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Influence of microwave treatment on honey quality

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Abstract

Microwaves application could be a potential tool to investigate the influence on different honey quality parameters since it is commonly used in modern kitchen. In this study, microwave sharp R-202 W serial 009157338 was used to analyse the effect of microwave treatment on flower and honeydew honeys by testing the most important honey quality parameters: invertase, diastase, glucoseoxidase and hydroxymethylfurfuraldehyde (HMF). It was examined that the change of these parameters are dependent on further parameters like the moisture content and the pH. However, all the tested enzymes are sensitive to heat. Glucoseoxidase turned out to be the most sensitive followed by invertase and diastase. The analysis showed that the pH of honeys affects the decrease of the enzymatic activities; the lower the pH the stronger the enzyme reduction. HMF, after microwave treatment there was no correlation to the pH. However, there was a slight tendency that honeys with low pH produces more HMF amount than those with higher pH but within both flower and honeydew honeys some samples showed opposite reaction. It was a clear indication that the HMF formation is not only influenced by the pH, there could be other possible factors that might influence the compound and that demand a research work. The effect of the moisture content on the enzyme activities and the HMF amount could be detected after microwave treatment.

Key words: Honey quality parameters; enzymatic activities, invertase, diastase, glucoseoxidase, HMF

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Introduction

Microwaves are an electromagnetic spectrum that range in frequencies between 300 MHz to 30 GHz (Dibben, 1995). The microwave region of the electromagnetic spectrum lies between infrared and radio frequencies (Lauf et al., 1993). The considerable advantage of microwave application was recognized in the 1980s (Decareau and Peterson,1986) and presently accepted nationwide due to its high heating efficiency, less cooking time, homogenous heating, safe handling and easy to operate with low maintenance (Salazar-Gonzalez, et al., 2012; Zhang, et al., 2006). The largest consumer of microwave energy is the food industry specifically for the cooking, tempering; thawing, drying, freeze-drying, sterilization, pasteurization, baking, heating (Ayappa et al. 1991a) with the used of modern heating techniques such as ohmic, infrared, radio frequency, pressure and other assisted microwave heating application (Sun, 2005). Microwave heating of food has been studied by many researchers (Dolande and Datta, 1993) and to investigate the different parameters that affect microwave heating (Ryyn¨amen and Ohlsson, 1996).

Crystallized honey is unappetizing to consumers compared to liquefied honeys (Horn and Hammes, 2002). Nevertheless, heating of honey is essential. Many studies showed that loss of freshness of the

honey is the result of overheating (White et al., 1964c) and recommend gentle heating for short time to avoid destroying of sensitive substances in the honey such as starch and sugar digesting enzymes (Subramanian et al., 2007). For instance, heating honey at high temperatures could result in inactivation of enzymes (Whitehurst and Law, 2001). Nonetheless, the parameters for the determination of honey quality are controversial as the tendency to honey crystallization is directly linked to sensitive parameters (Manikis and Thrasyvoulou, 2001). Similarly quality deterioration of honey could be observed by investigating the quality control parameters (Bodganov and Lüllmann 1997). Honey enzymes have become concerned to many scientific studies over the years due to its significant role for differentiating between fresh honey from adulterated honeys (von der Ohe and von der Ohe, 1992) and it is one of the features that make honey unique from other sweetner products (Huidobro et al. 1995). These include; invertase (α-glucosidase), amylase or diastase (α-amylase), glucoseoxidase, small quantities of catalase and acid phosphatase constituting major honey enzymes (White, 1975). Nonetheless, besides the enzymes, other quality parameters such as hydroxymethylfurfuraldehyde (HMF) could be used to determine adulterated honey (White, 1994). Although, initially not present in fresh honey but its content increases during storage (Nafea (2011). It is well known that heating or storage of honey has different negative on the reduction of diastase activity and the increase of HMF-contents, irrespective of the same samples been from the same botanical origin (Thrasyvoulou, 1986). Research findings suggested that HMF occurs naturally with time in most fresh honey, however high levels could be attributed to inadequate storage, adulteration with sugar additives or uncontrolled heating (Moralles, 2009) and its formation is the result of the breakdown of fructose considered to be toxic and carcinogenic, hence must be regulated in all honey standards (Michail et al. 2007). It is estimated that HMF amount in fresh honey could be approximately 5 to 30 mg/kg (Bogdanov et al. 2001) and in the Codex Alimentarius a maximum limit of 40 mg/kg was recommended in the Council directive of the European Union (Anonymous, 2002). Invertase has been discussed controversial by the European Honey Commission that, it could be used to determine the freshness of the honey, adulterated or heated at high temperature (Bogdanov et al., 1997). Nevertheless, the European commission does not specify a standard value for the enzyme but for the regulation from the German Beekeepers Association (Deutscher Imkerbund, 2004) fixed a minimum value is stated for instance, one kilogram of honey must not contain less than 64.0 units, measured after Siegenthaler (1977) (Deutsche Imkerbund, 2004). It has been observed that decrease in invertase value was a clear indication that honey is exposed to heat treatment (Bogdanov and Gallmann, 2008). Although invertase is among the most heat sensitive enzyme of honey due to its natural variations other researchers strongly argued that it is also not a dependable parameter to guarantee freshness of honey (Oddo et al., 1999; Karabournioti and Zervalaki, 2001). Nonetheless, the enzyme is often found in smaller quantity (Sibel and Arthur 2005). Although, there are honey types which have naturally caused low enzyme activities. For instance, honey from *Robinia pseudoacacia*, the invertase activity may not pass a minimum limit of 45 units /kg and the HMF content may not be more than 5 ppm (Deutscher Imkerbund, 2004). The diastatic activity reduces not only during storage but also during heating of the honey, usually measured and expressed as diastase number (DN) (Hopper, 1983) determined after the methods of SCHADE (Schade et al., 1958, Bogdanov et al., 2002) or after PHADEBAS (Sakač and Sak-Bosnar, 2012). The honey quality and International Regulatory Standards of the International Honey Commission state that the diastase activity may not be lower than 8, when expressed as diastase number (DN) (Bogdanov, 2002). However, the European Regional Standard for honey (1969) allows a minimum DN value of 8 on treated honeys. The diastase number (DN) according to Schade units is a measure of one

diastase unit that corresponds to the enzyme activity of 1 g of honey that can hydrolyse 0.01 g of starch in 1hour at 40°C (Schade et al., 1958). In Europe, little attempt has been made to investigate the influence of microwave treatment on different honey quality parameters. This is the motivation to conduct the research since devise has become a common appliance in the kitchen (Dibben, 1995), There is the need also to exploit the effect of microwave treatment on HMF concerning different type of honeys and its dependence on pH and moisture content.

