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THE QUALITY OF HONEYS INFLUENCED BY THE TRADITIONAL HEATING METHOD

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ABSTRACT

In this study, three parameters (hydroxymethylfurfural, diastase activity and invertase activity) were used to evaluate the quality of twenty samples of multifloral honey from northern Algeria heated by a traditional method (conventional heating in a water bath at 100 °C) for five treatment periods (2, 4, 6, 8 and 16 min). The assay was carried out with honey samples whose initial HMF, diastase activity and invertase activity values were within the recommended food authority limit. HMF was determined by HPLC, diastase was measured with Phadebas and invertase was determined using the Siegenthler method. During heating, it was observed an increase in the HMF related to an increase in temperature, but still below the international standard limit (40 mg.kg^{-1}). Diastase activity and invertase activity also decreases during heating. However, invertase is more heat-sensitive and heating time than diastase and HMF, and it's an important parameter to determine if honey has been submitted to heating. Therefore to liquefy honey we can use this method but with a low temperature and a short time because time has a deep impact on the quality of heating treatment of honey.

Keywords: honey, traditional heating, hydroxymethylfurfural, diastase activity, invertase activity, Algeria

INTRODUCTION

Honey is a product that contains a blend of complex carbohydrates, mostly monosaccharides glucose and fructose. Others are present in lower amounts, according to the botanical origin. Moreover, other compounds such as organic acids, lactones, amino acids, mineral salts, vitamins, enzymes, pollen, wax and pigments are present (**Fallico *et al.*, 2004**).
The enzymes are secreted by bees (invertase, glucose oxidase and amylase) or by plants (amylase, catalase and phosphatase) (**Vorlova and Čelechovská, 2002**). Honey contains small amounts of different enzymes, the most important of which are diastase (∝-amylase), invertase (∝-glucosydase), glucose oxidase, catalase and acid phosphatase (**White, 1975**). They are sensitive to heat and therefore are able to indicate overheating of the product and the degree of conservation (**Ahmed *et al.*, 2013**). The activity of enzyme in honey also depends on age of the bees, stage of the colony, nectar flow, environmental conditions and the beekeeping practices (**Karabournioti and Zervalaki, 2001**).
According to several authors the activity of invertase in honey is used mainly in Europe for the evaluation of monofloral honeys, as well as for the determination of the characteristics related to the geographical origin of the different types of honey (**Oddo *et al.*, 1999; 2004; Bartakova *et al.*, 2007; Serrano *et al.*, 2007**).
Honey freshness is generally evaluated by determining the value of parameters that increase or decrease with overheating and/or ageing. The most commonly used are hydroxymethylfurfurale, diastase and invertase (**Oddo *et al.*, 1999**). However, excessive heat treatment leads to the formation of 5-hydroxymethylfurfuraldehyde (**Nozal *et al.*, 2001**). Hydroxymethylfurfural (HMF) is a cyclic aldehyde produced as a result of sugar degradation (**Cervantes *et al.*, 2000**). HMF value is virtually absent or very low in fresh honey and is high in honey that has been heated, stored in non-adequate conditions and old honey (**Nozal *et al.*, 2001; Khalil *et al.*, 2010**). At room temperature, the action of normal honey acidity on reducing sugars can possibly produce HMF. It has a toxic effect and also induces reactive oxygen species (**De Smet *et al.*, 2015**).
The Alimentarius Codex (2001) and International Honey Commission (**Bogdanov *et al.*, 1997**), set the maximum concentration of HMF to 40 mg.kg^{-1}for honey from non-tropical regions and high values of HMF (80 mg.kg^{-1}) from countries or regions with tropical ambient temperatures. Extremely high values of HMF (>500 mg.kg^{-1}) demonstrate adulteration with invert syrup (**Coco *et al.*, 1996**).
Usually the heating process is used to reduce viscosity, and to prevent crystallization or fermentation (**Singh *et al.*, 1988**). According to **Bakier (2006)**, the effective liquification of honey requires heating for at least 10 min at 52–55 °C. Honey heating is carried out in two different ways: in air-ventilated chambers, at 45–50 °C for 4 – 7 days or by immersion of honey drums in hot water. Although, the second heating method is more efficient, the first is the most common (**Belitz and Grosch, 1999**). Algeria is a broad territory extends over an area of 2,381,741 km2 and is the second largest country in Africa (Haouam *et al.*, 2016), in this country immersion of honey bottle in hot water for liquefaction is more used.
In this context, the aim of this study is to test the quality of honey and the efficiency of the traditional method of heating by analyzing the HMF content, the diastase activity and invertase activity in twenty honey samples from north of Algeria, treated by the traditional heating method (immersion the bottle of honey in hot water) and compare their levels by while five treatment periods.

