit the symptoms of fermentation, and most of all, its chemical composition cannot change as a result of heating. For consumers, organoleptic properties of honey, such as taste, color, consistency and aroma, are the most important features defining its quality. Consumers prefer honey with liquid honey, even if they are aware that granulation is a natural process and that it proves the authenticity of honey. Studies carried out by numerous researchers indicate that the form of honey which is mostly desired by consumers is liquid, translucent and light-colored [38, 32, 30, 21, 19, 24, 41, 35]. Due to the desire to meet consumers' requirements, new solutions are sought, which allow for extending the storage time of liquid honey without adverse effects on its quality.

Producers commonly use the methods of decrystallization and liquefaction of solidified honey to restore its fluidity. However, actions undertaken while collecting honey also can prevent quick solidification of honey. Treatments to be undertaken before honey becomes solid are of interest to scientists, beekeepers and honey producers, who search for new advanced solutions to prevent quick honey granulation.

During prolonged storage, physicochemical transformations leading to crystallization occur in honey [17, 26]. Honey transforms from a liquid form into a solid form (called crystallized, granulated or candied honey) [2]. Crystallization occurs when glucose precipitates from the oversaturated honey solution. It is connected with the lower solubility of glucose than that of fructose. Glucose crystals form into a lattice which keeps the other components in a suspension, thus forming a semisolid form of honey [46, 3, 20]. The rate of the crystallization process depends on many various factors. They include internal factors, such as the origin of honey, its composition or inverted sugars content, and external factors, with temperatures of both processing and storage of honey being of decisive importance [33].

In order to liquefy honey again, thermal treatment called decrystallization should be used [4, 37]. Most frequently, honey is decrystallized by heating in thermal chambers, using electrical decrystallizers or in systems equipped with stirrers [4]. As an alternative for thermal heating, ultrasonic waves may be used. During exposure to ultrasound, the size of glucose crystals decreases, inhibiting the crystallization process. Apart from breaking the glucose crystals, yeast cells are destroyed, which may pose a risk for health safety of honey [5, 14, 37]. Another method for the rapid decrystallization of honey consists in the application of microwaves for its treatment. Apart from the solubilization of glucose crystals, yeast is also eliminated [21].

The storage time during which honey remains liquid can be extended for honey before it becomes solid. As honey is much more appreciated by consumers when it is in a non-crystallized state, the most common methods of preventing crystallization, which are widely used by producers, are as follows: heat treatment at high or very low temperatures, ultrasound, microwave, filtration, ultrafiltration. Research also mentions the use of food additives as a way to prevent transformation from liquid to solid form of honey [2]. Heat treatment is common and easy to carry out, but it can have an impact on quality, since the use of high temperature to honey processing cause 5-hydroxymethylfurfural growth [40]. Storing honey in a freezer at a temperature below -40 °C prevents honey granulation, but it is not economically viable. A granulation rate depends mainly on a fructose-glucose (F/G) ratio and a water content. The more glucose and the higher F/G ratio < 1.2 is, the faster honey granulation takes place [34, 2, 26]. Adding fructose to honey to adjust an F/G ratio can prevent quick crystallization. Similar results can be achieved by adding water. Honey crystallization is prevented by adding granulation inhibitors such as isobutyric and sorbic acid [40]. Amariei et al. [2] added trehalose, a non-reducing disaccharide, a sugar naturally occurring in honey, and a differential scanning calorimetry (DSC) analysis revealed the absence of crystallization process in trehalose-added honey samples at a wide range of temperatures, including also a typical storage temperature. Nevertheless, adding food additives/foreign substances to honey is in contravention of European legislation and such procedures count as honey adulteration [13]. This prompts scientists to look for physical methods to prevent honey crystallization.

The goal of the study was to determine the impact of a thermal treatment to prevent honey crystallization on the selected qualitative features of honey. The treatment should result in the extension of the period during which honey remains liquid. The purpose of this work was to test whether treatments commonly used to liquefy honey can be used to prevent the crystallization of honey when applied on liquid honey before granulation starts.

Materials and Methods

Material

The research material was raw non-purified multifloral honey originating from P.W. Bartnik Kujawski located in Maksymilianowo, Poland. The raw honey was purified by separating impurities on a filter cloth. Next, 50 g of honey was weighed into 100 cm^3 glass beakers. Five specimens were prepared forevery method of honey treatment. Those specimens were subjected to selected operations delaying crystallization. The parameters of the treatments used for the tested specimens are shown in Table 1. The purified honey was used as a control sample. All honey samples were stored at room temperature in the dark for a year.

For all honey samples, the moisture content, the 5-hydroxymethylfurfural (HMF) content, and the amount of light backscattered (BS) by the sample were determined. Thermal properties were tested for the selected variants of honey treatment.

Treatment for the extension of the period during which honey remains in liquid phase

Hot air heating

The honey samples in opened vessels (50 g) were thermally treated in a forced-airflow oven operating at 45 and 50 ºC (SLW 115 STD, Pol-EKO, Poland). Continuous hot air heating was carried out for different periods from 72 to 168 h. A thermal treatment for longer periods was carried out differently, i.e. the samples were heated with hot air for 24 h, and next, stored at room temperature for 24 h repeatedly. Sequential thermal heating was carried out in periods of 168 h and 264 h. At the end of heating, the samples were mixed and the temperature reported as the peak temperature was measured.

