Diastase Number Changes During Thermal and Microwave Processing of Honey

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Abstract

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The presented paper covers the preliminary studies on microwave inactivation of honey enzymes described as diastase number (DN). All the investigations were done on commercially available honey from Polish local market. Microwave processes were compared to the conventional ones. In the case of conventional conditions, the constant rate of diastase enzyme inactivation was estimated using the first order kinetics. In the case of microwave heated samples, it was impossible to establish the rate constant; however, the investigation proved the suitability of such kind of processing for short-term thermal treatment of honey.

Keywords: honey; microwave; enzymes; heating

Honey belongs to the group of complex foodstuffs with a high nutritional potential. The quality of honey is strictly linked to its chemical composition (Devillers *et al.* 2004). On the other hand, the tendency to carbohydrate crystallisation in honey is very often unacceptable by consumers and causes same technological hindrance.

It is well known that honey as a natural product may be processed by means of thermal treatment for two main reasons: first of all, to destroy the micro-organisms that may contaminate it and to modify its tendency to crystallisation or delay the appearance of monosaccharide crystals (Tosi *et al.* 2002). During crystallisation that leads to phase separation, both liquid and non-liquid phases may coexist. Simultaneously, water activity of the remaining liquid phase begins to increase. This is the result of water release during crystallisation and subsequent decrease of carbohydrates concentration in the liquid phase. This phenomenon makes the honey suitable for the growth of microorganisms like yeasts and fungi and leads to sensory properties modifications and quality damage (Tosi *et al.* 2004).

In order to solve this problem, a thermal treatment of honey is needed. On the other hand, any thermal processing of honey may also result in product quality deterioration. Uncontrolled heating influences the parameters such as hydroxymethylfurfural (HMF) content and enzymatic activity and results in increasing or decreasing these parameters, respectively (Subramanian *et al.* 2007).

The conventional honey processing includes preheating (to about 40°C), straining, clarification by means of filtration, and indirect heating of the product at 60–65°C for 25–30 min (Subramanian

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et al. 2007). On the other hand, higher temperature may cause enzyme inactivation (Whitehurst & Law 2001). Any type of honey possesses several kinds of enzyme that play both nutritional and analytical role in the product. One of the most important honey enzymes is diastase that is capable to break down glycosidic linkages in oligo- and polysaccharides. The activity of this enzyme decreases with the time of storage and that of heating. Diastase activity is measurable and is expressed as the Diastase Number (Hooper 1983).

The starch-digesting enzymes of honey are used as an indicators of honey quality because of their heat sensitivity (Subramanian *et al.* 2007). There is a lot of information on the influence of higher temperatures on HMF formation in honey (Tosi *et al.* 2002; Fallico *et al.* 2004; Tosi *et al.* 2004; Turhan *et al.* 2008) as well as diastase activity changes (Tosi *et al.* 2004, 2008). According to the Honey Quality and International Regulatory Standards from the International Honey Commission, the diastase activity must be not lower than or equal to 8 when expressed as diastase number (Bogdanov 2002).

Tosi *et al.* (2004) have found that it is a possible to inhibit crystallisation for up to 4 weeks or even 9 weeks in honey with a high tendency to crystallisation by applying high temperature for a short time, i.e. 80°C during 60 s in the transient stage, and 30 s in the isothermal stage, respectively. Under the experimental conditions, the temperatures from 80°C to 140°C applied for very short time did not cause any unfavourable effects on honey in view of HMF and DN changes (Tosi *et al.* 2004).

In the field of food technology, modern techniques of heating are widely used. These include ohmic, radio frequency, and infrared heating, pressure and pH assisted thermal processing, as well as microwaves irradiation (Sun 2005).