MATERIAL AND METHODS

Honey samples

Twenty multifloral honey samples of *Apis mellifera intermissa* were produced in various regions from north of Algeria (Table 1) and (Figure 1) and were collected from beekeepers. All samples were collected in airtight plastic containers while the same year and then they have been stored in a refrigerator at 4 – 5 °C until analysis.

Heating procedure

Each honey sample was divided into six sub-samples of about 5g in glass bottles. One portion was immediately analyzed and five portions in closed bottles were undergo a thermal processing (conventional heating in a water bath- isothermal heating) without stirring, during five periods of treatment (2, 4, 6, 8 and 16 min). The conventional heating procedure was as follows: 2, 4, 6 and 16 min at 100 °C and cooled down in a room temperature. The temperature of the analysis was selected on the basis of the traditional heating (dilution the bottle of honey in the saucepan with water boiling). Heat-treated samples were subjected to Hydroxymethylfurfural (HMF), diastase activity and invertase activity analyses.

Table 1 Botanical and geographical origin of honey samples

Samples	*Honey type*	*Geographical origin*	*Region*
H1	Multifloral	M'Sila	Bossada
H2		Djelfa	Ain Wessera
H3		Lagoute	Aflou
H4		Mila	Mila
H5		Khenchla	Chachare
H6		Oum- El- Bouaghi	Meskianna
H7		Tebessa	Oinza
H8			Ain Zargua
H9			Yokous
H10			Limit Tebessa-Tunisie
H11		Guelma	Bouati mahmoud
H12		Skikda	El Marsa
H13		Annaba	Edough Seraidi
H14			Oued El Aineb
H15			Berhel
H16			Triaate -Berahel
H17			Bomaiza
H18		El-Taref	El-Taref
H19			West Ben Mhidi
H20			Bounamousa- Ben Mhidi