Microwave heating

Microwave treatments were conducted in a microwave oven (model: R-93ST-AA, 2450 MHz, maximum power 900 W, Sharp, Germany). The treatment of the honey samples (50 g) was carried out under two power levels – 450 W and 900 W for different periods ranging from 10 to 45 s, which corresponded to power intensity ranging from 9 to 18 W/g. After microwave heating, the samples were mixed and the temperature was measured, which was reported as the peak temperature. The samples were left until reaching room temperature.

Ultrasonic treatment

The ultrasound system was composed of a chamber (Sound Protection Box SB-2-16, Hielscher, Germany), an ultrasonic processor (UP100H, maximum power 100 W, Hielscher, Germany) and a sonotrade (MS 10, 10 mm diameter, maximum amplitude

70 μm Hielscher, Germany). The ultrasonic treatment was carried out at the constant frequency of 30 kHz for different treatment periods and amplitudes (60 %, 80 %, 100 %). At the end of the ultrasonic treatment, the samples (50 g) were mixed and the temperature reported as the peak temperature was measured.

Table 1. Honey treatment variants
Tabela 1. Warianty obróbki miodu

	Treatment type / typ obróbki						
	Heating with hot air, air temperature / Ogrzewanie z wykorzystaniem gorącego powietrza, temperatura powietrza (°C)		Microwave heating, microwave power / Ogrzewanie mikrofalowe, moc mikrofal (W)		Sonication, wave amplitude / Sonikacja, amplituda fali (μm)		
	45	50	450	900	42	56	70
Exposure time / Czas obróbki	120 h	72 h	15 s	10 s	90 s	90 s	90 s
	168 h	120 h	30 s	20 s	180 s	180 s	180 s
	264 h*	168 h*	45 s	30 s	–	–	–

Explanatory notes / Objaśnienia:
*sequential heating at a given temperature for 24 h and another 24 h at 20 °C
*ogrzewanie sekwencyjne w danej temperaturze przez 24 h i kolejne 24 h w temperaturze 20 °C

Methods

Moisture analysis

Honey moisture content (%) was measured with a refractometer (Rudolph J257, USA) at 20 °C. Average refractive index values were converted to honey moisture content using the Chataway Table [9], as cited by Wedmore [45].

HMF analysis

For the determination of 5-hydroxymethylfurfural (HMF) in honey, a spectrophotometric method by Winkler, according to the methodology described by Zappalà et al. [48], was employed. A 10 g portion of honey was transferred to a 50 cm^3 flask and dissolved with distilled water. The 2 ml solution and 5.0 ml of *p*-toluidine solution were put in two different test tubes; 1 ml of distilled water was added to one tube (reference solution) and 1 ml of 0.5 % barbituric acid solution was added to second tube (sample solution). The absorbance of the solutions at 550 nm was determined using a HP/Agilent 8453 UV/Vis Spectrophotometer (USA). HMF measurements were carried out immediately after the treatment (Table 1) and reported in milligrams per kilogram (mg/kg) of honey.

Honey granulation analysis

For the observation of the course of honey crystallization process, a Turbiscan Lab®Thermo (Formulaction, France) was used. The honey crystallization process was followed via an analysis of the backscattered light proportion in the measured sample. When the crystallization process progresses, honey loses its transparency, therefore not only the amount of backscattered light increases, but also the number of crystals in honey is higher. The preparation of samples to determine a backscattered light proportion consisted in placing honey in 50 cm^3 glass vials immediately after the treatment. Measurements were made right after the treatment (Table 1) and after 28 and 56 days of storage. They resulted in a curve showing the ratio of backscattered light (BS) *vs.* vial length. An average increase in the proportion of backscattered light ΔBS was determined (ΔBS/week).

Thermal properties analysis

To investigate the impact of the treatment methods applied on the homogeneity and structure of honey, tests by differential scanning calorimetry (DSC), using a DSC 204 F1 Tarus apparatus from Netzsch (Germany), were carried out on the selected samples of honey. A honey sample having a mass of approx. 30 mg was placed in a punctured aluminum crucible, and measurement was carried out in a nitrogen atmosphere in the temperature ranging from -50 to 180 °C, in the following cycles: cooling to -60 °C at a rate of 10 °C/min, maintaining in an isotherm for 15 min, heating to 180 °C at a rate of 10 °C/min, cooling to 20 °C at a rate of 10 °C/min. On the basis of the course of the thermogram obtained during a heating cycle, glass transition temperature T_g defined as T_{mid} the point on the DSC baseline curve corresponding to 1⁄2 the heat flow difference between the extrapolated onset and extrapolated end (is the midpoint between onset and endset of the inflectional tangent) and T_{onset} defined as a temperature at which the first deviation from the DSC baseline on the low-temperature side was determined. Also, the change in specific heat ΔCp accompanying the glass transition was determined. The values of enthalpy of the endothermic peak were read in the temperature range of 40 ÷ 90 °C (enthalpy of fusion ΔH (J/g)). The DSC measurement of every sample was done in triplicate, and DSC thermograms of the studied honey samples were subjected to various treatments, as shown in Fig. 7 and 8.

Statistical analysis

A one-way variance analysis ANOVA was used to evaluate significant statistical differences among the studied parameters and storage periods. The means were compared using Tukey´s method. Statistical calculations were performed in Microsoft Excel 2016. Significance was defined as $p<0.05$. The correlation coefficient as a measure of linear relationship between ΔBS and ΔH enthalpy of fusion was determined.