Materials and Methods

The experiment was conducted at the Apicultural State Institute Laboratory, University of Hohenheim. Forty grams of each flower honey and honeydew honey were filled into mini honey jars and treated 2 min and 4 min at 80 watt using microwave.

***Determination of the refractive index*:** The refractive index of the honey samples was determined according to Chataway (1932), DIN 10752 using an Abbé leica mark II plus refractometer at 20^0 C.

***Determination of the moisture content*:** The water content of the flower honey and honeydew honey samples were analysed by applying the refractometric method as described in the standard DIN 10752 (1992).

***Determination of the pH*:** The pH of the flower and honeydew honey samples were analysed according to the instructions mentioned in the DIN 10756 (1995).

***Determination of the invertase activity*:** The invertase activity was measured according to the Siegenthaler method (1977), DIN 10759-1. The procedure requires the preparation of Buffer, substrate, stopper and honey solutions before starting the analyses. The invertase activity was calculated as: Units/ kg honey $=E_{400}$ * 198.68, where E_{400} = Extinction of the test solution at 400nm while 198.68 is a factor for the dilution and extinction coefficient.

Determination of the diastase activity: To measure the diastatic activity, the procedure given by the Megazyme International Ireland Limited (2007) calculated in Schade units per gram of honey (DN) using the equation: Schade Units/kg = (26.4 * ΔE 590) + 0.06.

***Determination of the glucoseoxidase activity*:** White and Subers (1963) observed a colour formation in an oxidation chromogen (o-dianisidine) in presence of hydrogen peroxide based on calorimetric method whereby hydrogen peroxide was used in diluted honey to investigate the glucoseoxidase in Apis mellifera. Similar procedure was followed in order to determine the glucoseoxidase activity in flower honey and honeydew honey. The glucoseoxidase activity was calculated as follows:

$$Gox = 35.59 * \Delta E_{402\ nm}$$

***Determination of hydroxymethylfurfuraldehyde (HMF)*:** The determination of HMF value was based on the spectrophotometric determination described by Winkler (1955) and the procedure was followed as mentioned in DIN 10 751 The HMF amount was calculated as follows:

$$HMF = \frac{192.00 * A * 10}{mE}$$

Where A is the absorbance; 192.00 is the factor for dilution and extinction coefficient while mE is weight of honey in grams.

Results

Moisture content: The moisture content of honey is considered as an important quality parameter. In comparison of the water content of the honeys, the flower honeys measured a minimum value of 14.4% (FH13), and a maximum value of 18.6% (FL2) with a mean of 16.3% and a standard deviation of 1.1% (Table 1 and Figure 1). The honeydew honeys measured 15.0% (HD1 and HD5) as the minimum value and the maximum value of 16.2% (HD15), by a mean of 15.5% with a standard deviation 0.5% (Table 1 and Figure 2).

Table 1. Mean and standard deviation of moisture content and pH for Flower honey and Honeydew honey.

Samples	Moisture content (%)		pH	
	Mean	Standard deviation	Mean	Standard deviation
Flower honey	16.33	1.13	4.41	0.40
Honeydew honey	15.45	0.47	4.76	0.32

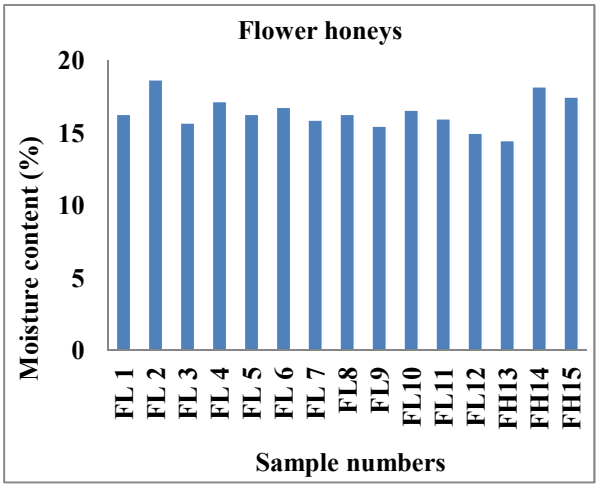

Figure 1. Moisture content of single flower honeys.

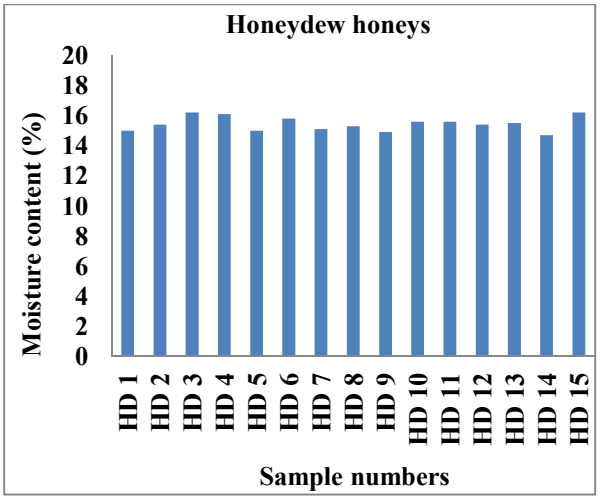

Figure 2. Moisture content of single honeydew honeys.

***pH value*:** The pH of honey can help to distinguish between the different types of honeys. In general, the pH of flower honey is lower than those of honeydew honeys. The flower honey samples based on the results measured a minimum pH of 3.68 (FL2) and a maximum value of 5.1 (FL12). The mean was found to be 4.4 with a standard deviation of 0.4. Honeydew honey samples varied between 4.14 (HD8) and 5.24 (HD10) showing the minimum and maximum pH values. The mean was 4.7 by a standard deviation of 0.3 (Table 1, Figure 3 and Figure 4).

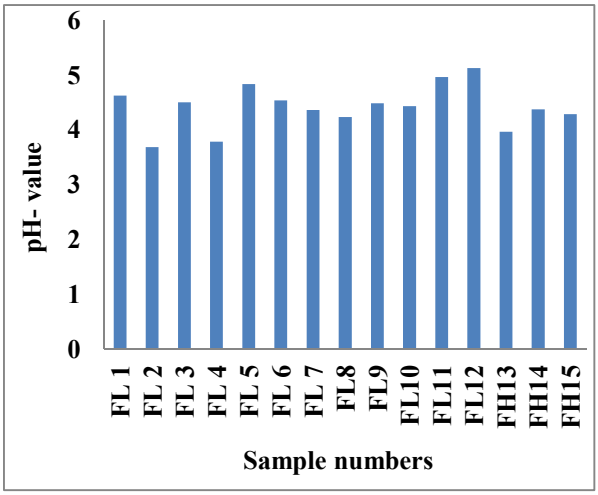

Figure 3. pH values of single flower honeys [n= 15].

