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Dima Al-Diab

Associate Professor, Department
of Food Chemistry, College of
Pharmacy, Tishreen University,
Latakia, Syria

Bushra Jarkas

Ms. student, Department of
Food Chemistry, College of
Pharmacy, Tishreen University,
Latakia, Syria

Effect of storage and thermal treatment on the quality of some local brands of honey from Latakia markets

Dima Al-Diab, Bushra Jarkas

Abstract

This study was conducted to assess the influence of and thermal treatment and storage conditions on the content of 5-hydroxymethylfurfural (HMF) in some types of Latakia honey citrus, anise, black seed (*Nigella sativa*), polyfloral blossom A, polyfloral blossom B. stored at room temperature for six months Based on White's method, the five types of honey (citrus, mountain, anise, Erica, and oak) were thermally treated through microwave as well as conventional methods at 50, 80, 100 °C for hour, two and three hour intervals. HMF content was spectrophotometrically determined at 284 and 336 nm In accordance with the Syrian standard No. 412/of/2004/.

Four samples citrus, mountain, anise, black seed (*Nigella sativa*) were stored at room temperature for six months. It was found the higher temperature and time the higher content of HMF was obtained. Comparatively, microwave-based heating was the least influential on HMF content in the four crystallized samples of citrus, mountain, anise, black seed (*Nigella sativa*)

Keywords: Honey, observing, 5-hydroxymethylfurfural, White's method

1. Introduction

Honey is a dietary supplement ^[1]. Honey is essentially a concentrated aqueous solution of sugars with pH values of (3.4-6.1). Sugars such as glucose and fructose form a high ratio of its formulation. It's a mixture of nutritional such as proteins, amino acids, organic pigments, aromatics, waxes, minerals, pollen and enzymes ^[2, 3].

Honey is very common in traditional medicine. Numerous studies revealed the antimicrobial and antifungal activity. Besides, these studies supported the increased use of honey in treatment skin lesions ^[1]. Honey is also considered a significant source of antioxidants since it's rich in phenolic acids, flavonoids, ascorbic acid and carotenoids ^[4] Honey also serves as an approach in controlling diabetes ^[5]. Some studies have shown that honey possesses anti-cancer properties, specifically in regard to inhibiting the growth of tumor cells ^[6].

But, several harmful and toxic compounds such as HMF may be present in honey and possibly effect when consumed by humans. And several studies have shown that the compound has adverse effects of causing mutation, toxic genetically and carcinogenic to mice ^[7], and it was also has adverse effects on blood cells and it was found that it induced tumors and colon cancer also ^[8]. Due to potential toxic effects, HMF is essential for assessing the conformity of honey, 9chua

Honey quality is significantly influenced by storage time and heating. Thermal treatment of honey is a critical issue for preserving honey integrity during manufacture. Thus honey pasteurization can be safely used to get rid of fermentation ^[10]. Honey should not be heated or treated thermally that lead to a change in its basic formulation or affects its quality, and chemical or biochemical treatments must not be performed to effect on the crystallized honey ^[11].

It was observed that it can improve the appearance and texture of honey and stop its crystallization ^[12]. And, honey nutrients such as sugars, proteins...etc. are prone to thermal decomposition in which much of its nutritional value is lost. Further, HMF is a thermal byproduct as well as a major marker of honey quality ^[13]. HMF results from the caramelization of carbohydrates especially hexa- polysaccharides in an acidic medium ^[3, 14], and it also results from the degradation of products of Maillard reaction (MR) ^[15].

(HMF) compound can be formed from Ascorbic acid decomposition ^[16]

In accordance with the Syrian standard specifications No. 412/of/2004, it's recommended that

Correspondence:**Dima Al-Diab**

Associate Professor, Department
of Food Chemistry, College of
Pharmacy, Tishreen University,
Latakia, Syria

the maximum level of HMF is 40 mg/kg for most varieties of honey and 15 mg/kg for citrus honey.