Microwave heating is a new, selective technique for short and intensive thermal processing. The acceleration of heating by microwaves has been proven, and in many cases, microwave techniques appears more effective than the conventional processing. The phenomenon originates in the interaction of the electromagnetic field with the matter by means of dielectric and/or conducting mechanisms (Kingston & Haswell 1997). Microwave heating is greatly affected by the presence of water in foods. Materials containing polar molecules, like water, are rapidly heated when exposed to microwave radiation due to molecular friction generated by dipol rotation in the presence of an alternating electric field. It is also reported that dissolved sugars are the main microwave susceptors in many foodstuff like syrups etc. (Subramanian *et al.* 2007). The application of microwave heating is well known in the food industry, particularly for tempering, blanching, drying, and pasteurisation of the respective materials (Decareau & Schweigert 1985).

Microwave processing can be considered a fast method of foodstuffs heating. On the other hand, some reports indicate the inhibition of enzymes during such processing (Günes & Bayindirli 1993; Kermasha *et al.* 1993; Tajchakavit & Ramaswamy 1997; Huang *et al.* 2007; Matsui *et al.* 2007, 2008), which is highly recommended in many cases in food technology. Using peroxidase and lipoxygenase as indicators of microwave blanching of peas, green beans and carrots, it has been found out that the inactivation of these enzymes follows the first order kinetic model. It has also been stated that microwave processing is more appropriate for the retention of ascorbic acid then blanching in water bath (conventional method) (Günes & Bayindirli 1993). Microwave processing is also suitable for the inactivation of enzymes responsible for vitamin C and tea polyphenols oxidation as well as chlorophyll decomposition in green tea. In these cases, the authors obtained better quality parameters of the product during storage then after the traditional thermal treatment (oven heating) (Huang *et al.* 2007). Kermasha *et al.* (1993) described a process of soybean lipoxygenase inactivation using first-order reaction kinetics. Microwave treatment resulted in sharp inactivation of the enzyme. The authors explained the higher enzyme inactivation rates in the microwave processing by possible non-thermal effects (Kermasha *et al.* 1993). Under the microwave heating conditions, non-thermal effects probably took a part in the inactivation of pectin methylesterase in orange juice. In this case, the first-order reaction kinetics was also suitable for describing the process (Tajchakavit & Ramaswamy 1997). Microwave heating was also incorporated for the inactivation of peroxidase and polyphenol oxidase in coconut water in order to hinder the decrease of nutritional value and colour losses (Matsui *et al.* 2007, 2008).

The aim of the present work was to find out if microwave processing can affect one of the main honey quality parameters – diastase enzymes activity.

Table 1. Coding of the all investigated honey samples

Honey type	Country of origin	Code
Honeydew	Poland	M1
Honeydew	Poland	M2
Forest	Italy	M3
Honeydew	Poland	M4
Aegean	Turkey	M5

MATERIAL AND METHODS

Five samples of honeydew honey were obtained from different producers at the local market in Poland and were encoded from M1 to M5 (Table 1).

Analytical procedures.The determinations of specific rotation, electrical conductivity, moisture (by means of refractometric method), pH, and free, total and lactones acidity were carried out according to Harmonized Methods of the International Honey Commission (Bogdanov 2002).

High Performance Liquid Chromatography was used to determine carbohydrate composition as well as HMF content in all the samples (Lupano 1997; Bogdanov 2002). For carbohydrate determination, Knauer chromatograph equipped with Lichrospher 100-10 NH_2 column and RI detector was used. The flow rate of the mobile phase was set to 1.3 ml/min of acetonitrile:water (87:13, v/v). The sample volume was 10 µl. Honey solution (50 mg/ml) was injected after filtration through membrane of filter – pore size 0.45 µm. In the case of HMF content determination, Lichrospher RP-18 column and UV detector (285 nm) were applied. The flow rate was set up to 1 ml/min using the mobile phase of methanol:water (1:9, v/v). The sample volume was 10 µl and honey solution (200 mg/ml) was injected after filtration through membrane of filter – pore size 0.45 µm.

