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Honey diastase activity modified by heating

E. Tosi *, R. Martinet, M. Ortega, H. Lucero, E. Ré

Centro de Investigación y Desarrollo en Tecnología de Alimentos, Facultad Regional Rosario, Universidad Tecnológica Nacional, E. Zeballos 1341, Rosario, CP: S2000 BQA, Argentina

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Abstract

Diastase activity is a honey quality parameter used to determine if honey has been extensively heated during processing. Honey heating effect on the diastase activity was studied in two steps. Heating was carried out in the transient heating step, with final temperatures between 60 and 100 °C and 14 s elapsed time, and in the isothermal heating step, in which temperature was held between 60 and 100 °C at heating times between 120 to 1200 s. Six honey samples with initial diastase activity between 25.8 and 11.2 Schade units were tested. During the transient heating, it was observed a decrease in the diastase activity related to an increase in temperature in all assays. The activity becomes zero at 100 °C for both transient and isothermal heating. During isothermal heating, all samples showed a decrease of the diastase activity at short heating times. However, an activity recovery occurs in medium temperature treatments at longer times. The initial activity value, corresponding to the control sample, was not achieved in any case. This changing behaviour makes diastase activity an uncertain parameter to determine if honey has been submitted to heating.

Keywords: Honey diastase; Diastase activity reversibility; Heating; Honey quality

1. Introduction

Distinctive characteristics of honey are due to a large number of minor components that come from the nectar and the bees themselves. Many of these substances, which provide honey its specific flavor and some of its biological activities, are thermolabile. During honey processing, heating is frequently used for decreasing viscosity or, for melting crystallized honey which causes troubles in fractioning and packaging. In some crystallised honey, a liquid phase may exist, thus making the development of microbial osmophile flora possible (Malan & Marletto, 1974). This is caused by an increase in water activity, which becomes greater than that of the original honey, due to the release of water from the solid phase.

Heating of honey motivates the loss of thermolabile, aromatic substances. Losses are proportional to temperature and heating time. Damages caused by heating can be evidenced by measuring quality control parameters, such as diastase activity and hydroxymetylfurfural (HMF) (5-(hydroxymetyl-)furan-2-carbaldehyde) content (Bodganov, Martin, & Lüllmann, 1997). These properties are used together as their values are indicative of the heating intensity to which honey has been subjected (Ramírez Cervantes, González Novelo, & Sauri Duch, 2000).

According to the Honey Quality and International Regulatory Standards, from the International Honey Commission, the diastase activity must not be less than or equal to 8, expressed as diastase number (DN). DN in Schade scale, which corresponds to the Gothe scale number, is defined as g starch hydrolysed in 1 h at 40 °C per 100 g honey. Diastase activity should be determined after processing and blending (Codex draft) or for all retail honey (USDA draft). The Codex Alimentarius (1998) has established the

* Corresponding author. Tel./fax: +54 341 4484909.
E-mail address: tosienzo@ciudad.com.ar (E. Tosi).

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minimum diastase activity value of 3, for honeys with natural low enzyme content. In honeys with a DN less than 8 and higher than or equal to 3, the HMF must not be higher than 15 mg/kg. If DN is equal to or higher than 8, HMF limit is 60 mg/kg.

Gonnet, Lavie, and Louveaux (1964) suggest 78 °C and 6–7 min as the best pasteurising condition to avoid damage to honey quality. The same authors mention a 0.25 DNreduction due to pasteurisation. Bogdanov (1993) points out that thermal treatments, which may destroy the diastase activity, should be as long as 31 days at 40 °C, but they can be shortened to 1.2 h at 80 °C. On the other hand, Ramírez Cervantes et al. (2000) reported a 6.8 or 2.5 DN unit decrease in two honeys treated at 55 °C for 15 min.