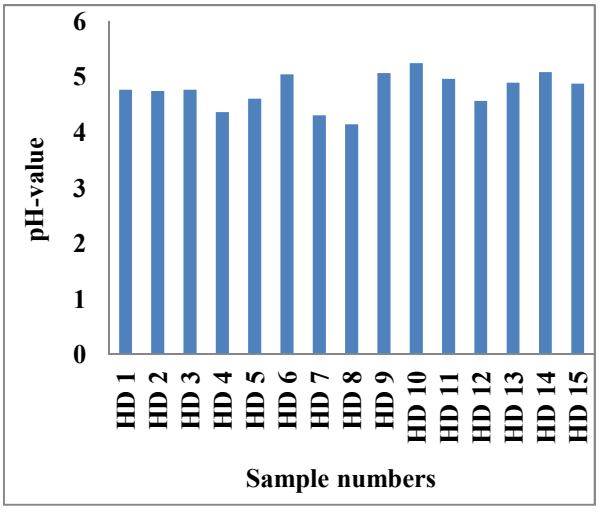

Figure 4. pH of single honeydew honeys [n= 15].

***Invertase activity*:** The minimum invertase activity of flower honeys (control) was measured at 101.2 U/kg (FL11), by a maximum of 239.4 U/kg honey (FH13) with a mean of 149.4 U/kg and a standard deviation of 47.0 U/kg. In honeydew honeys (control), 98.6 U/kg honey (HD3) was determined as the minimum invertase activity with a mean value of 130.0 U/kg honey and a maximum of 175.0 U/kg. The standard deviation measured was 23.4 U/kg honeys (Table 2, Figure 5 and Figure 6).

Table 2. Mean, standard deviation, maximum and minimum of enzyme activity and HMF for Flower honey and Honeydew honey at different treatments.

		Flower honey				Honeydew honey			
Enzymes/ compound	**Lab. Treatment**	**Mean**	**Std. dev.**	**Max.**	**Min.**	**Mean**	**Std. dev.**	**Max**	**Min**
Invertase	Control	149.36	47.00	239.4	101.2	130.05	23.39	175.0	98.6
	2 Minutes	118.98	36.04	216.0	40.6	124.15	24.08	173.3	95.0
	4 Minutes	36.64	15.14	62.3	9.7	54.88	13.40	83.2	33.3
Diastase	Control	29.06	8.29	48.7	17.5	23.91	6.57	39.5	16.4
	2 Minutes	27.47	7.51	45.3	16.7	23.00	6.46	37.4	16.3
	4 Minutes	23.32	4.15	31.4	15.5	21.36	5.53	36.4	15.9
Glucoseoxidase	Control	4.80	4.11	8.70	0.3	6.72	2.17	10.1	3.20
	2 Minutes	3.77	3.89	8.4	0.1	4.94	2.06	7.9	2.5
	4 Minutes	0.72	0.68	2.2	0.0	0.89	0.67	2.6	0.0
HMF	Control	4.38	2.66	10.1	0.7	5.53	3.99	15.5	1.7
	2 Minutes	5.79	3.19	12.4	3.2	6.87	4.82	19.1	2.6
	4 Minutes	7.71	4.34	15.6	3.0	11.11	10.32	42.5	3.0

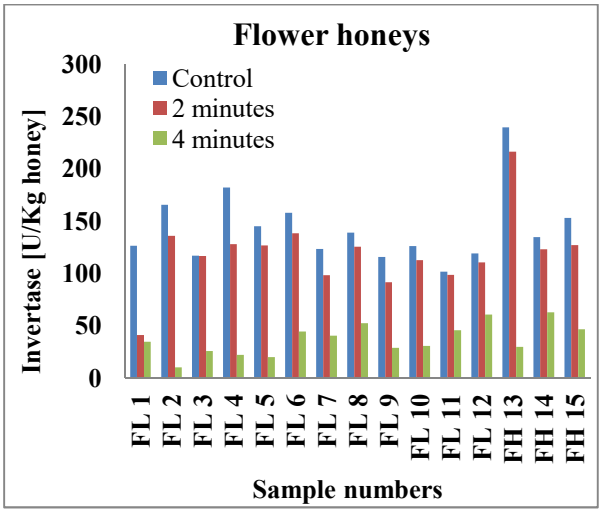

Figure 5. Invertase activities (U/kg) of flower honeys [n=15] for control and microwave treated honeys after 2 and 4 min at 80 watt.

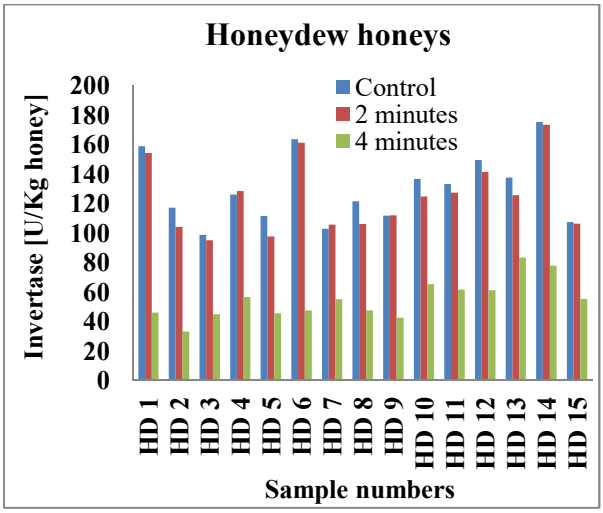

Figure 6. Invertase activities (U/kg) of honeydew honeys [n=15] for control and microwave treated honeys after 2 and 4min at 80 watt.

During microwave treatment at 2min/80 watt, the minimum invertase activity of flower honeys was 40.6 U/kg (FL1). The maximum value was 216.0 U/kg honey (FH13) with a mean of 118.9 U/kg honey and a standard deviation of 36.0 U/kg honey. Within the honeydew honeys 95.0 U/kg honey (HD3) was measured as the minimum invertase value, the maximum was 173.3 U/kg honey (HD14). The mean invertase activity was 124.2 U/kg by a standard deviation of 24.1 U/kg (Table 2). The following invertase activity of flower and honeydew honeys was measured after microwave treatment of 4 min at 80 watt. The minimum value obtained for flower honeys was 9.7 U/kg honey (FL2), by a maximum of 62.3 U/kg honey (FH14). The mean was 36.6 U/kg honey by a standard deviation of 15.1 U/kg honey. In comparison to honeydew honeys, the minimum invertase activity was 33.3 U/kg honey (HD2), maximum invertase measured was 83.2 U/kg honey (HD13) with a mean of 54.9 U/kg honey while the standard deviation was 13.40 U/Kg honey (Table 2). It needs to be mentioned here that the control honey was represented by dark blue colour, microwave treatment 2min/80 watt was red accent colour while microwave treatment 4min/80 watt was depicted as light green colour (Figure 5 and Figure 6).