The increasing concentration of the (HMF) compound on the limit refers to the increasing age of the honey [17] or subjecting it to thermal processing during getting and/or filling it [18] or during heating in order to eliminate the problem of crystallization [10].

HMF content increase in a way beyond the set limits indicates to adulteration of honey whether by selling the old honey as a new, replacement of honey class with another cheaper one or by adding cheap materials such as syrup corn starch glucose-rich or corn, starch syrup and fructose-rich syrup to honey. Other types of honey falsification is feeding bees with sucrose syrup [19].

The quality parameters of honey can be classified into physical, chemical and biological groups. With physical methods important parameters such as sensory, color, taste and smell, moisture and viscosity could be determined. such while the water activity and [20] and [19]. The quality control has several chemical opportunities for the analysis. Some of the chemical parameters of honey acidity, ash, sugar identification as glucose, fructose, and sucrose. Besides, screening of the effectiveness of the Diastase enzyme. In this context detecting and measuring (HMF) could be included [21].

Therefore and depending on White's method, we aim in this study at investigating the presence and the content of hydroxymethylfurfural HMF levels of some honey samples in local market in Latakia on one hand, and at demonstrating the influence of thermal treatment and storage on hydroxymethylfurfural levels in honey on the other hand.

In correlation with variations of hydroxymethylfurfural content due to thermal processing and storage. HMF is a significant indicator for honey quality

2. Materials and Methods

2.1 Honey samples

Different Honey samples were obtained from different local markets in Latakia. The research was conducted on many honey samples stemming from different botanical origin (citrus sp., anise sp, Nigella sativa, Erica, oak, and mountain). The two remaining ones were polyfloral blossoms (A, B) in origin.

2.2 Chemical and Reagents

In this study many solvents and reagents were used such as:

- standard hydroxymethylfurfural (Sigma-Aldrich),
- Carrez solution1 (Potassium Hexacyanoferrate (II) 3-hydrate 15%) (Riedel-de Haën)
- Carrez solution2 (zinc acetate 30%)(Rectapur),
- Sodium bisulphite (Riedel-de Haën).

2.3 Apparatus and Instruments

- sensitive balance (Precisa XB 220 A),
- spectrophotometer (UV -530V Jasco)
- water bath (ESM-3711-H)

2.4 HMF Determination

According to the study course honey samples were sorted on basis of impact factors on HMF content

1. Storage
2. citrus, anise, black seed (Nigella sativa), polyfloral blossom A, polyfloral blossom B Water bath Thermal treatment citrus, mountain, anise, Erica, and oak Water bath and microwave thermal treatment citrus, mountain, anise, black seed (Nigella sativa)

2.4.1 Influence of storage on HMF development

Five honey samples of citrus, anise, black seed (Nigella sativa), polyfloral blossom A, polyfloral blossom B were stored in tightly sealed containers for six months storage at room temperature (<25 °C) in dark place (with less sun shine and therefore with cold conditions) analyzed prior to and after storage where each sample test was performed in triplicate and was expressed as mean value ± standard deviation.

2.4.2 Effect of temperature on HMF

Five honey samples of citrus, mountain, anise, Erica, and oak were subjected to thermal treatment heated in a water bath at temperatures of (50, 80, 100 °C) for time intervals (an hour, two and three hours).

The effect of temperature and duration of heat treatment on the formation of HMF were studied, and the HMF concentration has been identified before and after heating by using White's method. Each sample test was performed in triplicate and was expressed as mean value ± standard deviation.

2.4.3 Comparison between water bath and microwave heating

A sample from each honey type of citrus, mountain, anise, black seed (Nigella sativa) was divided into three sets subsamples. As two of them were crystallized by refrigeration the third set was left without crystallization as a reference. While, the second set of subsamples were individually melted by microwave heating for 30, 45, 60, and 90 seconds at the selected power of 270 W, the third set was melted by a conventional heating method (water bath) at temperatures of (50 °C, 80 °C, 100 °C) that liquefaction would completely occur in a given time period. HMF was immediately determined in all subsamples by the White's method.