The activity of the diastase is closely related to its structure and can be modified by denaturation, brought about by heating. Denaturation may be considered as a discontinuous phenomenon with various intermediate or transition states between the natural or native state and the completely denatured state (Cheftel, Cuq, & Lorient, 1989). These intermediate states, which are in equilibrium at a given temperature, correspond to different steps in the modification of protein structure. Each of these equilibrium situations implies the possibility of a total or partial reversibility of the denaturation phenomenon. According to the theory of the transition-state of Eyring, the enzyme in native state is in equilibrium with a transition state, which is found in an energetic level higher than the native and the denatured states. Transformation rate of native state (NS) on transition state (TS) by temperature effect is given by

$$\frac{d(NS)}{dt} = B(TS) \quad (1)$$

and

$$B = \frac{k_B T f}{h} \quad (2)$$

where, k_B is the Boltzman constant; f fraction of molecules which go through the energetic barrier and h is the Plank's constant.

Eq. (1) indicates that the decrease on enzyme concentration in native state depends on the rate at which molecules in transition stage exceed the energetic barrier. Such energetic barrier corresponds to the free energy of the transition state. The molecules in transition state, which do not exceed such barrier, remain in equilibrium with those in native state. According to Kuwajima (1989) the transition stage, named "molten globule state", is characterized by a relatively compact globule with native-like secondary structure and a disrupted tertiary structure. Consequently, in thermal treatments, especially in those carried out at high temperatures and short times, the enzyme may not be destroyed, but only reversibly inactivated. Alkaline phosphatase, a natural milk enzyme used to control milk pasteurization, undergoes such reversible inactivation (Richardson & Hyslop, 1992). Taking that into account, it is possible that honeys treated at high temperatures do not show a significant decrease in DN if the diastase activity is recovered after heating.

The aim of this work was to evaluate the incidence of honey heating variables: treatment temperature (T) and time (t) on the modification of the diastase activity measured as diastase number.

2. Materials and methods

2.1. Materials

Six multifloral honey samples were provided by beekeepers from the province of Santa Fe, Argentina. Samples were taken not more than two weeks after their extraction from the hives and were not heated. Maturity, purity, deterioration and adulteration criteria indicated that their quality was good, and all of them showed no signs of fermentation or crystallisation.

2.2. Methods

2.2.1. Honey properties

Honey physicochemical properties (Table 1) were determined according to the Harmonized Methods of the European Commission of Honey (Bodganov et al., 1997). Moisture was determined by refractometry, free acidity by titration with 0.1 M sodium hydroxide solution to pH 8.30. Fructose and glucose were determined according to the method of Bogdanov and Baumann (1988). After filtering the solution, sugar content is determined by HPLC with a column of 4.6 mm internal diameter, 250 mm length, containing amine modified silica gel with 5–7 μm particle size, Supelco Inc. (Supelco Park, Bellefonte, PA). Column and detector temperature 30 °C; mobile phase acetonitrile:water (80:20, v/v), degassed; flow rate 1.3 mL/min; sample volume 10 μL. Peaks were identified on the basis of their retention times. The standard of fructose and glucose were from Sigma (S. Louis, MO, USA). Quantification was performed according to the external standard method on peak areas or peak heights. Determinations were performed on a Shimadzu LC 10AS HPLC (Shimadzu Co. Kyoto, Japan) with a Shimadzu RID 10A Refractive Index detector.

HMF (5-(hydroxymetyl-)furan-2-carbaldehyde) was determined by reverse phase HPLC in a clear, filtered, aqueous honey solution using a C-18 reversed phase column Supelco Inc. (Supelco Park, Bellefonte, PA) 250 mm length, 4.6 mm diameter, 5–7 μm particle size. Flow rate 1.0 mL/min, mobile phase water:methanol (90:10 v/v) both HPLC quality. Sample volume 20 μL. Detection UV 285 nm range 0.2 AUFS. Standard of HMF from Merck (Darmstadt, Germany). Determinations were performed on a Shimadzu LC 10AS HPLC with a Shimadzu SPD 10A UV–Vis detector.