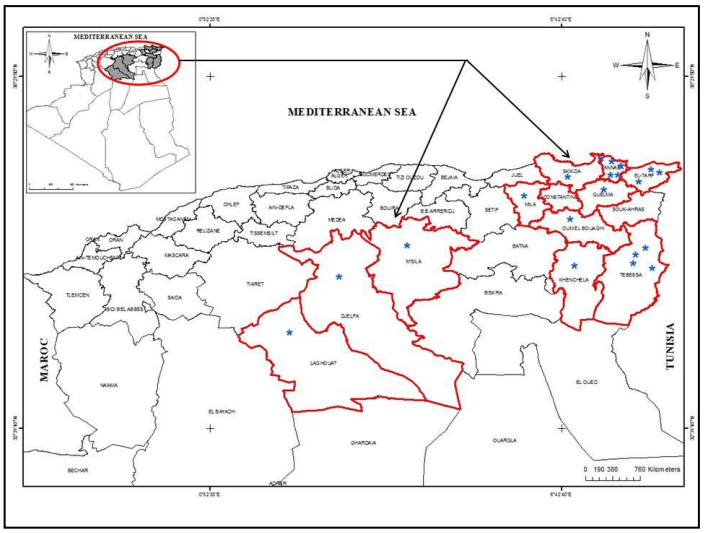

Figure 1 Distribution of honey samples from north of Algeria

Hydroxymethylfurfural analysis

HMF (5-(hydroxymetyl-) furan-2-carbaldehyde) was determined by reverse phase HPLC Agilent 1200 (Ramsey, Minnesota, USA) equipped with UV detector, according to the harmonized by the European Honey Commission (**Bogdanov *et al.*, 1997**). Five gram of honey sample was weighed into a 50 mL beaker and dissolved in 25 mL HPLC grade water. The solution was transferred into 50 mL volumetric flask and filled to the mark with HPLC grade water. Then the solution was centrifuged and poured into sample vials for chromatographic separation. The HPLC condition was the following: mobile phase, 90% water and 10% acetonitrile; flow rate 1ml/min; injection volume 20 µl. HMF content of the sample was calculated by comparing the corresponding peak areas of the sample and those of the standard solutions, taking into account the dilution factor. Results were expressed in $mg.kg^{-1}$.

Enzyme analysis

Diastase activity analysis

Diastase activity was measured by Phadebas, according to the harmonized Methods of the European Commission of Honey (**Bodganov *et al.*, 1997; Tosi *et al.*, 2008**), using spectrophotometric method. Diastase activity is the one unit corresponds to the enzyme activity of 1 g honey that can hydrolyse 0,01g of starch in 1 h at 40^0 C (**Oddo *et al.*, 1999**). According to **Oddo and Pulcini (1999)** the number of diastases (ND) is calculated with the following equation: $ND = - 4.37 \times (\Delta A_{620})^2 + 3.38 \times (\Delta A_{620}) + 0.03$ and the results were expressed in Schade units.

Invertase activity analysis

Invertase was determined using the Siegenthler method, according to the harmonized by the European Honey Commission (**Bogdanov *et al.*, 1997**). The enzyme activity is evaluated photometrically, by measuring the decomposition of the substrate p-nitrophenyl α-D glucopyranoside into the product p-nitrophenol (which has a maximum absorbance at 400 nm). The results were expressed as invertase number (IN). The IN indicates the amount of sucrose per gram hydrolysed in 1h by the enzymes contained in 100g of honey under test conditions (**Oddo *et al.*, 1999**).

Statistical analysis

A one-way analysis of variance (ANOVA) was performed to examine the effects of heating at five period of treatment on HMF, diastase activity and Invertase activity with their initial values. F-test was used to estimate the statistically significant differences (P-value <0.05) among honey samples. The differences among the means were determined for significance at the 5% level using Tukey's test. All the analyses were carried out at least in duplicate, and the results are expressed as mean values ± standard deviations (SDs). All data were analyzed using the Statistica 8.0 software for windows from Statsoft.

RESULTS AND DISCUSSION

The variation of HMF contents, diastase activity and invertase activity according to initial value and different period of treatment of honey are reported in Table 2. As well as the number of samples exceeding limit presented in Table 3.

Table 2 Variation of HMF, diastase activity and invertase activity during the period of treatment (n = 20)

Time	HMF ($mg.kg^{-1}$)			Diastase activity (Schade units)			Invertase activity (IN)		
	p -value	rang	mean±sd	*p* -value	range	mean ± sd	*p* -value	range	mean ± sd
0 min	-	0.69 - 09.50	3.72 ± 2.45^{a}	-	9.62 - 29.47	19.37 ± 5.35^{a}	-	38.01 - 163.91	101.93 ± 37.23^{a}
2 min	ns	0.15 - 10.08	4.69 ± 3.22^{b}	ns	10.12 - 28.72	18.95 ± 5.01^{a}	**	11.79 - 162.39	71.41 ± 39.04^{b}
4 min	ns	0.58 - 11.23	4.18 ± 3.31^{b}	ns	7.60 - 28.26	15.96 ± 4.74^{a}	***	0 - 115.15	23.54 ± 36.12^{c}
6 min	ns	0.52 - 12.13	4.82 ± 3.46^{b}	***	0.47 - 28.26	9.12 ± 8.17^{b}	***	0 - 53.91	$1.10 \pm 18.63^{c,d}$
8 min	ns	0.18 - 12.59	4.60 ± 3.69^{b}	***	0.00 - 25.47	$5.62 \pm 7.50^{b,c}$	***	0.0	0 ± 0^{d}
16 min	**	0.55 - 23.85	8.20 ± 4.97^{b}	***	0.11 - 15.45	2.16 ± 3.81^{c}	***	0.0	0 ± 0^{d}