***Diastase activity*:** The diastase number measured for flower and honeydew honeys control, microwave treatment 2 min and 4 min/80 watt depicted the following results. The flower honeys (control) had a minimum diastase number of 17.5 (FL12), maximum 48.7 (FL2) with an average of 29.1 and a standard deviation of 8.3. Similarly for the honeydew honeys (control), a minimum diastase number of 16.4 (HD15)was measured with a maximum of 39.5 (HD14) and an average of 23.9. The standard deviation was 6.6 (Table 2).

After microwave treatment at 2 min/80 watt, flower honeys measured a minimum diastase number of 16.7 (DN) (FL12), maximum of 45.3 (DN) could be determined (FL2). The average was 27.5 (DN) with a standard deviation of 7.5 (DN). The minimum value for honeydew honeys was 16.3 (DN) (HD15) with maximum 37.4 (DN) (HD14). The mean was 23.0 (DN) and a standard deviation of 6.5 (DN). This is showed in Figure 7 and Figure 8.

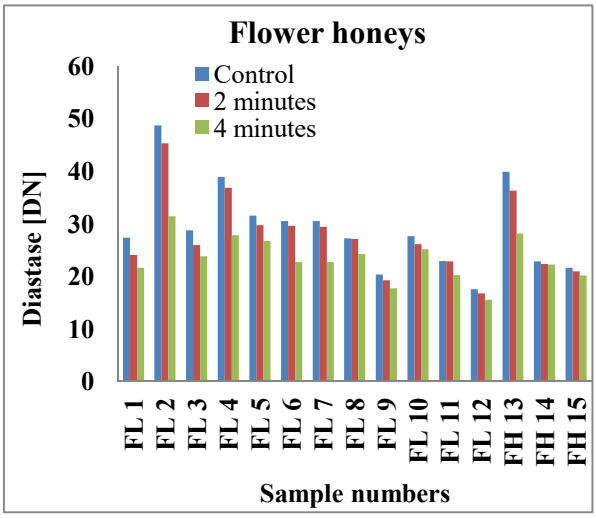

Figure 7. The diastase number (DN) of flower honeys for control and microwave treated honeys after 2 and 4 min/80watt.

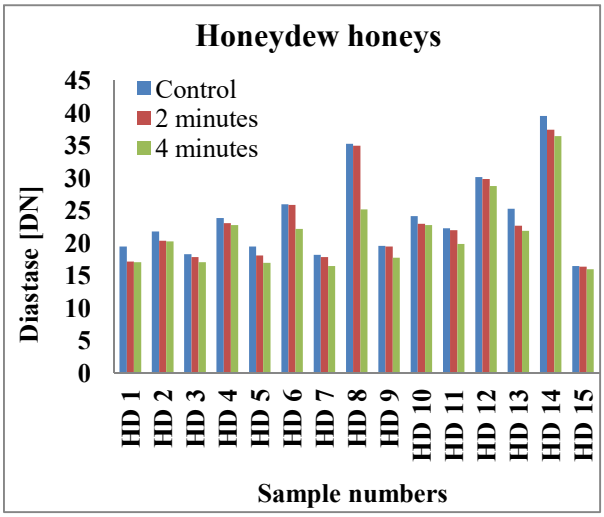

Figure 8. The diastase number (DN) of honeydew honeys for control and microwave treated honeys after 2 and 4 min/80watt.

The results obtained after microwave treatment for 4 min/80 watt in the case of flower honeys showed a minimum diastase number of 15.5 (DN) (FL12). The maximum was 31.4 (DN) (FL2) with mean and standard deviation with 23.30 (DN) and 4.2 (DN) respectively. Similarly, the minimum diastase number (DN) for honeydew honey was 15.9 (DN) (HD 15), maximum was recorded as 36.4 (DN) (HD 14) with a mean of 21.4 (DN) and a standard deviation of 5.5 (DN) (Table 2).

Glucoseoxidase activity: The minimum value for flower honeys (control) was 0.3 µg H_2O_2/min (FL7) and the maximum was measured as 8.7 µg H_2O_2/min (FL6) with a mean value of 4.8 µg H_2O_2/min and a standard deviation of 4.1 µg H_2O_2/min. Honeydew honeys (control) was measured as 3.2 µg H_2O_2/min (HD1) as the minimum value and a maximum of 10.1 µg H_2O_2/min. The mean was 6.7 µg H_2O_2/min with a standard deviation of 2.2 µg H_2O_2/min (Table 2).

The microwave treatment after 2 min/80 watt for the flower honeys was measured a minimum glucoseoxidase of 0.1 µg H_2O_2/min (FL1), maximum of 8.4 µg H_2O_2/min (FH13) with a mean value of 3.8 µg H_2O_2/min while the standard deviation was 3.9 µg H_2O_2/min. Similarly, the honeydew honeys varied between 2.5 µg H_2O_2/min (HD1) as the minimum and 7.9 µg H_2O_2/min (HD14) as a maximum, by a mean value 4.9 and a standard deviation 2.1 µg H_2O_2/min (Table 2).

After microwave treatment at 4 min/80watt between the two honeys types, flower honeys measured 0.0 µg H_2O_2/min (FL7) as the minimum. This means, that the glucoseoxidase activity had been completely destroyed. The maximum value recorded was 2.2 µg H_2O_2/min (FL6), by a mean of 0.7 µg H_2O_2/min and a standard deviation of 0.7 µg H_2O_2/min. Like flower honeys, honeydew honeys showed an average of 0.9 µg H_2O_2/min and standard deviation of 0.7 µg H_2O_2/min (Table 2).

The Figure 9 and Figure 10 showed the reduction of glucoseoxidase activity (µg H_2O_2/min) of the different honey types (flower and honeydew honeys after microwave treatment for 2 and 4 min/80 watt.