Table 1
Composition[a] of six honey samples

Parameters	Sample number					
	1	2	3	4	5	6
Diastase activity (Schade number[b])	11.2 ± 0.6	12.5 ± 0.8	14.2 ± 0.6	18.6 ± 0.7	20.5 ± 1.1	25.8 ± 0.9
Moisture (%)	20.1 ± 0.8	19.4 ± 0.5	19.3 ± 0.8	20.3 ± 0.6	19.6 ± 0.3	19.7 ± 0.5
Ash (%)	0.05 ± 0.01	0.08 ± 0.01	0.07 ± 0.01	0.4 ± 0.01	0.11 ± 0.02	0.07 ± 0.01
Fructose (%)	32.5 ± 0.9	39.8 ± 0.7	41.6 ± 0.6	37.7 ± 0.8	39.5 ± 0.5	40.4 ± 0.6
Glucose (%)	23.1 ± 0.8	33.5 ± 0.5	28.8 ± 0.7	25.2 ± 0.6	33.3 ± 0.8	29.8 ± 0.4
Acidity (meq/kg)	17.5 ± 0.6	22.3 ± 0.8	25.4 ± 0.5	19.0 ± 0.6	29.8 ± 0.5	15.2 ± 0.6
Hydroxymetyl furfural (mg/kg)	13.2 ± 0.9	20.6 ± 0.5	10.3 ± 0.8	6.2 ± 0.8	9.0 ± 0.6	5.8 ± 0.7

[a] Determined according to the Harmonized Methods of the European Commission of Honey (Bodganov et al., 1997).
[b] Schade number, corresponds with Gothe number, or g starch hydrolysed · h^{-1} at 40 °C per 100 g honey.

2.2.2. Diastase activity determination

Diastase activity was measured with Phadebas, according to the Harmonized Methods of the European Commission of Honey (Bodganov et al., 1997). An insoluble blue dyed cross-linked type of starch is used as the substrate. This is hydrolysed by the enzyme, yielding blue water-soluble fragments, determined photometrically at 620 nm. The absorbance of the solution is directly proportional to the diastatic activity of the sample. A Jasco Model 7800 UV–Vis spectrophotometer (Japan Spectroscopic Co., Tokyo, Japan) was used. Diastase activity was obtained from the absorbance measurements by using the equations

$$DN = 28.2 \times \Delta A_{620} + 2.64; \quad (3)$$

and

$$DN = 35.2 \times \Delta A_{620} - 0.46; \quad (4)$$

for high (8 to 40 diastase units) or low (up to 8 diastase units), respectively. Eqs. (4) and (5) are suggested by the Harmonized Methods of the European Commission of Honey (Bodganov et al., 1997). Diastase activity was referred to as DN in Schade scale which corresponds to the Gothe scale number, or g starch hydrolysed × h^{-1} at 40 °C per 100 g honey. Phadebas tablets were from Pharmacia Diagnostics AB (Uppsala, Sweden). All other reagents were from Merck (Darmstadt, Germany) ACS quality.

2.2.3. Heating methodology

Honey heating was carried out by batch treatments. A stainless-steel coil 5 mm internal diameter, 150 mL capacity, was filled with honey warmed up at 40 °C for viscosity reduction. Coil filling was performed under a positive pressure at piston flow conditions to prevent air bubble development. A headspace was kept to prevent coil blowout by hydraulic pressure increase due to thermal expansion. Assays were performed following two heating sequences: (i) a transient heating step alone, in which honey temperature rises from 20°C standard initial temperature to the desired temperature T in a fixed time, or (ii) a transient heating step followed by an isothermal heating step in which T was kept unchanged during a certain treatment time. The temperature in the honey core axis was measured by a T-type micro-thermocouple probe (accuracy ± 0.5 °C) (Cole Parmer International, Vernon Hills, IL, USA).

2.2.3.1. Transient heating step. Transient heating was carried out by immersing the coil containing honey in a stirred thermostatic bath set at a certain temperature ($T_t \pm 0.5$) which assures honey can reach T at the end of the transient step. Time was standardized at 14 s. The following equation was used to calculate T_t.

$$T_t = A \cdot \ln T - B \quad (5)$$

where: T_t was the thermostatic bath temperature; T the desired isothermic treatment temperature; $A = 0.1525T + 58.703$ and $B = -0.5398T + 191.49$. Eq. (5) was obtained empirically for the heating system used.