Results and discussion

Moisture content

Water and HMF contents in the samples subjected to the treatment to prevent crystallization by heating, exposure to microwaves and sonication, are shown in Table 2. The moisture of honey depends on many factors, such as the harvesting season, a degree of maturity, climatic factors and a post-harvest treatment [16]. An increase or loss in product moisture after exposure to air depends on the temperature, moisture and relative humidity of air [14]. Moisture plays an important role in the resistance of honey to fermentation and granulation (crystallization) during storage. As stated by general recommendations pertaining to honey physicochemical properties, honey moisture should be below 20 % (with the exception of heather honey – 23 %). On average, the moisture of mature honey is below 18.6 % [47, 1]. In this work, the control honey contained 12.2 % of water. Heating a batch of honey with hot air continuously for 72 to 168 h caused the largest decrease in moisture among all tested treatments, in relation to the value determined in the control honey (even by 3.1 %). It results from the fact that the heating with hot air was the longest tested operation performed on the honey, and thus the largest water loss in the samples. Sonication, regardless of the exposure time and the sound wave amplitude, also caused a decrease in the moisture of the samples (Table 2). Similarly, the moisture content decreased after microwave heating. Such a result in relation to honey could be expected because of the heat effect of the treatments applied. Similar observations were made by other researchers, testing heating with air, microwaves and sonication [21, 14, 16, 23]. The proportion of water in honey increased only with long and sequential heating of honey with air having a temperature of 45 °C. It resulted from the hygroscopic properties of honey, which was being heated and cooled interchangeably in an open vessel for 11 days. At that time, honey was absorbing moisture from air. This property results from the presence of fructose, which is a hygroscopic carbohydrate, and the majority of honey is characterized by a fructose to glucose ratio higher than 1 [13].

HMF content

As it is commonly known, there is an adverse effect of food product thermal treatment consisting in the formation of hydroxymethylfurfural (HMF), which is a product of degradation of sugars in an acidic environment, caused by non-enzymatic browning or the Maillard reaction [23]. Prolonged exposure to hot air, microwave radiation and ultrasound was accompanied by an increase in the hydroxymethylfurfural content. The highest HMF content was found in the honey which was heated sequentially with hot air having a temperature of 50 °C for 168 h (20.3 mg/kg), and the content was 25 times higher than that in the control sample. The time of exposure to thermal energy is of importance to the HMF content. In comparison to the other treatments, conventional heating lasted for 168 h, while the time of exposure to microwave radiation and ultrasound was shorter than 180 s, translating into a lower hydroxymethylfurfural content in the honey subjected to short exposure to the heating agent. The HMF content ranged between 3.1 and 15.9 mg/kg. However, one should note that the HMF content in the honey heated conventionally fulfils the Council Directive 2001/110/EC [13] criteria (< 40 mg/kg). In his paper, Kowalski [25] compared the impact of convective and microwave heating on honey features, including the HMF content, and obtained similar dependencies. The longer the convection heating time is, the higher the HMF content in honey can be found. Since microwave heating has a smaller adverse effect on honey properties, it should be treated as an alternative to thermal heating. Many honey producers often treat honey with hot air having a temperature above 60 °C for a prolonged period of time and do not care about overheating. Overheating has an adverse impact on the chemical composition and bioactivity of honey [7]. In studies on the decrystallization of honey using ultrasound and convection heating, indeed, honey subjected to the latter contained more HMF [42].

Out of the samples subjected to the tested treatment, the smallest amount of HMF was formed as a result of the sonication of honey at the lowest wave amplitude. Exposure to ultrasound for 90 and 180 s led to HMF contents in honey, equal to 3.4 and 4.1 mg/kg, respectively (Table 2). As a result of ultrasound waves generated by an ultrasonic head with a diameter of 10 mm and an amplitude of 42 μm, the amount of energy transferred into honey was 8.1 and 16.2 kJ. In his studies, D'Arcy [14] proved that decrystallization using an ultrasonic head generating ultrasound with an amplitude of 12 μm, carrying 70 kJ, an increase in the HMF content was small (up to 4.2 mg/kg) and comparable with the values of this parameters obtained in honey heated conventionally at 55 °C for 16 h or at 72 °C for 120 s. Scripcă and Amariei [37] also tested HMF in honey treated with ultrasound waves, and the treatment did not increase the HMF concentration above 40 mg/kg, with honey remaining in a liquid state for a long time.

The concentration of hydroxymethylfurfural is one of the most important quality factors of honey. Even if honey remains liquid, its composition may change during storage, which might affect the overall quality of honey. During storage, the enzymatic activity and content of polyphenols may decrease. The change of those properties, together with HMF concentration, can show the adverse effect of thermal treatment or storage. The diastatic activity can be used as an indicator similar to HMF. As is the case with HMF, it can demonstrate aging and the increase of temperature during storage or intentional thermal treatment. Heating above 60 °C and for a long period of storage may reduce the enzymatic activity [15]. Storage affects the antioxidant properties of honey. Šarič et al. [36] observed a decrease in the total phenolic and flavonoid content of honey, as well as its antioxidant activity, during one year of storage.