All concentrations by applying the following equation:

$$\text{HMF}_{(\text{mg/kg})} = (A_{284} - A_{336}) \times 149.7 \times 5 / W$$

Where:

HMF_(mg/kg): a number of milligrams of (HMF) compound in (1 kg) of honey.

A₂₈₅: absorbance at wavelength (284 nm).

A₃₃₆: impurities absorbency at wavelength (336 nm)

W: weight of the honey sample (grams).

As shown in formula the absorbency caused by impurities (336 nm) is taking into account and is subtracted from the total absorbency of sample (284 nm) in order to minimize the error resulting from the presence of impurities as possible [22] (International Honey Commission, 2002).

2.4.4 Statistical analysis

The data obtained in the study were analyzed statistically using student t-test. Differences between honey types were determinate by Student's t-test (P ≤ 0.05).

3 Results and Discussion:

3.1 Influence of storage on HMF development

Fresh honey contains practically no HMF but its concentrations increase during storage. Honey quality can be affected by ageing during storage. The initial content of HMF was obviously low in the five samples of the honey. HMF content in the study honey samples rose slightly during six months of storage in tightly sealed containers for six months storage at room temperature (<25 °C) in dark place, which can be considered suitable conditions for preserving the quality of honey, although according to different ratio are summarized in Table 1 and Fig 1

During the six month-storage the HMF increased respectively 14.7%, 11.1%, 8.2%, 5.4%, 2.5% in Poly floral1, citrus, anise,

black seed (*Nigella sativa*)

Polyfloral blossom B honey samples. Thus, the maximum increase in HMF after storage for six months was found highest in Poly floral blossom a, citrus while it was found lowest in Polyfloral blossom B honey the HMF content progressively increasing. Thus, HMF content formation progressively increased as per to storage time. These findings are similar to the reported increases values of HMF (1 mg/kg) or (16%), (2 mg/kg) or (33%) respectively, were found to happen in Turkey and Nepal within seven and eight months^[23,24] It should be noted however that high permissible levels of HMF 38 mg/kg, thus HMF content beyond 100 mg/kg can still be an indicator of adulteration with inverted sugars due to inadequate storage conditions that do not induce caramelization and Millard interactions which lead to the formation of HMF compound especially when storage was in the ninth month of the year (2014) and the third month of the year (2015), i.e. the period of autumn and winter away from high temperatures in summer. Amadori compound in the

intermediate stage of the Millard interaction Hydroxymethylfurfural (HMF) content is one of the most important quality parameters of the quality and health safety of honey.^[25] this cyclic aldehyde develops in honey either by hexose dehydration (glucose and fructose) in acidic environment or as a result of Millard's reaction^[9, 26]. HMF content in fresh honey is very low or nonexistent, its concentration increases in the course of storing (in relation to pH, the length of storing) and also in the course of the honey heating.

The Student's t-test was performed to evaluate the significance of the differences between the HMF concentrations in honeys obtained with White method All of the result values were less than 4.303 at 95% confidence limit with 2 degrees of freedom; After storage, t- student test the values were found to be 2.04, 2.23, 0.8, 2.31, 2.83 for honey samples of citrus, anise, black seed(*Nigella sativa*), Poly floral blossom A, Polyfloral blossom B respectively as shown in the table 1 Fig1

Table 1: HMF content of honey in function of storage (<25 °C, 6 months) with the values of the calculated statistical T

Honey sample	(HMF) content before storage (mg/ kg)	(HMF) content after storage (mg/ kg)	HMF ratio%	T
citrus	26.9 ± 1.56	29.9±2.52	11.1%	2.04
anise	30.4 ± 0.98	32.9±1.9	8.2%	2.23
black seed (<i>Nigella sativa</i>)	29.2 ± 2.82	30.8±3.38	5.4%	0.8
Polyfloral blossom A	34.6 ± 2.17	39.7±3.3	14.7%	2.31
Polyfloral blossom B	35.2 ± 3.22	36.1±0.95	2.5%	2.83