2.2.3.2. Isothermal heating step. Once honey temperature reached the isothermal treatment temperature T, the coil was immediately transferred from the transient bath to the isothermal bath set at $T \pm 0.5$ °C. Nominal **T** values were 60, 70, 80, 90 and 100 °C. The coil was held in the isothermal bath during the isothermal treatment time t, with values of 120, 300, 600 and 1200 s. Assays were carried out in triplicate, reporting the mean value and standard deviation.

2.2.4. Cooling

Once heating time was elapsed in both heating sequences, the heated coil was removed from the hot bath and immediately immersed in a cooling bath, with water set at 18 °C until honey temperature reached 30 °C. The cooling time was not fixed, but dependent on the initial temperature T. The coil transfer from one bath to the other did not exceed 2 s.

3. Results

Table 2 shows the modification of the diastase activity (expressed as DN) as a function of T for the transient heating step. A DN decrease, with reference to control DN value, was verified in transient heating for all tested temperatures during a constant (14 s) heating time. This

Table 2
Diastase activity, expressed as diastase number (*DN*)[a,b], of six honeys samples, as regards honey final temperature *T* at the end of transient heating

Transient heating		Sample number					
Final temperature *T* (°C) (Nominal)	Time (s) (Nominal)	1	2	3	4	5	6
		Diastase number DN [a,b] $\pm \delta$					
Control[c]	–	11.2 ± 0.6	12.5 ± 0.8	14.2 ± 0.6	18.6 ± 0.7	20.5 ± 1.1	25.8 ± 0.9
60	14	8.6 ± 0.6	9.2 ± 1.2	11.2 ± 0.7	15.8 ± 0.8	17.6 ± 0.7	20.5 ± 0.9
70		7.3 ± 0.7	8.1 ± 0.8	8.9 ± 1.1	12.5 ± 0.7	14.4 ± 1.0	17.8 ± 0.7
80		5.2 ± 0.8	6.5 ± 0.8	7.2 ± 0.9	9.6 ± 0.6	11.8 ± 0.7	14.1 ± 1.1
90		3.5 ± 0.8	3.8 ± 1.2	4.2 ± 1.0	5.2 ± 0.9	5.8 ± 0.8	6.8 ± 0.9
100		Non-detectable diastase activity for all treatments					

[a] Expressed as mean of three replications plus standard deviation.
[b] Schade units, refers to Table 1.
[c] Sample without treatment, corresponds to original honey diastase number.

decrease was more evident when final *T* was higher. For *T* equal to 100 °C, DN was zero.

Table 3 shows the variation of diastase activity as a function of *T* and heating time during the isothermal heating step. The initial DN value ($t = 0$), corresponds to the value reached at the end of transient heating. An initial decrease of diastase activity, as heating time increased, was noted in all samples. However, when the isothermal heating was longer, a DN increase was verified (values in black in Table 3). Such DN increase exceeded the initial DN value (at $t = 0$), though it did not reach the value of the control sample. Some increased DN values were statistically significant at $p < 0.05$ (*). Treatments at 100 °C give zero activity at all heating times.

HMF, determined on samples treated at 90 °C and 1200 s, gave values between 90.5 and 24.3 mg/kg.

Table 3
Diastase activity, expressed as diastase number (DN)[a,b], of six honeys samples, as regards of honey isothermic heating temperature (*T*) and isothermic heating time (*t*)