Table 2. Physicochemical properties of honey after different treatments
Tabela 2. Właściwości fizykochemiczne miodu po różnych obróbkach

Treatment type / Typ obróbki	Peak temperature / Temperatura maksymalna (°C)	Moisture / Wilgotność	HMF (mg/kg)
Control / Kontrolna	22.1	12.2^{b}	0.8^{h}
HA/45 °C/120 h	45.0	9.7^{e}	14.4^{c}
HA/45 °C/168 h	45.0	9.1^{f}	16.3^{b}
HA/45 °C/264 h*	45.0	14.2^{a}	16.9^{b}
HA/50 °C/72 h	50.0	9.0^{f}	18.5^{a}
HA/50 °C/120 h	50.0	10.5^{e}	18.9^{a}
HA/50 °C/168 h*	50.0	12.3^{b}	20.3^{a}
M/450 W/15 s	58.3	12.3^{b}	6.5^{e}
M/450 W/30 s	65.9	11.9^{b}	10.2^{d}
M/450 W/45 s	97.1	11.7^{bc}	12.9^{c}
M/900 W/10 s	41.2	11.9^{b}	5.4^{eg}
M/900 W/20 s	76.8	11.8^{bc}	8.9^{d}
M/900 W/30 s	96.2	11.2^{c}	10.1^{d}
S/42 µm/90 s	24.3	11.6^{c}	3.1^{g}
S/42 µm/180 s	25.8	10.9^{d}	4.3^{g}
S/56 µm/90 s	27.0	11.2^{c}	6.8^{e}
S/56 µm/180 s	28.7	10.7^{d}	7.0^{e}
S/70 µm/90 s	32.5	10.0^{e}	10.4^{d}
S/70 µm/180 s	33.2	10.5^{e}	15.7^{bc}

Explanatory notes / Objaśnienia:
a, b, c... – average values in columns denoted with the same letters do not differ statistically significantly at $p < 0.05$; *sequential heating at a given temperature for 24 h and another 24 h at 20 °C; **capital letters identify the treatment type (HA – heating with hot air; M – microwave heating; S – sonication), the second symbol indicates treatment conditions (temperature, microwave power, wave amplitude), the third mark is time of exposure to treatment;
a, b, c… – wartości średnie w kolumnach oznaczonych tymi samymi literami nie różnią się istotnie statystycznie przy $p < 0,05$; *ogrzewanie sekwencyjne w danej temperaturze przez 24 h i kolejne 24 h w temperaturze 20 °C; **wielkie litery oznaczają rodzaj zabiegu (HA – ogrzewanie gorącym powietrzem; M – ogrzewanie mikrofalami; S – sonikacja), drugi symbol oznacza warunki zabiegu (temperatura, moc mikrofal, amplituda fali), trzeci znak to czas ekspozycji na obróbkę.

Peak temperature

The value of the lowest temperature reached by a sample depended on the applied power level and treatment duration. The temperature slightly increased as a result of sonication. The honey subjected to ultrasonic waves reached the maximum temperature of 33.2 °C (Table 2). These values are lower than those described in the literature on honey treated with ultrasound [42]. It results from other parameters and equipment used for the heating of samples.

Prolonged heating with hot air caused an increase in the temperature of honey to 45 ÷ 50 °C. The highest temperatures were reached by the honey samples subjected to microwave radiation. Immediately after microwave heating for 10 to 45 s, the honey samples had temperatures of 41.2 to 97.1 °C. Hebbar et al. [21] used microwave radiation with the power of 425 W and 850 Wfor the same treatment time, and their samples reached a temperature lower by approx. 8 °C. It should be taken into account that the microwave treatment caused an increase in the sample temperature above the maximum recommended treatment temperature, but because of the short exposure time, it did not result in exceeding the limit values of the HMF content in the samples (Table 2). A slowdown of the crystallization of honey by thermal treatment at a high temperature is not accepted as a current practice within the quality standards [44, 40]. There is a small number of studies on the application of high-temperature short-time (HTST) treatment in relation to honey, but they indicate the validity of this type of treatment because of yeast eradication and a small impact on the qualitative features of honey [44].

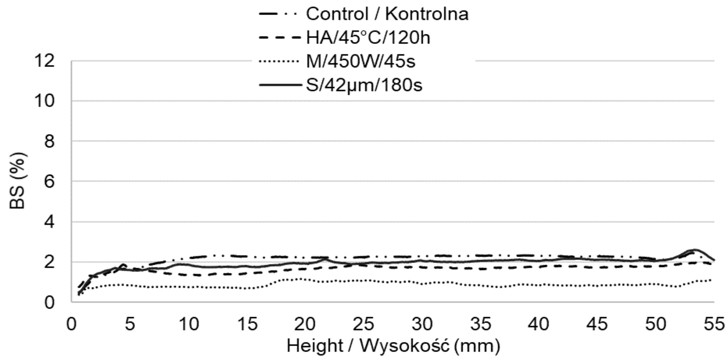
Prolonged storage time is accompanied by an increase in the backscattered light value for all variants of honey (Fig. 1). It means that during storage, more and more crystals of monosaccharides form in honey, or honey transforms from its liquid form into a solid form. The heating of the honey in hot air for 120 h to 168 h influenced the clarity of the honey at the beginning of the storage period significantly, which is reflected in lower BS values. The values of the average proportions of backscattered light of the air-heated honeys after 28 and 56 days of storage were higher compared to those of the control sample. This indicates a higher proportion of monosaccharide crystals present in the honey (Table 3). The sonication of honey did not affect the proportion of backscattered light so significantly in comparison with the BS amount for the control honey, therefore it may be concluded that the crystallization of both types of honey occurred at the same rate. It is proved by calculated weekly increases in the proportion of backscattered light, having values close to the control sample and the sonicated honey (Table 3). The lack of significant impact of sonication on the extension of time during which honey remains in liquid form indicates a necessity for further studies on the parameters of the liquefaction process using ultrasound. The lowest BS amounts characterized the honey heated with microwaves. It pertains to the whole storage period. After one-year storage, the honey subjected to microwave heating for a time longer than 20 s remained liquid (Fig. 4 and 5), while the honey samples treated with hot air and sonicate changed their state and became solid (Fig. 2, 3 and 6).