HMF – hydroxymethylfurfural, n – number of samples, ns –not significant, sd – standard deviation, **significant at p < 0.01, ***significant at p < 0.001, With different letters are significantly different

Table 3 Variation of HMF, diastase activity and invertase activity during the period of treatment (n = 20)

Time	*HMF* (mg.kg^{-1})			*Diastase activity (Schade units)*			*Invertase activity (IN)*		
	International standard limit	Samples exceeding limit	Samples conforming limits (%)	International standard limit	Samples below limit	P Samples conforming limits (%)	International standard limit	Samples below limit	Samples conforming limits (%)
0 min	max.40	0	100	max.8	0	100	max.10	0	100
2 min		0	100		0	100		0	100
4 min		0	100		1	95		12	40
6 min		0	100		11	45		14	30
8 min		0	100		13	35		20	0
16 min		0	100		18	10		20	0

Hydroxymethylfurfural (HMF)

HMF content in untreated honey

Hydroxymethylfurfural (HMF) is absent or present in trace amounts in fresh honeys, since it is a parameter of honey freshness **(Sodre *et al.*, 2011)**. HMF level in fresh honey samples (at time 0 min) varied between 0.69 and 9.50 mg.kg^{-1}, these results were indicated HMF contents were below 10 mg.kg^{-1}, similar results were observed by **Getu and Birhan (2014)** for Ethiopian honey (HMF ranged between 0.5 and 3.2 mg.kg^{-1}). According to **Al-Farsi *et al.* (2018)** high quality honey should present low HMF contents. All fresh honey samples studied contained HMF within the recommended food authority limit (40 mg.kg^{-1}). According to **White (1994)** proposed the HMF level as the only reliable heating/storage index in honey.

Effect of heating on HMF contents

HMF, this product of fructose decomposition, increases with storage and prolonged heating of honey **(Al-Farsi *et al.*, 2018)**. During heating from initial value (0 min) to 8 min all honey samples showed not formation of HMF their values maximum varied from 9.50 to 12.59 mg.kg^{-1} (Figure 2). These results are in agreement with those of **Fallico *et al.* (2004)**, at high temperature (100 °C) no difference, related to HMF formation, can be measured among honeys of different origin. At time 16 min all samples had a slight increase to the maximum 23.85 mg.kg^{-1}. In this time there is a remarkable HMF formation, moreover a significant difference ($p<0.001$) was also observed between honeys, but still below the international standard limit (40 mg.kg^{-1}). The same authors **Fallico *et al.* (2004)** showed that the HMF levels in honey samples, heated at 100 °C, were significant correlated only with time of heating. **Singh and Bath (1997)** reported that with increasing heating time of 0–30 min, an increased in intensity of HMF formation for three monofloral honeys from India at 65 °C.