Diastase number (DN), after Schade, was determined spectrophotometrically using UVS-2800 Labomed spectrophotometer. Diastase activity, in Schade scale, is defined as the amount of starch (g) hydrolysed during 1 h at 40°C per 100 g of honey (Bogdanov 2002).

Heating procedure and deactivation kinetics. Conventional heating of the honey samples (50 g) was conducted in a water bath at 90°C for 20, 40, and 60 min (isothermal heating) without stirring.

Microwave processing of the honey samples (50 g) was performed without stirring in a multimode microwave reactor – RM 800 Plazmatronika (Wroclaw, Poland) operating with continues power (1.26 W/g of honey) for 2, 4, and 6 min, respectively.

After all thermal treatments and cooling of the samples to room temperature, DN were determined as a function of time. Thus, the inactivation curve was obtained.

In the case of conventional heating, the first order deactivation kinetics was obtained:

$$\ln DN_r = -k_{inact} \times t$$

where:

DN_r – relative diastase activity (°Goethe)

k_{inact} – rate constant of first-order deactivation process (1/min)

t – time at given temperature (min) (Liu 2008)

RESULTS AND DISCUSSION

The physicochemical properties of the investigated honeys were collected into Table 2. On the basis of psychochemical analysis, we stated that all honey samples used in the experiment fulfilled European Union standards with regard

Table 2. Physicochemical properties of honeys

Sample	Water content (%)	Fructose	Glucose	Maltose	Sucrose	Free acidity	Lactones acidity	Total acidity	Specific rotation (°)	HMF (mg/kg)	Initial diastase number (°Goethe)
		(% in DM)				(mmol/kg)					
M1	15.5^{a}	42.45^{c}	36.03^{a}	5.16^{ab}	1.02^{b}	23.25^{b}	2.50^{b}	25.75^{b}	4.49^{a}	2.51^{b}	22.6^{ab}
M2	16.7^{b}	38.64^{ab}	36.57^{a}	5.40^{ab}	0.36^{a}	22.75^{b}	2.50^{b}	25.25^{b}	12.73^{b}	7.1^{a}	18.0^{c}
M3	15.7^{a}	41.28^{bc}	32.26^{b}	6.31^{b}	0.20^{a}	29.00^{a}	2.75^{ab}	31.75^{a}	7.08^{c}	11.88^{c}	21.8^{a}
M4	17.3^{c}	43.37^{c}	35.82^{a}	4.80^{a}	0.30^{a}	22.50^{b}	2.25^{b}	24.75^{b}	2.17^{d}	6.69^{a}	23.2^{b}
M5	15.4^{a}	38.11^{a}	29.57^{c}	6.32^{b}	0.21^{a}	28.75^{a}	3.25^{a}	32.00^{a}	12.32^{e}	9.33^{d}	15.5^{d}

Values in columns with the same letter do not differ significantly at α = 0.05

to the quality parameters. Especially the values of Diastase number (higher than 8) suggested that the honey samples had not been subjected to any long or intensive heating. After the basic analysis, all the samples were heated conventionally for specific periods of time in order to estimate the rate constant of diastase inactivation (Table 3). As shown in the Figure 1, the deactivation reaction of diastase followed the first-order reaction with all the honey samples investigated.

Based on the statistical analysis, no correlation was found between the rate constant (k) and the main carbohydrates and water contents, acidity, and HMF. A statistically significant correlation (correlation coefficient – 0.66; $P < 0.05$) was observed only between the rate constant and maltose content. With almost all the honey samples, it was observed that the higher was maltose concentration, the lower was the rate constant. Additionally, a correlation between k and specific rotation (correlation coefficient – 0.66; $P < 0.05$) was found. Specific rotation, which may be interpreted as a derivative of total sugar content, confirmed the correlation observed for maltose.

According to the results obtained, it is worth stating that the reduction of enzymatic activity below the normative value of DN = 8 followed after 43.0 (M1), 39.5 (M2), 61.0 (M3), 33.5 (M4), and 33.0 (M5) min of heating, respectively. This testifies that 30 min thermal processing at temperature 90°C did not cause any above-average decrease in honey diastase activity. In an extreme example, even one hour of heating at 90°C would not reduce diastase activity below the normative value.