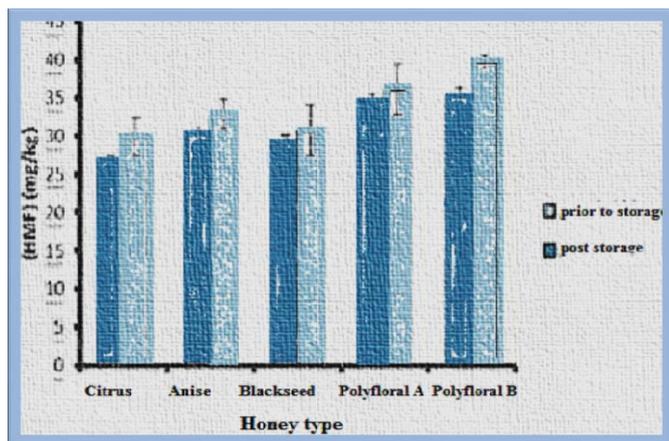


Fig 1: HMF content of honey in function of storage

3.2 Influence of Bath water heating on HMF development

It is well known that honey heating results in the formation of HMF. Hence the chosen temperature degrees (50, 80, 100 °C) at time intervals of an hour, two and three hours were suitable for screening the impact of conventional thermal treatment with water bath WB on five selected honey samples of citrus, mountain, anise, Erica, and oak. Obviously, Table 2 Data summarize the influence of heat treatments on the HMF of the five tested honey samples. When submitted to 50 °C temperature for an hour, citrus, mountain, anise, Erica, and oak honey samples HMF ranged from 39 to 45.3mg/kg. The HMF content depends on the temperature thus, at 80°C temperature for an hour, it was found that HMF of the tested honey samples ranged from 45 to 62.1mg/kg. Further, at 100 °C temperature for an hour, HMF of the tested honey samples progressively increased and ranged from 55 to 91.1mg/kg similarly as reported by^[9, 27, 28] It was found that HMF content

also depends on the duration heating. So, HMF content after two hours of heating at 50 °C ranged from 39 to 45.3mg/kg and progressively increased to range from 49.9 to 68.1mg/kg, after three hours of thermal treatment at the same temperature. Besides, the higher temperature and the longer duration of heating the higher HMF we get. Hence, a low heating temperature resulted in low HMF increase in the given periods of time. As noted in Figure 2, 3, 4, 5, 6 the increase proportion of HMF strongly depends on temperature and heating duration. The ranges of the recorded values of HMF were: When the tested samples submitted to 80 during 2-3 hours (66.3-88) (80-94 respectively comparing with (3.22-99.9) and (89-116.7) mg/kg when submitted to 100 °C during two -three hours.

The heating time and temperature are the main parameters that influence the enzymatic activity and HMF formation in honey. The heating time range and temperature seems to have comparable impact in case of HMF formation. At 50 °C the HMF increase ratio is 0% in the first 0.5 hour and 47.19% after 3 hours and in this time range only heating at 80 °C for 0.5 hour has lower increase ratio (27.9%). The highest HMF formation ration is obtained at 100 °C in time range 1-5 hours. In terms of diastase activity loss, the heating time seems to have a larger impact on enzyme inactivation rather than temperature. Heating at 50 °C for 0.5 hour reduce the enzymatic activity with 7.9% and with 21% after 1 hour, while heating at 80 °C reduces the diastase activity in 0.5 hour with 15.3%. After 3 hours at 50 °C, the enzymatic activity is more drastically reduced (53.71%) than in cases of heating at higher temperature but shorter time ranges: 48.29% for 0.5 hour at 100 °C or 49.55% for 1 hour heating at 80 °C. The complete inactivation of enzymatic activity appears in range 3-5 hours at 50 °C and 80 °C and in range 2-3 hours at 100 °C 31 Tosi (2008).