Table 3. Proportion of light backscattered by samples of honey subjected to various treatments
Tabela 3. Udział światła rozproszonego wstecznie w próbkach miodu poddanych różnym obróbką

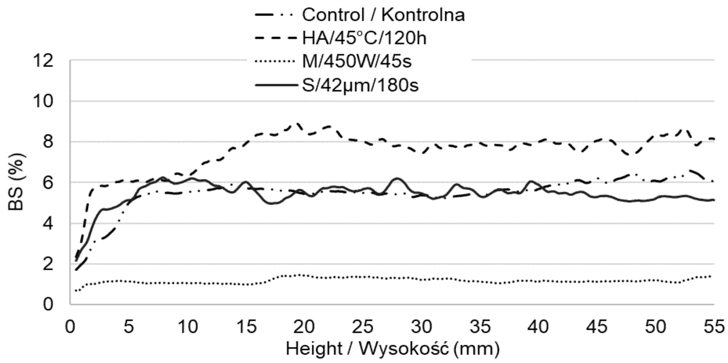
Treatment type / Typ obróbki	Average proportion of backscattered light during storage / Średni udział światła rozproszonego wstecznie podczas przechowywania (%)			
	Storage time (days) / Czas przechowywania (dni)			Average increase in the proportion of backscattered light ΔBS/week / Średni wzrost udziału światła rozproszonego wstecznie ΔBS/tydzień
	1	28	56	
Control / Kontrolna	2.13^{d}	5.46^{c}	6.50^{c}	0.55
HA/45 °C/120 h	1.65^{c}	7.52^{de}	8.56^{e}	0.86
HA/45 °C/168 h	1.62^{c}	7.25^{d}	8.28^{e}	0.83
HA/45 °C/264 h*	2.99^{e}	9.40^{f}	10.78^{f}	0.97
HA/50 °C/72 h	2.19^{d}	6.11^{cd}	7.29^{d}	0.64
HA/50 °C/120 h	1.81^{c}	5.67^{c}	6.75^{cd}	0.62
HA/50 °C/168 h*	2.04^{d}	8.22^{e}	9.15^{e}	0.89
M/450 W/15 s	0.86^{ab}	1.19^{b}	1.59^{b}	0.09
M/450 W/30 s	0.44^{a}	1.12^{b}	1.58^{b}	0.14
M/450 W/45 s	0.54^{ab}	0.64^{a}	0.85^{a}	0.04
M/900 W/10 s	1.28^{bc}	1.58^{b}	1.74^{b}	0.06
M/900 W/20 s	0.92^{b}	1.25^{b}	1.41^{b}	0.06
M/900 W/30 s	0.35^{a}	0.50^{a}	0.92^{a}	0.07
S/42 μm/90 s	1.94^{d}	5.43^{c}	6.47^{c}	0.57
S/42 μm/180 s	1.87^{c}	5.47^{c}	6.41^{c}	0.57
S/56 μm/90 s	1.84^{c}	5.41^{c}	6.34^{c}	0.56
S/56 μm/180 s	1.88^{c}	5.63^{c}	6.63^{c}	0.59
S/70 μm/90 s	2.07^{d}	5.76^{c}	6.63^{c}	0.55
S/70 μm/180 s	1.73^{c}	5.73^{c}	6.63^{c}	0.86

Explanatory notes / Objaśnienia:
a, b, c... – average values in columns denoted with the same letters do not differ statistically significantly at $p < 0.05$; *sequential heating at a given temperature for 24 h and another 24 h at 20 °C; **capital letters identify the treatment type (HA – heating with hot air; M – microwave heating; S – sonication), the second symbol indicates treatment conditions (temperature, microwave power, wave amplitude), the third mark is time exposure to treatment;
a, b, c… – wartości średnie w kolumnach oznaczonych tymi samymi literami nie różnią się istotnie statystycznie przy $p < 0,05$; *ogrzewanie sekwencyjne w danej temperaturze przez 24 h i kolejne 24 h w temperaturze 20 °C; **wielkie litery oznaczają rodzaj zabiegu (HA – ogrzewanie gorącym powietrzem; M – ogrzewanie mikrofalami; S – sonikacja), drugi symbol oznacza warunki zabiegu (temperatura, moc mikrofal, amplituda fali), trzeci znak to czas ekspozycji na obróbkę.

A



B



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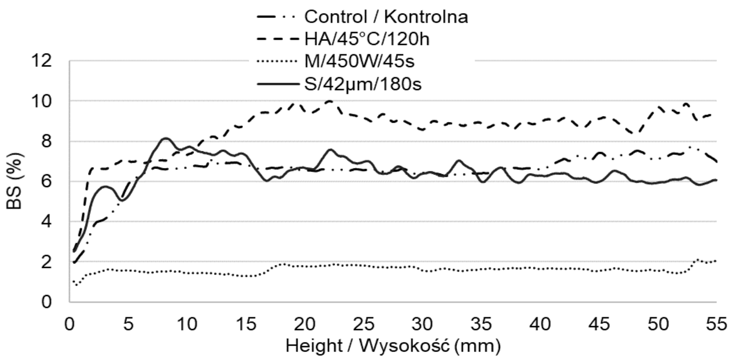

Fig. 1. Examples of plots of backscattered light *vs.* the height of the honey vial (A – day 1 of storage, B – day 28 of storage, C – day 58 of storage).