Table 3. Rate constants of diastase inactivation during conventional heating at 90°C

Sample	k (min^{-1})
M1	0.021
M2	0.017
M3	0.015
M4	0.027
M5	0.019

Opposite to the results obtained by other authors (Tosi *et al*. 2008), we did not observe any increase or unpredictable changes in diastase activity of the honeys exposed to high temperatures. Tosi *et al*. (2008) observed a variable behaviour of diastase activity during conventional heating. They found out that DN after 20 min of heating was higher (8.1) than that after 2 min of heating (6.7) at the temperature of 90°C. We observed a continuous decrease of diastase activity of the selected honeys and this decline was less sharp than in the case of the works mentioned.

In the next step of the research the honey samples were exposed to microwave irradiation. In the case of the honeys with the lowest initial diastase numbers (M5, M2) we observed a continuous decrease in the activity of amylases (Figure 2).

The results of our experiments clearly showed that in those cases the diastase inactivation did not fit the first order kinetics and its changes showed rather a complicated mechanism of enzyme inactivation. Such phenomenon might be the result of nonisothermic heating conditions

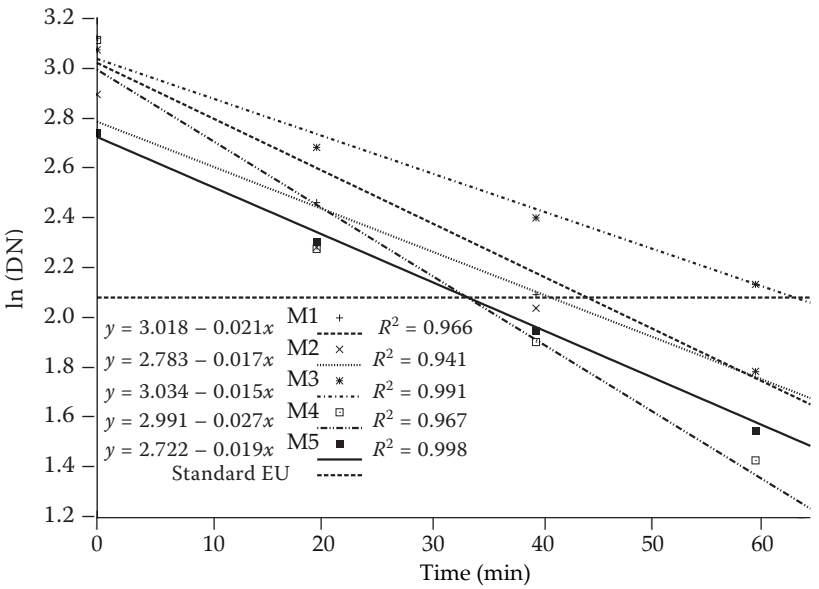

Figure 1. Changes of ln of DN as a function of time at 90°C

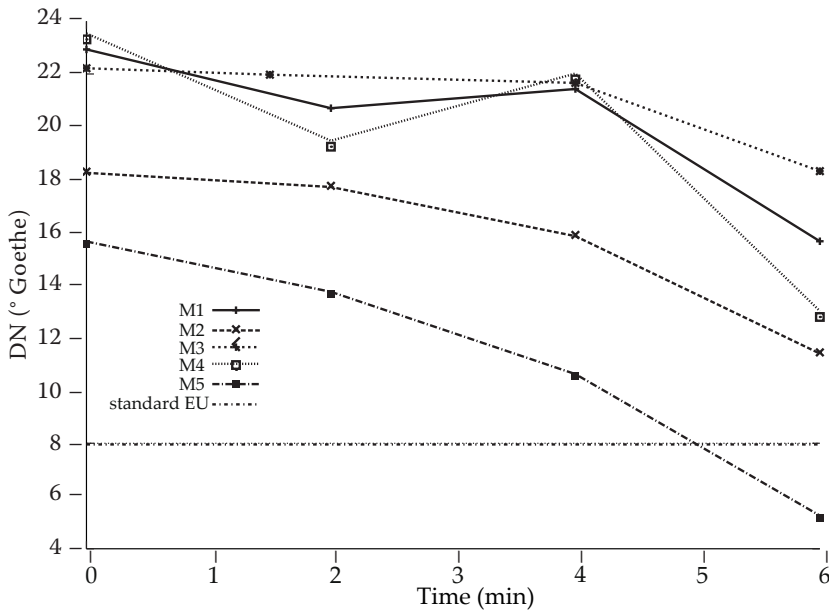

Figure 2. Changes in honeys diastase activity (DN) after microwave processing at power level of 1.26 W/g

(Table 4). It is also possible to explain this phenomenon by thermal (e.g. hot-spots in a highly viscous system) or non-thermal specific microwave effects (influence of electromagnetic field on thermodynamic parameters like free energy of activation or changes in the system entropy) or changes in enzyme inactivation including parallel or stepwise reactions. However, microwave effects need further examination.

Going into details, it is worth pointing out that in honey with average DN like M3 no significant decrease of its value occurred at the initial stages of the process. DN dropped to 18.0 after 4 min of microwave heating. In the case of honey with the highest DN, an increase of DN was observed after 4 min following the decrease at the initial stages of up to 2 minutes. A similar phenomenon was observed with M1 but the increase of DN was not statistically important (Table 4).