Table 2: spiked HMF content of honey in function of temperature degree and duration of heating: HMF levels present in thermally treated honey samples under time intervals, and percent recoveries of spiked samples

Honey type	(HMF) concentration before Heating (mg/kg)	Heat treatment time)h(Heat treatment					
			50 °C	(HMF) Recovery%	80 °C	(HMF) Recovery (%)	100 °C	(HMF) Recovery (%)
			Mean value ±Sd		Mean value ±Sd		Mean value ±Sd	
citrus	26.9	1	39± 2.62	44	45 ± 2.61	67	55 ± 4.01	104
		2	55±2.95	104	75 ±1.54	178	3.22±2.33	193
		3	60±3.82	123	80 ± 4.64	197	89 ± 3.34	230
mountain	36	1	39±1.74	8.3	53 ± 2.29	47	88.7 ± 0.9	146
		2	58.9±3.3	63	66.3 ± 2.9	84	99.9 ± 4.4	177
		3	68.1±3	89	89.5 ± 1.4	148	116.7 ± 3.1	224
Anise	39.5	1	45.3± 2.1	14	55.7±2.1	41	63.8±5	61
		2	68.1±2.4	72	88±2.7	122	91.1±3.4	130
		3	79.9 ±2.3	102	91±2.1	130	105.5±1.9	167
Erica	37.4	1	39.1±1.4	4.5	62.1±2.9	66	91.1±4.2	143
		2	62±3.3	65	70±3.6	87	99.8±5.7	166
		3	81±4.1	116	94±3	151	115±1.7	207
oak	33.2	1	42.3±2.4	27	48.3±5.8	45	60.7±5.6	80
		2	49.9±2.3	50	72.9±1.7	119	75.9±2.9	128
		3	78.2±4.6	135	88.1±2.4	165	100.9±2.5	203

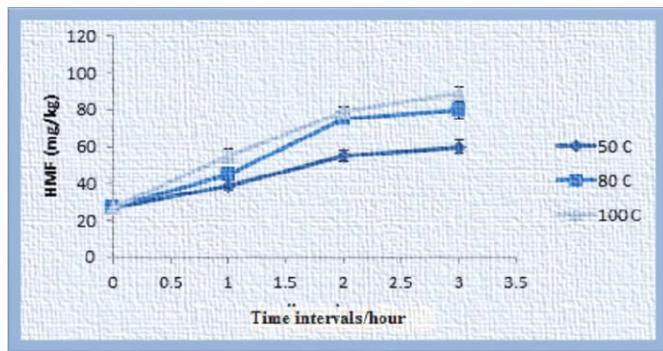


Fig 2: spiked HMF content of citrus honey in function of temperature degree and duration of heating

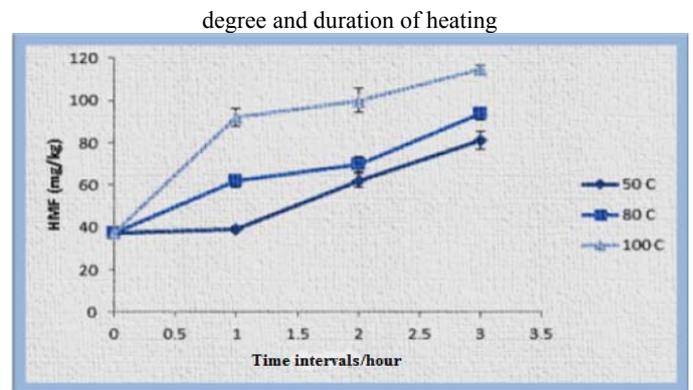


Fig 5: spiked HMF content of Erica honey in function of temperature degree and duration of heating:

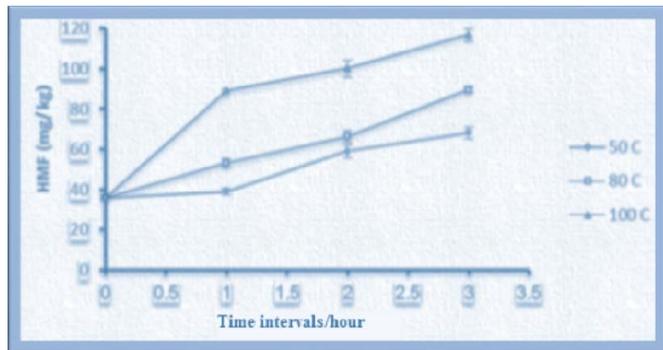


Fig 3: HMF content of mountain honey in function of temperature degree and duration of heating