Rys. 1. Przykładowe wykresy światła rozproszonego wstecznie w zależności od wysokości fiolki z miodem (A – 1 dzień przechowywania, B – 28 dzień przechowywania, C – 58 dzień przechowywania).

Explanatory notes / Objaśnienia:

*capital letters identify the treatment type (HA – heating with hot air; M – microwave heating; S – sonica-

tion), the second symbol indicates treatment conditions (temperature, microwave power, wave amplitude), the third mark is the time of exposure to treatment /
*wielkie litery określają rodzaj zabiegu (HA – ogrzewanie gorącym powietrzem); M – ogrzewanie mikrofalowe; S – sonikacja), drugi symbol oznacza warunki zabiegu (temperatura, moc mikrofal, amplituda fali), trzeci znak to czas ekspozycji na obróbkę.

Fig. 2. The comparison of honey samples heated with hot air (HA) at a temperature of 45 °C in different time exposure and stored for 12 months (*sequential heating at a given temperature for 24 h and another 24 h at 20 °C)

Rys. 2. Porównanie próbek miodu ogrzanych gorącym powietrzem (HA) o temperaturze 45 °C w różnym czasie ekspozycji i przechowywanych przez 12 miesięcy (*ogrzewanie sekwencyjne w danej temperaturze przez 24 h i kolejne 24 h w 20 °C)

Fig. 3. The comparison of honey samples heated with hot air (HA) at a temperature of 50 °C in different time exposure and stored for 12 months (*sequential heating at a given temperature for 24 h and another 24 h at 20 °C)

Rys. 3. Porównanie próbek miodu ogrzanych gorącym powietrzem (HA) o temperaturze 50 °C w różnym czasie ekspozycji i przechowywanych przez 12 miesięcy (*ogrzewanie sekwencyjne w danej temperaturze przez 24 h i kolejne 24 h w 20 °C)

Fig. 4. The comparison of honey samples treated with microwaves (M) having a power of 450 W in different time exposure and stored for 12 months

Rys. 4. Porównanie próbek miodu poddanych działaniu mikrofal (M) o mocy 450 W w różnym czasie ekspozycji i przechowywanych przez 12 miesięcy

Fig. 5. The comparison of honey samples treated with microwaves having a power of 900 W in different time exposure and stored for 12 months

Rys. 5. Porównanie próbek miodu poddanych działaniu mikrofal o mocy 900 W w różnym czasie ekspozycji i przechowywanych przez 12 miesięcy

Fig. 6. The comparison of honey samples treated with ultrasound (S) at a different wave amplitude and time exposure and stored for 12 months

Rys. 6. Porównanie próbek miodu poddanych działaniu ultradźwięków (S) o różnej amplitudzie fali i czasie ekspozycji, przechowywanych przez 12 miesięcy

Thermal properties of honey investigated by DSC

Fig. 7 shows DSC thermograms of honeys subjected to various thermal treatments, recorded by heating from -50 to 180 °C. The value of glass transition temperature of the honeys studied, determined as T_{onset}, changed in the range from –40.9 to -34.9 °C, and defined as T_{mid}, changed in the range from -38.6 to -31.7 °C. Glass transition occurs during the transformation of material from a rubbery like state into a hard state and *vice versa*. This phenomenon is well-known in polymer materials [39, 6] and we find it also in honey [11]. The occurrence of glass transition is connected with a disordered amorphous form of the sample studied. The determined T_g values are consistent with the literature data, determined for similar samples [11, 12, 18, 43]. The T_g value is affected by the presence or lack of sugar crystals. The control sample was characterized by a glass transition temperature, which was 6 ÷ 7 °C lower than that of the honey subjected to the microwave treatment, which had a liquid form when examined. Similar T_g changes were shown in the report by Tomaszewska-Gras et al. [43], where changes in the glass transition temperature of honeys before and after decrystallization were described. T_g of solid honey was 6 ÷ 11 °C lower than the liquid one.

Cordella et al. [11] reported that glass transition is often linked with a relaxation effect observed as a value of ΔCp, which depends on the thermal history of samples. In Fig. 7., high ΔCp values refer to the samples with a high temperature peak, especially a microwave treatment (Table 2.). It seems that an analysis of DSC thermogram, particularly the curve section with glass transition, might be helpful in tracking adulterations, especially those related to the overheating of honey.

