Diastase Number Changes During Thermal and Microwave Processing of Honey

Stanisław KOWALSKI¹, Marcin LUKASIEWICZ¹, Szczepan BEDNARZ²
and Marzena PANUŚ¹

¹Department of Carbohydrate Technology and ²Department of Engineering and Machinery
in Food Industry, Faculty of Food Technology, University of Agricultural Krakow,
Krakow, Poland

Abstract

Kowalski S., Lukasiewicz M., Bednarz S., Panus M. (2012): Diastase number changes during thermal

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 some 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

Supported by the Polish State Committee for Scientific Research, Grant No. N N312 441237.
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; Fallisco 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 methyltransferase 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.
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:

\[ \ln \text{DN}_r = -k_{\text{inact}} \times t \]

where:

- \( \text{DN}_r \) – relative diastase activity (°Goethe)
- \( k_{\text{inact}} \) – 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 physicochemical analysis, we stated that all honey samples used in the experiment fulfilled European Union standards with regard
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 \( r = 0.66; P < 0.05 \)) 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 \( r = 0.66; P < 0.05 \)) 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

<table>
<thead>
<tr>
<th>Sample</th>
<th>( k ) (min(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>M1</td>
<td>0.021</td>
</tr>
<tr>
<td>M2</td>
<td>0.017</td>
</tr>
<tr>
<td>M3</td>
<td>0.015</td>
</tr>
<tr>
<td>M4</td>
<td>0.027</td>
</tr>
<tr>
<td>M5</td>
<td>0.019</td>
</tr>
</tbody>
</table>

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

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.

References


Received for publication April 12, 2010
Accepted after corrections February 15, 2011