Isothermic heating		Sample number					
Temperature *T* (°C) (Nominal)	Time *t* (s) (Nominal)	1	2	3	4	5	6
		Diastase number DN[a,b] $\pm \delta$					
Control[c]	–	11.2 ± 0.6	12.5 ± 0.8	14.2 ± 0.6	18.6 ± 0.7	20.5 ± 1.1	25.8 ± 0.9
60	0[d]	8.6 ± 0.6	9.2 ± 1.2	11.2 ± 0.7	15.8 ± 0.8	17.6 ± 0.7	20.5 ± 0.9
	120	8.4 ± 0.7	9.0 ± 0.8	10.8 ± 0.8	14.2 ± 0.9	17.4 ± 0.6	19.8 ± 0.8
	300	8.4 ± 0.8	9.1 ± 0.9	10.2 ± 0.7	14.0 ± 0.7	17.2 ± 1.1	19.6 ± 0.8
	600	8.6 ± 0.9	**9.3** ± 0.8	**11.3** ± 0.9	15.3 ± 1.0	**17.8** ± 0.8	**21.6** ± 0.6*
	1200	**8.7** ± 1.0	**9.5** ± 0.7	**11.8** ± 0.7	**16.5** ± 0.9	**18.2** ± 0.7	**21.7** ± 0.8*
70	0[d]	7.3 ± 0.8	8.1 ± 0.9	8.9 ± 0.8	12.5 ± 0.7	14.4 ± 0.9	17.8 ± 0.7
	120	7.1 ± 0.7	7.8 ± 0.9	8.6 ± 0.7	12.1 ± 0.9	14.1 ± 1.0	17.2 ± 0.9
	300	**7.1** ± 0.9	**8.2** ± 0.7	8.4 ± 0.9	11.8 ± 0.7	13.8 ± 0.9	**17.1** ± 0.7
	600	**7.4** ± 1.1	**8.3** ± 0.8	**9.1** ± 0.9	12.5 ± 0.8	**14.6** ± 0.9	**18.4** ± 0.8
	1200	**7.8** ± 0.8	**8.6** ± 0.7	**9.9** ± 2.5*	**13.2** ± 0.8	**15.6** ± 0.8*	**18.9** ± 0.8*
80	0[d]	5.2 ± 0.8	6.5 ± 0.7	7.2 ± 0.8	9.6 ± 0.7	11.8 ± 0.9	14.1 ± 0.7
	120	4.7 ± 0.8	6.0 ± 0.9	6.8 ± 0.7	9.3 ± 0.8	11.2 ± 0.8	12.8 ± 0.8
	300	4.5 ± 0.9	5.8 ± 1.0	6.9 ± 0.9	9.3 ± 0.7	11.0 ± 0.7	13.9 ± 0.7
	600	**5.3** ± 0.9	**6.7** ± 0.8	**7.4** ± 0.7	**9.8** ± 0.9	**11.9** ± 0.8	**15.2** ± 0.8*
	1200	**5.9** ± 0.8	**7.2** ± 0.8	**8.3** ± 0.8*	**10.7** ± 0.8*	**12.9** ± 0.7*	**15.6** ± 0.8*
90	0[d]	3.5 ± 0.8	3.8 ± 0.8	4.2 ± 0.7	5.2 ± 0.9	5.8 ± 0.9	6.8 ± 0.9
	120	3.2 ± 0.9	3.4 ± 1.0	4.0 ± 0.9	4.9 ± 0.9	5.4 ± 0.8	6.7 ± 0.9
	300	3.4 ± 0.9	3.9 ± 0.9	4.1 ± 0.8	5.1 ± 0.8	5.7 ± 1.1	**6.9** ± 1.0
	600	**3.8** ± 0.8	**4.3** ± 0.8	**4.6** ± 0.7	**5.9** ± 0.9	**6.2** ± 0.8	**7.9** ± 0.7*
	1200	**4.6** ± 0.7*	**4.9** ± 0.8*	**5.3** ± 0.8*	**6.4** ± 0.8*	**7.0** ± 0.9*	**8.1** ± 0.8*
100	0–1200	Non-detectable diastase activity for all treatments					

Numbers in black indicate DN values higher than values at the beginning ($t = 0$) of isothermal heating, (corresponds to the end of transient heating).
[a] Expressed as mean of three replications plus standard deviation.
[b] Schade units, refers to Table 1.
[c] Sample without treatment, corresponds to original honey DN.
[d] Zero isothermic heating time. DN corresponds to DN values at the end of the transient heating (Table 2).
* Statistically significant ($p < 0.05$).