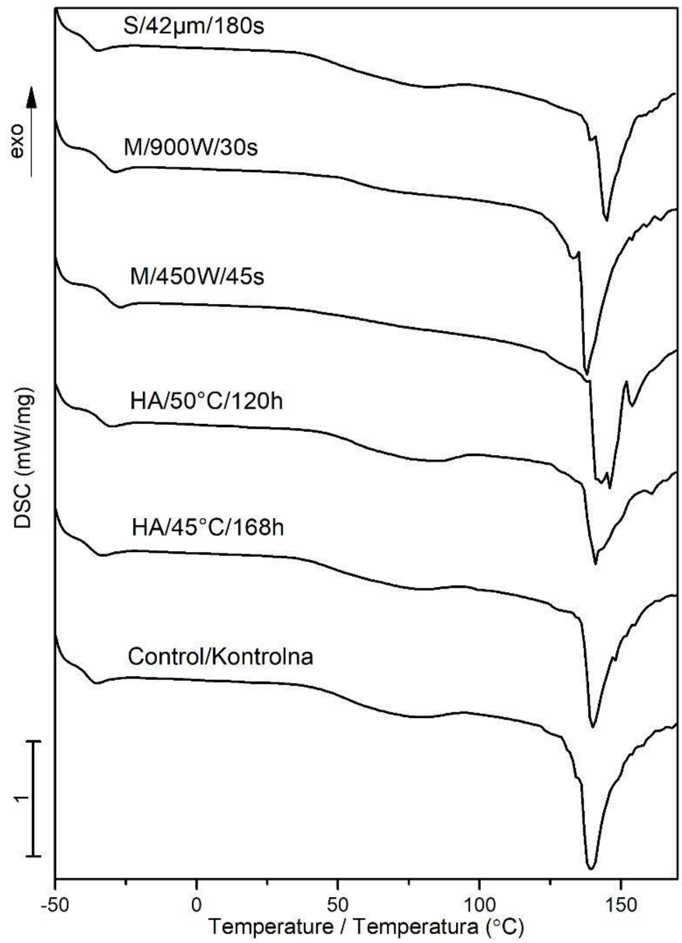

Fig. 7. A differential scanning calorimetry (DSC) heating thermogram of honeys
Rys. 7. Termogram ogrzewania miodów z różnicowej kalorymetrii skaningowej (DSC)
Explanatory notes / Objaśnienia:
*capital letters identify the treatment type (HA- heating with hot air; M- microwave heating; S- sonication), the second symbol indicates treatment conditions (temperature, microwave power, wave amplitude), the third mark is the time of exposure to treatment /
*wielkie litery określają rodzaj zabiegu (HA – ogrzewanie gorącym powietrzem; M – ogrzewanie mikrofalami; S – sonikacja), drugi symbol oznacza warunki zabiegu (temperatura, moc mikrofal, amplituda fali), trzecim znakiem jest czas ekspozycji na obróbkę

Table 4. Thermodynamic properties of honeys
Tabela 4. Właściwości termodynamiczne miodów

Treatment type / Typ obróbki	Thermal phenomena / Zjawiska termiczne			
	Glass transition / Przejście szkliste			Enthalpy of fusion / Entalpia topnienia ΔH ($J \cdot g^{-1}$)
	T_g (°C)		ΔC_p ($J \cdot g^{-1} \cdot K^{-1}$)	
	T_{onset}	T_{mid}		
Control / Kontrolna	-40.9	-38.6	0.470	-22.62
HA/45 °C/168 h	-40.3	-36.3	0.643	-19.31
HA/50 °C/120 h	-37.2	-33.4	0.782	-20.63
M/450 W/45 s	-34.9	-31.7	0.809	-0.49
M/900 W/30 s	-37.1	-32.6	1.273	-6.47
S/42 µm/180 s	-40.7	-37.2	0.731	-21.19

Explanatory notes / Objaśnienia:
*capital letters identify the treatment type (HA- heating with hot air; M- microwave heating; S- sonication), the second symbol indicates treatment conditions (temperature, microwave power, wave amplitude), the third mark is the time of exposure to treatment /
*wielkie litery oznaczają rodzaj zabiegu (HA – ogrzewanie gorącym powietrzem; M – ogrzewanie mikrofalami; S – sonikacja), drugi symbol oznacza warunki zabiegu (temperatura, moc mikrofal, amplituda fali), trzeci znak to czas ekspozycji na obróbkę

The difference of T_g of the honeys studied is affected by the treatment type. One may note the dependence of the T_g value on the peak temperature, or temperature reached by honey after a treatment (Table 2). The honey which reached the highest peak temperature during a treatment, is characterized by the higher values of T_g and ΔCp (Table 4 and Fig 8). The increase in the peak temperature is accompanied by increases in T_g and ΔCp values.

The thermogram section between 40 and 100 °C responds to the melting of sugar crystals [11, 17]. Enthalpy ΔH is higher, the lower peak temperature after treatment was obtained. This reference corresponds to the BS amount as well (Table 2.). The correlation between ΔBS and ΔH was determined, and the correlation coefficient was r = -0.9, which shows a strong negative linear relationship. The value of enthalpy indicates a progressing process of crystallization. The higher ΔH is, the more intense melting of sugar crystals is seen, hence the higher enthalpy value refers to a more intense crystallization process, which took place in the honey samples during the storage period [2].

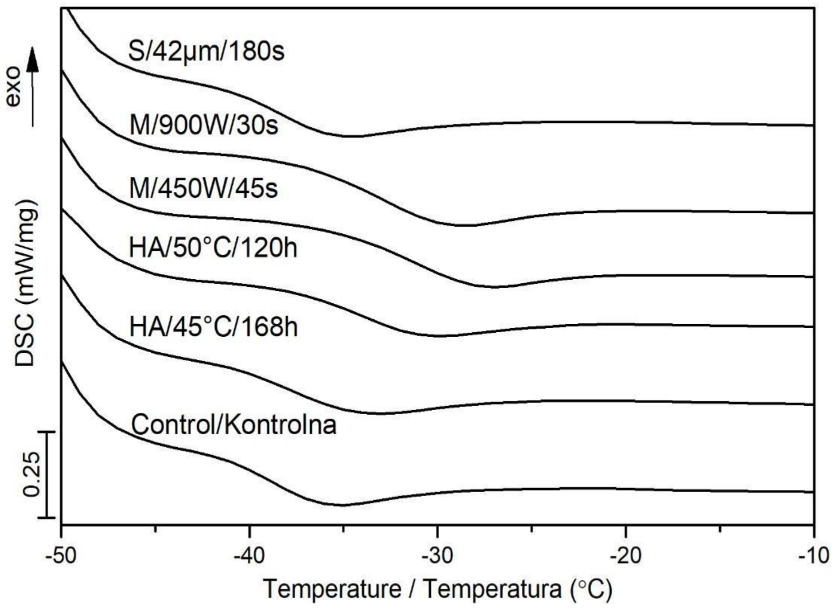

Fig. 8. A view on glass transition obtained by the differential scanning calorimetry (DSC) heating thermogram of honeys