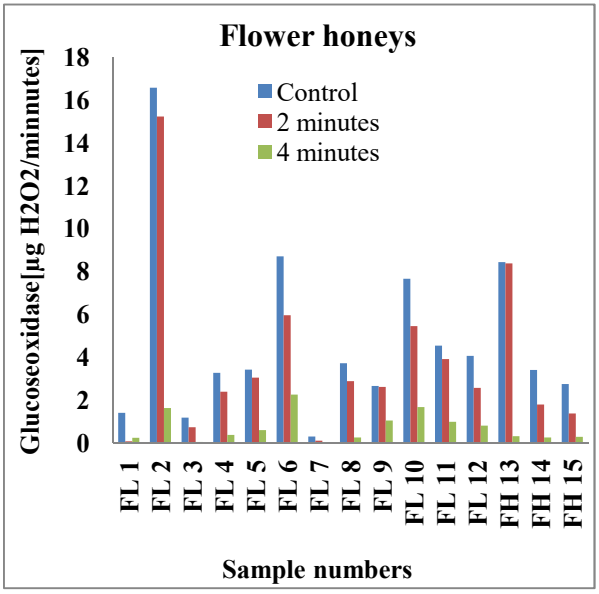

Figure 9. Glucoseoxidase activity (µg H_2O_2/min) of flower honeys (control, 2 and 4 min treatment /80 watt).

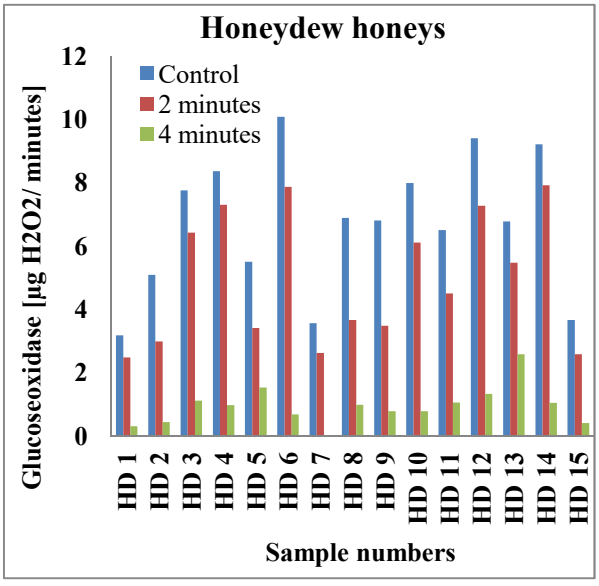

Figure 10. Glucoseoxidase activity (µg H_2O_2/min) honeydew honeys (control, 2 and 4 min treatment /80 watt).

***Hydroxymethylfurfuraldehyde (HMF)*:** The HMF value for untreated control flower honeys was measured within a range of 0.7 mg/kg (FH14) and 10.1 mg/kg (FL2). The average value was 4.4 mg/kg with a standard deviation of 2.7 mg/kg. In comparison to honeydew honeys, the minimum was 1.7 mg/kg (HD13) and a maximum of 15.5 mg/kg (HD3). The mean value was measured 5.5 mg/kg with a standard deviation of 3.9 mg/kg (Table 2).

After microwave treatment for 2 min/80 watt, the flower honey's minimum HMF value was 3.2 mg/kg (FL12) with maximum of 12.4 mg/kg (FL1), an average of 5.8 mg/kg and a standard deviation of 3.2 mg/kg. For honeydew honeys the minimum HMF value was 2.6 mg/kg (HD13) with a maximum of 19.1 mg/kg (HD3). The mean value was 6.9 mg/kg by a standard deviation of 4.8 mg/kg (Table 2).

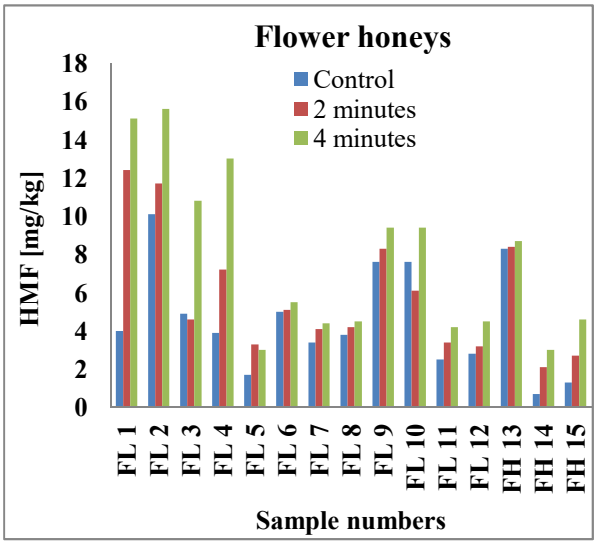

Figure 11. HMF values (mg/kg) of flower honeys for the control and after microwave treatment for 2 and 4 min/80 watt.

The microwave treatment of flower and honeydew honeys for 4 min/80 watt was also measured and compared with their average and standard deviation in Table 2. The flower honeys had a minimum of 3.0 mg/kg (FL5), the maximum HMF value was 15.6 mg/kg (FL2) by an average of 7.7 mg/kg. The standard deviation was measured as 4.3 mg/kg. Similarly, the honeydew honeys showed a minimum HMF value of 3.0 mg/kg (HD9), maximum value of 42.5 mg/kg (HD2) by an average of 11.1 mg/kg while the standard deviation recorded was 10.3 mg/kg (Table 2, Figure 11 and Figure 12).

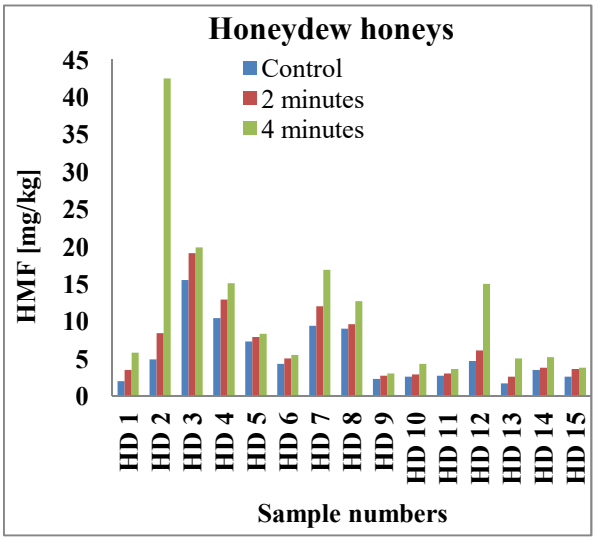

Figure 12. HMF values (mg/kg) of honeydew honeys for the control and after microwave treatment for 2 and 4 min/80 watt.