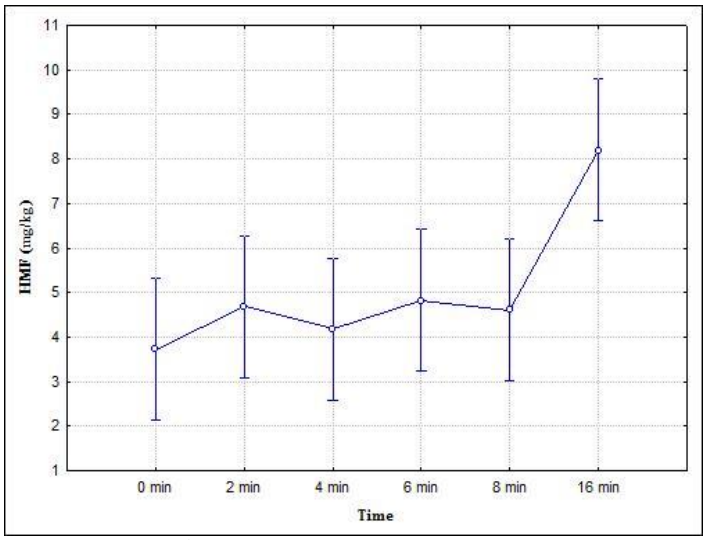

Figure 2 Variation of HMF during heating

Enzymes

Diastase activity in untreated honey

The diastase activity is a very interesting enzyme to know the freshness of honey **(Oddo *et al.*, 1990)**. The diastase content in our samples ranged between 9.62 and 29.47 Schade units. Similar values for diastase reported in Argentina honey which averaged 19.7 Schade units. **(Cantarelli *et al.*, 2008)**. According to the European Codex Honey Standards, a well-processed and ready to be consumed honey must contain diastase number ID ≥ 8 Schade units. We noted that 100% of fresh honey samples studied contained ID ≥ 8 Schade units. The enzyme activity in honey from the same floral origin can possibly vary, due to the plant's nectar secretion quality and quantity, which was influenced by the contribution of the environment and the presence of different geographical races of bees, which is mainly governed by biotic and abiotic factors **(Adgaba *et al.*, 2017; Belay *et al.*, 2017)**.

Effect of heating on diastase activity

During heating from initial value (0 min) to time 4 min (Figure 3), the diastase activity of all honey samples showed some differences were seen. The mean values decrease from 19.37 ± 5.35 Schade units to 15.96 ± 4.74 Schade units, but is not significant because only one sample reports a value below the limit. At time 6 min all samples had a slight decrease to 9.12 ± 8.17 Schade units, in addition a very highly significant difference ($p<0.001$) was observed, therefore 11 samples of multiflora were unconfirmed to the European Codex Honey Standards limit (diastase number "ID" ≥ 8 Schade units). According to **Bogdanov *et al.* (1999)** and **White (1994)** the honeys are also used as a quality parameter even though some have a lower level of enzymes intrinsically. Honeys with lower level of enzyme, needs to consist essentially a maximum of 15 mg.kg^{-1}of HMF **Zappala *et al.* (2005)**. At time 8 and 16 min the mean values of the diastase activity was also decrease from 5.62 ± 7.50 Schade units to 2.16 ± 3.81 Schade units respectively therefore lower of 8 Schade units. In addition a very highly significant difference ($p<0.001$) was also been noticed in these times (8 and 16 min), therefore 13 samples and 18 samples of multifloral respectively were below to limit, with the exception of two samples that have values above the limits at 16 min.

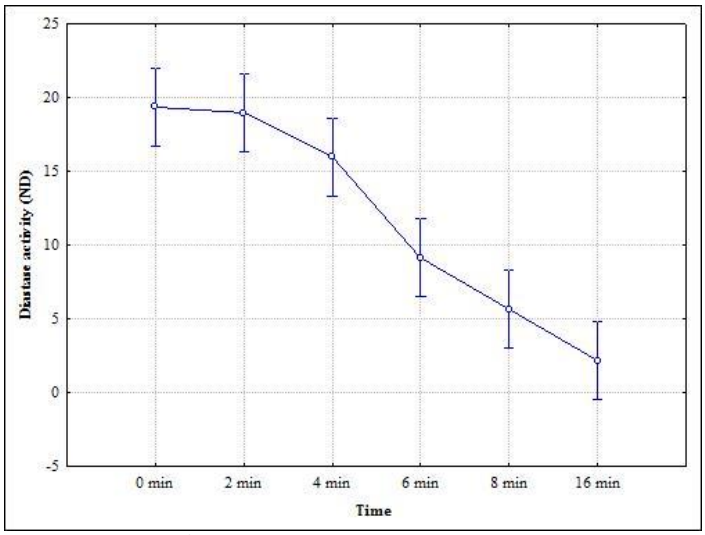

Figure 3 Variation of diastase activity during heating

Invertase activity in untreated honey

In particular, invertase is the enzyme responsible for converting sucrose, maltose, mélézitose, raffinose, cellobiose and tréhalose to fructose and glucose which are the main sugars in honey **(White, 1975; Parvanov, 2012)**. The mean value of invertase activity in fresh honey simples is 101.93 ± 37.23 IN with a minimum value observed is 38.01 IN. The invertase activity is variable in the different types of honey, the minimum values of its activity have been proposed by the