Rys. 8. Widok przejścia szklistego uzyskany metodą różnicowej kalorymetrii skaningowej (DSC) termogram ogrzewania miodów

Explanatory notes / Objaśnienia:

*capital letters identify the treatment type (HA- heating with hot air; M- microwave heating; S- sonication), the second symbol indicates treatment conditions (temperature, microwave power, wave amplitude), the third mark is the time of exposure to treatment

*wielkie litery oznaczają rodzaj zabiegu (HA – ogrzewanie gorącym powietrzem; M – ogrzewanie mikrofalami; S – sonikacja), drugi symbol oznacza warunki zabiegu (temperatura) , moc mikrofal, amplituda fali), trzecim znak jest czas ekspozycji na obróbkę

Conclusions

1. All the treatment methods carried out delayed the honey crystallization compared to the untreated control sample. However, the sequential heating of honey with dry hot air is the least effective method to prevent honey crystallization. Prolonged exposure to hot air, microwave radiation and ultrasound is accompanied by an increase in the hydroxymethylfurfural content. None of the treatments increased the HMF concentration above 40 mg/kg, allowing the honeys to meet the Council Directive 2001/110/EC [13] criteria.
2. The measurement of backscattered light (BS) is a good indicator of the rating of honey crystallization process. During the BS measurement, formed sugar crystals can be observed. That is why the BS measurement is a good tool to observe changes in the honey structure.

3. Changes in the honey structure can be also studied by a DSC analysis. The T_g value is affected by the presence or lack of sugar crystals. The T_g value, being strongly dependent on the amorphous phases of a sample, is correlated with the modification of physical properties and chemical composition, and the disclosed structural modification caused by the unsuitable industry conditions (overheating). The analysis of DSC thermogram, particularly the curve section with glass transition, might be helpful in tracking adulterations, especially those related to the overheating of honey. The proven strong negative linear relationship between ΔBS and ΔH shows the possibility of the potential use of backscattered light measurements instead of DSC measurements for the observation of honey crystallization progress.
4. The most favorable operation to prevent honey crystallization in relation to the HMF content and delay the granulation process, which may be performed on honey, is microwave heating. The microwave-heated honey remains in a liquid state for a long time, which is confirmed by the low values of the backscattered light proportion and the lowest glass transition temperature, while the HMF level is not increasing as rapidly as in the case of heating honey with dry hot air.

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WPŁYW ZABIEGÓW OPÓŹNIAJĄCYCH KRYSTALIZACJĘ NA WYBRANE WŁAŚCIWOŚCI MIODU PSZCZELEGO

S t r e s z c z e n i e

Wprowadzenie. Miód jest popularnym produktem spożywczym ze względu na swoje walory smakowe, odżywcze i zdrowotne. Ceniony jest również za bogaty skład. W trakcie przechowywania miód zmienia swoją konsystencję z płynnej na stałą. Długość tego procesu różni się w zależności od botanicznego pochodzenia miodu. Celem badań było określenie wpływu obróbki termicznej na wybrane cechy jakościowe miodu i wydłużenie okresu jego utrzymywania się w stanie płynnym. Materiał badawczy stanowił nieoczyszczony, surowy miód wielokwiatowy. Aby zapobiec szybkiej krystalizacji miodu, przeprowadzono obróbkę gorącym powietrzem, promieniowaniem mikrofalowym i ultradźwiękami. W celu określenia zmian w strukturze miodu oznaczono jego wilgotność, zawartość hydroksymetylofurfuralu (HMF), stopnia krystalizacji miodu podczas przechowywania z wykorzystaniem pomiaru ilości światła rozproszonego wstecznie (BS) oraz właściwości termiczne z wykorzystaniem dyferencyjnej kalorymetrii skaningowej (DSC).

Wyniki i wnioski. Każdemu z tych zabiegów towarzyszył wzrost zawartości hydroksymetylofurfuralu (HMF). Jednakże zawartość HMF w miodach podgrzewanych spełniała kryteria Dyrektywy Rady 2001/110/WE (< 40 mg/kg). W celu pomiaru postępu granulacji miodu podczas przechowywania sprawdzano ilość światła rozproszonego wstecznie (BS). Miód podgrzewany mikrofalowo długo pozostawał w stanie ciekłym, co potwierdza niskie wartości udziału światła rozproszonego wstecznie. Z drugiej strony zastosowanie powszechnego zabiegu zapobiegające granulacji miodu – gorącym powietrzem, nie było najskuteczniejsze w wydłużaniu czasu przechowywania miodu płynnego. Ponadto miód podgrzewany gorącym powietrzem charakteryzował się najwyższymi wartościami HMF i BS.

Słowa kluczowe: jakość miodu, krystalizacja miodu, obróbka termiczna, HMF, zapobieganie krystalizacji miodu