Discussion

Moisture content: The moisture content is an important honey quality parameter. Thus, it has been reported for its key role in maintaining the quality of the honey due to fermentation and crystallization. It is therefore obvious that high moisture content could be the possible course of fermentation and spoilage of honeys (Gleiter et al. 2006). Nevertheless, the Council Directive (European Commission, 2002) proposed a moisture content of 20.0% as the maximum. Most of the accredited beekeeping associations in the European countries such as Switzerland allows a maximum moisture content of 20g /100g honey and in Belgium, Austria, Italy, Spain recommend between 17.5 to 18.5g / 100g honey incase of special honey type (Bogdanov et al. 1999 a,b). The moisture content of

flower honeys ranges between 14.4% to 18.6% and that of the honeydew honeys ranges from 15.0% to 16.2%. The result obtained in the present study has conformed to the Baden-Württemberg local honey competition standard with a low average moisture content ranging from 15.6 and 15.9% (Horn, 1999, 2001). The researcher noticed that on the whole the results were within the standard limits recommended by the European honey commission. Similar to the results was the moisture content found by Lazaridou et al. (2004) that varies from 13.0% to 18.9%. It was reported in a research work from Lochhead (1933) that honey with moisture content less than 17.1% are not likely to ferment but 1.0% upwards is possible to ferment depending on the yeast counts presence in the honeys (White,1976 b). Additionally Stephen (1946) reported that, mainly honey with lower moisture content of 17.1% are considered safe and generally in ripened honey it is below 18.6% (White, 1978). The determination of the moisture content in the honey was based on the refractometric method described in the DIN 10752 (1992). There are further methods for the determination of the moisture content of honeys, like the Karl-Fischer titration. Zürcher and Hadorn, (1980) reported that normally the use of refractometry analysis results are lower compared to the Karl Fischer method. The results showed that the moisture content of different honey types neither reduces the enzyme activities (invertase, diastase, glucoseoxidase) nor increases the HMF-content. There were no significant statistical conditions between the moisture content and the single parameter.

The determination of pH in honey is an important quality parameter to investigate the presence of microbial contamination (Conti, 2000) and also during extraction and storage as the quality and storage life of honey is related to the pH (Terrab et al., 2004). The pH of flower honeys were in a range from approximately 3.7-5.0 while the honeydew honeys varied from 4.1 to 5.2. An earlier research carried out by Turhan et al. 2008 found that normally the flower honeys showed a lower pH than honeydew honeys).The composition of flower and honeydew honeys is differentiated between their pH, mineral content and sugar spectrum (Karkacier et al., 1995). The honeydew honeys often constitute of organic acids mainly malic, succinic and fumaric acids (Gray, 1952, Lamb, 1959). The pH of different honey types can vary and range from 3.6 and 5.4 and could have influence on the enzyme activities of flower and honeydew honeys (Hammes and Horn 2002).

Invertase: The invertase activity of flower and honeydew honeys was determined by Siegenthaler method (1977) and the protocol was followed according to the DIN 10759 for all the 30 samples. In order to visualize the relation between flower and honeydew honeys, they were statistically analyzed using a scattered plot. The flower honeys with lower pH experienced a strong reduction in invertase activity compared to the samples with higher pH. On closer examination of the scattered plot at pH of 3.6 after microwave treatment of 2 min/80watt, the invertase was 140 Units/kg after microwave treatment 4 min/80 watt, the invertase activity was reduced to 18 Units/kg (Table 3).

Table 3. Correlation between pH, enzyme activity and HMF after microwave treatment 2mins, 4mins/ 80watt.

	pH -value		**Microwave treatment**			
Enzyme/compound	**Flower honey**	**Honeydew honey**	**Flower honey**		**Honeydew honey**	
			2mins	**4mins**	**2mins**	**4mins**
Invertase	3.6	4.14	140.0	18.0	106.0	47.5
Diastase	3.7	4.14	45.0	32.0	35.0	25.0
Glucoseoxidase	3.7	4.18	3.8	0.7	4.9	0.9
HMF	4.5	4.74	4.6	10.8	12.0	42.5

This corresponds with a reduction of almost 80%. This result indicates that besides heating microwave treatment has a strong influence on the inveratse activity (Figure 13).

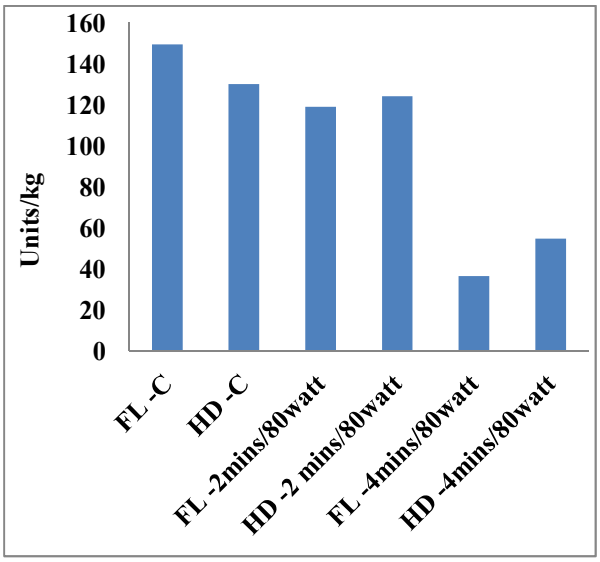

Figure 13. The invertase activity of flower and honeydew honeys after microwave treatment for 2 and 4 min at 80 watt.

In order to facilitate a better comparison, the pH of the honeydew honeys was also analyzed in a scattered plot with their corresponding microwave treatment. The honeydew honeys with lower pH experienced a strong reduction in invertase activity compared to the honeydew honeys with higher pH. In the scattered plot at the same pH of 4.14 after microwave treatment of 2 mins/80 watt, an invertase activity of 106 Units/kg could be determined, while after 4 min treatment the activity was reduced to 47.5 Units/kg (Table 3).

The average reduction of invertase was calculated manually from analytical results. The mean invertase activity within the control group of all (untreated) flower honey was determined at 149.4 Units/kg (100%). After microwave treatment for 2 and 4 min at 80 watt the mean activity reduced to 119.0 Units/kg (79.9% of the control) and 36.6 Units/kg (24.5%) of the control. In comparison to the flower honey, honeydew honeys showed the following mean inverstase activities: control group 130.0 Units/kg (100%), 2 min treatment/80 watt 124.4 Units/kg (95.5% of the control) and 4 min treatment 54.9 Units/kg (42.2% of the control). This indicates that concerning microwave treatment, the invertase activity of flower honey is more sensitive than that of honeydew honeys. In general could be proved, that the lower the pH-value of a honey, the stronger the reduction of the invertase activity, that means, the more sensitive the honey concerning microwave treatment. The results are in agreement with the results in literature who found that with increasing temperatures, saccharase in flower honeys becomes more inactivated than in honeydew honeys (Horn, Hammes, 2002). Thus, it has been reported that low invertase activities are an indication that the honeys have been subjected to heat treatment (Bogdanov and Gallmann 2008).