4. Discussion

Results show that the decrease of the diastase activity was not closely related to an increase of the heating temperature or treatment time. At all initial heating steps which correspond to transient heating, honey temperature rises from ambient temperature to T. A decrease of the diastase activity is observed if compared to the initial values corresponding to the control or untreated samples. Diastase activity loss occurred as temperature increases. The decrease in diastase activity was more evident at higher rates of temperature increase.

However, during the isothermal heating step, in which T remains constant during the complete heating time, the diastase activity decreased for short t treatments (typically for 120 s) but increased when t increased. This effect was closely related to all T with the exception of 100 °C treatments in which the diastase activity became null.

Such changes in diastase activity may be due to the modification of the enzymatic activity, brought about by the structural changes in enzyme molecules, promoted by heating. According to the theory of Eyring, during transient heating all molecules whose free energy exceeds the energy barrier undergo a complete and irreversible denaturation. Nevertheless, during the isothermal heating, in samples maintained at 60, 70, 80 and 90 °C, the number of activated molecules which could exceed the energetic barrier of the transition stage was low. As a consequence, the reaction towards complete denaturation did not occur to any large extent. When heating stopped, all activated molecules that did not surpass the energy barrier returned to a native-like state, and the enzymatic activity was recovered. Those transitory changes may be explained by a reversible loss of protein structure. This is in accordance with the report of Kuwajima (1989) for which in the transition stage, protein maintained its native-like secondary structure, but has its tertiary structure disrupted.

This variable behaviour agrees with that reported by Richardson and Richardson and Hyslop (1992) on the reversible inactivation of enzymes according to treatment conditions. Such is the case for alkaline phosphatase in milk and peroxidases and lipoxygenase in fruits and vegetables.

On the other hand, at 100 °C treatment, there was a total enzymatic inactivation. According to the obtained results, it is possible that, the irreversible inactivation of honey diastase occurred between 90 and 100 °C, for the samples and the heating parameter tested.

5. Conclusions

The variable behaviour of diastase activity makes it an unreliable parameter to examine thermally treated honey samples. For example, after 1200 s at 90 °C heating, a DN decrease from 25.8 to 8.1 occurs in honey sample number 6 (Table 3). Such honey would satisfy the current standards since the other control parameter, HMF, with initial concentration of 5.8 increased to 32.4 mg/kg but did not reach the 60 mg/kg limit.

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References

Bogdanov, S. (1993). Liquefaction of honey. *Apiacta, XXVIII*, 4–10.

Bogdanov, S., & Baumann, S. E. (1988). Bestimmung von Honigzucker mit HPLC. *Mitt Geb Lebensmittelunters Hyg, 79*, 198–206.

Bodganov, S., Martin, P., & Lüllmann, C. (1997). Harmonised methods of the European Honey Commission. *Apidologie* (pp. 1–59). France: Elsevier.

Cheftel, J., Cuq, J., & Lorient, D. (1989) *Proteinas Alimentarias* (pp. 37–47) Ed. Acribia, Zaragoza, España.

Codex Alimentarius (1998). *Codex Alimentarius standard for honey* Ref. CL 1998/12-S. FAO and WHO. Rome.

Gonnet, M., Lavie, P., & Louveaux, J. (1964). La pasteurisation des miels. *Annales Abeilles, 7*, 81–102.

Kuwajima, K. (1989). The molten globule state cis a clue for understanding the folding and cooperativity on globular-protein structure. *Proteins, 6*(2), 87–103.

Malan, C., & Marletto, O. (1974). Blastomiceti da mieli di diversi origini e provenienze. *Annali del' Accademica di Agricoltura di Torino, 116*, 1–18.

Ramírez Cervantes, M. A., González Novelo, S. A., & Sauri Duch, E. (2000). Effect of the temporary thermic treatment of honey on variation of the same during storage. *Apiacta, 35*(4), 162–170.

Richardson, T., & Hyslop, D. (1992). Enzimas. In: Fennema, O. (Ed.), *Química de los Alimentos* (pp. 451–456) Ed. Acribia SA. Zaragoza, España.