International Honey Commission (IHC): ≥50 IN for normal honeys, ≥20 IN for honeys with a low enzymatic activity and ≥10 IN for monofloral honeys (from *Arbutus sp.*, *Robinia sp.* and *Erica sp.* **(Bogdanov *et al.*, 1997)**. Therefore we noted that 100% of fresh honey samples studied contained the value of invertase activity IN ≥ 20 IN.

Effect of heating on invertase activity

During heating the invertase activity of all honey samples showed some differences were seen (Figure 4), the mean values decrease from 101.93 ± 37.23 IN to 71.41 ± 39.04 IN at time 2 min and to 23.54 ± 36.12 IN at time 4 min, according to **Karabournioti and Zervalaki (2001)**, the decrease of invertase is very fast and starts from the temperature of 35ºC, is the temperature that in many countries can be obtained during the summer. In addition the results of one-way analysis of variance (ANOVA) showed a highly significant difference ($p<0.01$) at 2 min and a very highly significant difference ($p<0.001$) at 4 min, but still above the International Honey Commission limit (invertase activity ≥ 10 IN). At time 6 to 16 min a very highly significant difference ($p<0.001$) was also observed and the mean values of the invertase activity are respectively 1.10 ± 18.63 IN, 0 ± 0 IN and 0 ± 0 IN however lower of 10 IN. Therefore the number of samples below limit was respectively decreased from 14 samples to 20 samples. These results showed that the values of the invertase activity are inversely proportional to the heating period. Invertase is considered the best indicator of freshness that diastase because it is more sensitive to heat **(Oddo *et al.*, 1999)**. According to the European Honey Commission the invertase activity could serve as a criterion for determining whether honey is stored long-term or heated at high temperatures **(Bogdanov *et al.*, 1997)**.

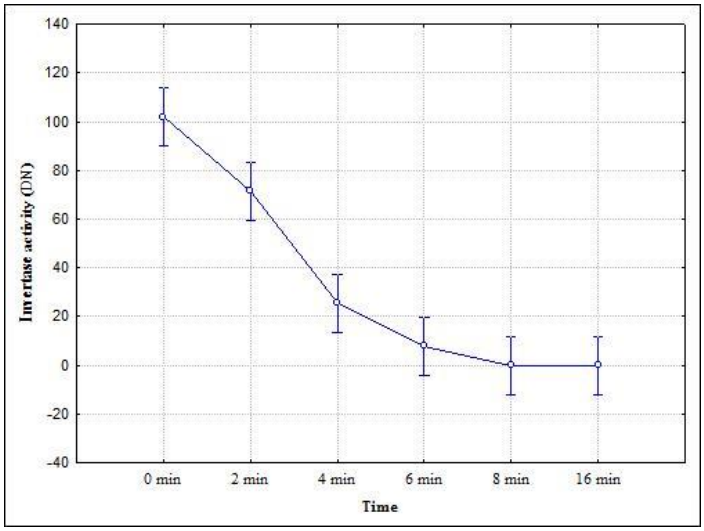

Figure 4 Variation of invertase activity during heating

CONCLUSION

We concluded that hydroxymethylfurfural (HMF), diastase activity and invertase activity concentrations in fresh multifloral honey samples from north of Algeria were within the internationally recommended range. The traditional heating (Conventional heating in the water bath at 100 °C) effect on the three parameters studied during five periods of treatment (0, 2, 4, 6, 8 and 16 min) were found to be significantly different in HMF at 16 min, diastase activity at 6 min and invertase activity at 4 min, but still within the international standard limit. The results show that invertase is more heat-sensitive and heating time than diastase and HMF. It is obvious that heating is not the only factor influencing HMF formation in honey and the destruction of enzymes, but the most important is the heating time.

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