The diastase number was determined after Schade et al. (1958), DIN 10750. The results of the analysis in Figure 14 showed that the diastase number of a specific flower honey at pH of 3.7 after microwave treatment of 2 min was 45 DN while the 4 min/80 watt was 32 DN. It could be found that the diastase is not so sensitive than the invertase. However honeys with low pH are more sensitive than samples with higher pH. For example, after a 2 min microwave treatment at 80 watt honey sample still showed a diastase number of 45 DN, after 4 min treatment at 80 watt a remaining diastase number of 32 DN.

On close examination of the honeydew honeys, the influence of the pH on the reduction of the diastase number could also be detected. However in comparison to the invertase activity, the reduction was not so distinctive. The difference between the diastase number of the honey samples at pH 4.14 after 2 and 4 min microwave treatment. The treatment led to a reduction from diastase number of 35 DN (2 min/80 watt) to a diastase number of 25 DN (4 min/80 watt). According to the results of the analyses, both honey types, flower- and honeydew honeys, showed a significant reduction

after microwave treatment but all honeys fulfilled the diastase limits of more than 8 DN, fixed within the European honey legislation for honeys for consumption.

In order to analyze the average reduction of the diastase number after microwave treatment, a manual calculation was performed between the different types of honeys. For the control group of flower honeys an average diastase number of 29.1 DN (100%) was calculated. After microwave treatment for 2 and 4 min/80 watt a diastase number of 27.5 DN (94.5%) and 23.3 DN (80.1%) could be determined. Concerning honeydew honeys the negative effect of the microwave treatment on the remaining diastase activity was less distinct. For the control group of honeydew honeys an average of 23.9 DN (100%) could be calculated, after 2 minutes and 4 minutes/80 watt, the DN was reduced to 23.0 DN (96.2%) and 21.4 DN (89.5%) (Figure 14).

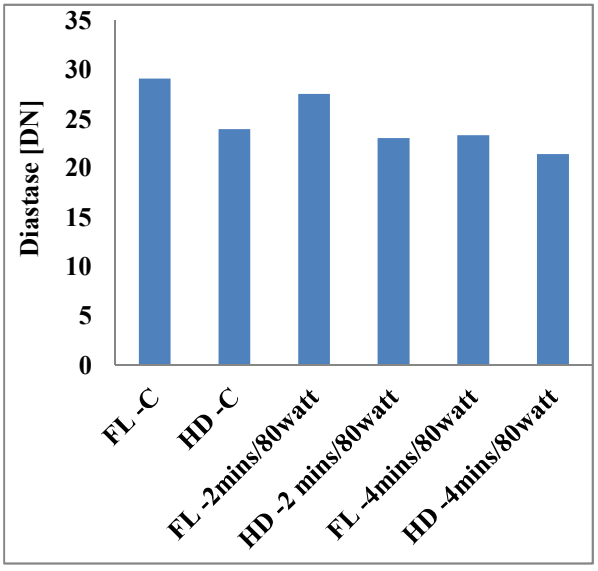

Figure 14. The effect of different microwave treatment (2, 4min/80 watt) on the diastase number (DN) of flower and honeydew honeys.

Just like the invertase activity there was a significant effect on the diastase reduction, dependent on the intensity of microwave treatment and linked to the pH-values of the different honey types. The use of diastase number as honey quality parameter has been discussed controversial. Therefore criticism of White (1992, 1994) to use diastase as honey quality parameter could be justified. Nevertheless, the assumption that diastase activity of flower honeys becomes more sensitive to heat than honeydew honeys was proved by the results (Horn and Hammes, 2002) after microwave treatment 4 minutes at 80 watt. Nevertheless, authors such as White et al., (1964); Horn and Hammes (2002) strongly argued that the enzyme is less sensitive to heat compared to invertase was also confirmed in the microwave treatment of the honeys.

Glucoseoxidase: The glucoseoxidase activity, express as µg H_2O_2/minutes has been determined after the method of Schepartz (1963). After analysing the results for flower- and honeydew honeys after microwave treatment for 2 and 4 minutes/80 watt, it can be seen, that in general the glucoseoxidase (Gox) is more sensitive to microwave treatment than the enzymes invertase and diastase. Concerning the influence of the pH on the Gox reduction after microwave treatment assume a weaker effect especially for honeydew honeys. For flower and honeydew honeys the Gox-concentration (µgH2O2) could be determined. Flower control 4.8(µg H2O2) (100%), microwave treatment for 2 min/80 watt 3.8 (µg H2O2) (79.2%) and 4 min/80 watt 0.71 (µg H2O2) (14.8%). Honeydew honeys control 6.7 (µg H2O2) (100%), microwave treatment for 2 min/80 watt 4.9 (µg H2O2) (73.1%) and 4 min/80 watt 0.9 (µg H2O2) (13.4%) (Table 3 and Figure 15). The enzyme is not fixed in the European Honey Standard. Honey contains small amounts of other different enzymes such as catalase and acid phosphatase which naturally could be observed in high amount in some honeys that destroy the glucoseoxidase (Horn and Hammes, 2002). Dustman (1967), observed that the presence of catalase maybe due to microorganisms in honeys already present in honeydew.

The assumption that glucoseoxidase is more sensitive to heat was confirmed by the results and therefore Gox

could considered as a good honey quality parameter. It is known that glucoseoxidase is highly sensitive than saccharase (White and Subers 1963). The same authors reported that it is sensitive to heat and light (White and Saubers, 1964a, 1964b). It has been argued that glucoseoxidase is not a good quality parameter to heat and storage when honey is stored under low condition (Horn and Hammes, 2002).