According to Hebbar *et al.* (2003), heating times between 60 s to 90 s at the power levels of 6.3 W/g, 9.1 W/g, and 11.9 W/g, respectively, reduced diastase activity of honey by about 50% of its initial value. At the power level of 16.0 W/g, heating above 45 s resulted in the reduction of diastase activity to the level lower then minimum permissible value (DN < 8.0) (Hebbar *et al.* 2003).

Our results clearly showed that even for honey with the lowest initial DN the decrease below 8.0 was detected after 6 min of heating. It may have been the result of a lower microwave power or the application of continuous power distribution. Such observation leads to the conclusion that a short microwave treatment of honey with a low power level did not influence the honey quality estimated by means of DN and allowed to imagine the industrial processing of honey with microwaves as a short and effective heating method.

CONCLUSION

Diastase inactivation under conventional conditions follows the first order kinetics, in contrary

Table 4. Temperature and DN changes during microwave processing

Time (min)	M1		M2		M3		M4		M5	
	DN (°Goethe)	T (°C)	DN (°Goethe)	T (°C)	DN (°Goethe)	T (°C)	DN (°Goethe)	T (°C)	DN (°Goethe)	T (°C)
0	22.6 ± 0.44^{b}	25	18.0 ± 0.07^{a}	25	21.8 ± 0.63^{a}	25	23.2 ± 0.07^{a}	25	15.5 ± 0.12^{a}	25
2	20.3 ± 0.13^{a}	39	17.5 ± 0.43^{a}	43	21.7 ± 0.59^{a}	56	19.2 ± 0.25^{b}	40	14.1 ± 0.7^{b}	31
4	21.1 ± 0.01^{a}	57	15.6 ± 0.23^{b}	66	21.4 ± 0.21^{a}	66	21.8 ± 1.30^{a}	60	10.8 ± 0.32^{c}	49
6	15.3 ± 0.38^{c}	78	11.2 ± 0.04^{c}	83	18.1 ± 0.44^{b}	68	12.8 ± 0.09^{c}	75	5.1 ± 0.13^{d}	68

Values in columns with the same letter do not differ significantly at α = 0.05

to the mechanism of diastase inactivation under microwaves conditions. The differences probably originate in specific microwave effects. The inactivation of diastase enzymes is much faster under microwaves treatment than in the conventional process. However, the possibility of microwave application as a fast, volumetric heating method has also been proven.

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