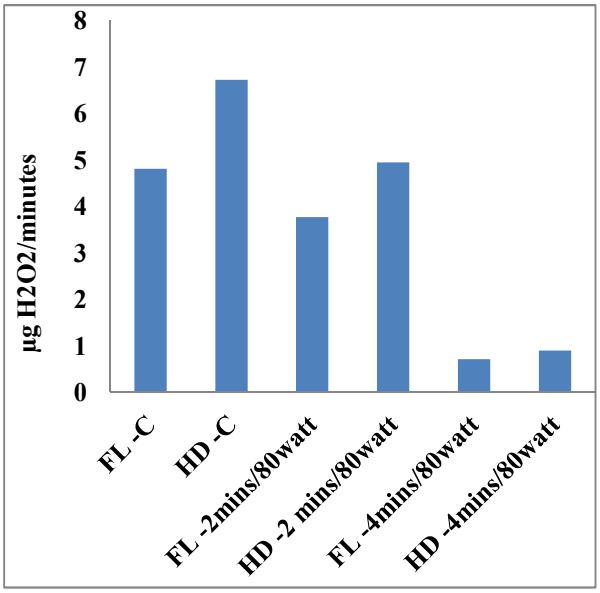

Figure 15. The Gox activity of flower- and honeydew honeys after microwave treatment for 2 and 4 min at 80 watt.

***Hydroxymethylfurfuraldehyde (HMF)*:** Besides the enzymatic activities of the honeys, the HMF content was also analyzed to investigate, if the change of the compound is dependent on the pH, as already proposed in the literature (Horn and Hammes, 2002). The results clearly confirmed this statement. For flower honeys it was observed that the tendency after microwave treatment, the increased HMF might be influenced by the pH. The lower the pH of the honey sample, the higher the HMF increase. This phenomenon could not be proved within the group of honeydew honeys, where no strict dependency between pH and HMF could be found. However even within the group of flower honeys some samples could be detected. In comparison to flower honeys with lower pH the sample number HP 23/13 with a pH of 4.5 showed a high increase of HMF (Table 3). In comparison to flower honeys with lower pH-values within a 2 minutes/80 watt it changed from 4.6 mg/kg to 10.8 mg/kg (after 4 min/80 watt). Other honey samples from this group with lower pH did not show comparable reactions. Concerning the group of honeydew honeys there is another sample, showing extreme reaction. The sample number HP 97/13 with a pH of 4.74 reached after 2 min/80 watt microwave treatment, a HMF content of 12.0 mg/kg and finished with 42.5 mg/kg after 4 min/80 watt. This is more than the three fold amount which was measured after 2 min treatment (Table 3). Other samples with lower pH did not show comparable reactions. The reasons for the fact, that the HMF- formation in different honey types is not only dependent on its pH. It could be explained that there exist still further parameters which influence the HMF- formation.

The average increase of the HMF contents was calculated from the analytical results. The mean HMF-content within the control group of flower honeys was determined at 4.4 mg/kg (100%), microwave treatment led to 5.8 mg/kg (131.8%) after 2 min/80 watt and 7.7 mg/kg (174.9%) after 4 min treatment/80 watt. Although the pH of honeydew honeys is normally higher than these of flower honeys, the increase of HMF production did not fulfill our expectations (Figure 16). Concerning the literature the average increase of HMF in the group of honeydew honeys should be lower than the average increase within the group of flower honeys. According to our analysis the mean HMF-content within the control group of honeydew honeys was determined at 5.5 mg/kg (100%), after 2 min treatment at 80 watt it reached 6.9 mg/kg (125.4%) and finished at 11.1 mg/kg (201.7%) after 4 min treatment at 80 watt. It is argued that honey with a high pH produces more HMF content compared to honeys with lower pH especially if they are stored in the same condition (Horn and Hammes 2002). According to (Fallico et al., 2004; Bath and

Singh, 1999; Karabournioti et al., 2001, White 1978) the increase of the compound maybe due to temperature and the time of heating were confirmed in the results. Picher et al. (1984) investigated on the factors that increase the HMF value in honey-like solution.

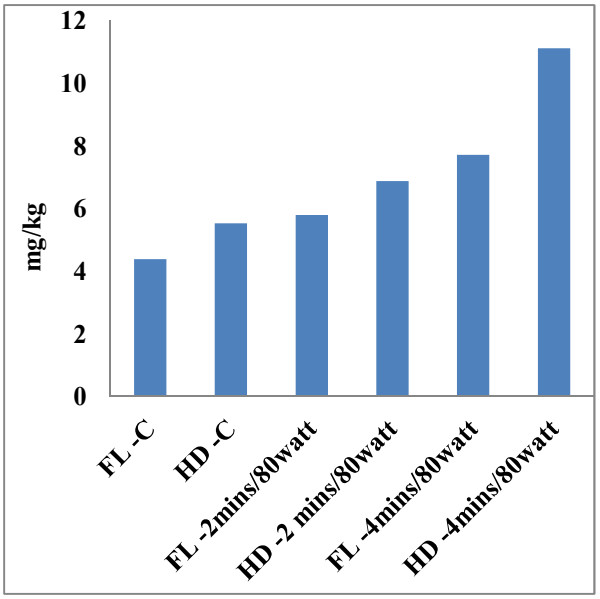

Figure 16. The HMF increase (mg/kg) in flower and honeydew honeys after microwave treatment of 2 and 4 min at 80 watt.

The researcher observed that the increase is mainly dependent on parameters such as pH, sugar spectrum, storage and moisture content. Horn and Hammes (2002) strongly argued that the demand of White (1994) to use HMF as a honey quality parameter could be further investigated. The same authors criticized that HMF could not be the right parameter to identify the freshness of honey due to heat and/or storage damage of honey.

Conclusion

The results confirm that negative effects on honey by microwave treatment even under low temperature conditions and could show for the first time that this damage is dependent on the sort of honey. It could be examined if the change of these parameters is dependent on further parameters like the moisture content and the pH. All the tested enzymes are heat sensitive. If the enzymes sensitivity is arranged, the Glucoseoxidase turned out to be the most sensitive, followed by invertase and diastase. It could be proved that the pH of the honeys affects the decrease of the enzyme activities, the lower the pH, the stronger the enzyme reduction. During microwave treatment the enzyme reduction is not a linear reaction. The average decrease in general between the control activities and the remaining activities after 2 min/80 watt is lower than the average damage between the remaining activities after 2 and 4 min/80 watt treatment. This reaction is caused by the progressive temperature rise during microwave treatment. After 2 min/80 watt treatment no significant increase in temperature could be ascertained. Concerning HMF, after microwave treatment, there was no correlation to the pH. There was a slight tendency, honeys with low pH set up more HMF than these with higher pH but within both honey types flower- and honeydew honeys a few samples showed opposite reaction. This indicates that the HMF formation is not only influenced by the pH. An effect of the moisture content on the enzyme activities and the HMF content after microwave treatment could not be detected. The results are important contribution to extension activities in order to prevent beekeepers from using microwave for the liquefaction of crystallized honey.

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