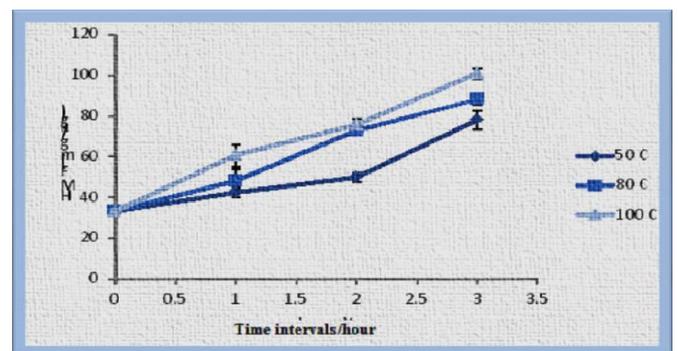


Fig 6: spiked HMF content of oak honey in function of temperature degree and duration of heating

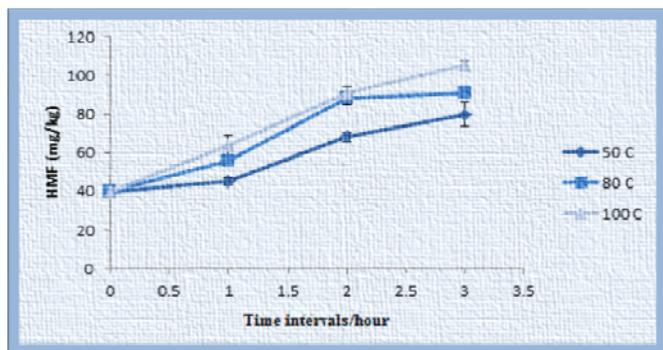


Fig 4: spiked HMF content of anise honey in function of temperature

Influence of microwave and conventional heating on HMF development

Microwave heating slightly influenced on hydroxymethylfurfural content in the tested honey samples in comparison with conventional heating approach. Measuring the changes that occur in the HMF content is primarily indicator of thermal processing, honey. HMF content in the analyzed honey samples due to microwave and conventional heating is presented in Table 3. Remarkable increase in HMF content was noticed as the conventional heating was applied on the four honey types of citrus, mountain, anise, black seed (*Nigella sativa*) Figs 7,8,9,10. HMF formation in the analyzed

honey samples due to microwave heating is presented in Figure 3. As these graphics show, no gradual increase in HMF content occurred as the heating interval was lengthened. Tab3 On the contrary, for some samples the HMF content was identical after two microwave heating, and then slightly increased or The lowest HMF content in crystallized honey was measured for Anise variety, 33.4±1.7mg/kg, while black seed honey had the highest level of this compound, 44.3±3.7mg/kg.

The recorded values of Hydroxymethylfurfural content of three other crystallized honey types were below 40 mg/kg, the maximum HMF content admitted in honey by the Syrian standard No. 412/of/2004/. Although it was very low or within the permissible limits in the unheated sample, the HMF content of all analyzed honey samples increased significantly after convention heating at 100 °C; it's recorded maximum 59.9±1.1mg/kg for citrus honey, 54.6±1.2 mg/kg and 48.5±1.2 mg/kg for black seed honey at 80 °C and at 50 °C respectively. Conventional heating of honey samples caused a significant increment in HMF contents. The results revealed also that heating was not the only factor influencing HMF formation in honey, but also honey composition, pH value and honey composition of sugars can contribute to these variations. Consequently, the amount of HMF may be an insufficient sole indicator of honey quality . the honey should not be subjected to microwave heating. While fridge based crystallization of honey samples leads to an increase in the water content of the

honey which causes an increase in the water activity. The energy transmitted by the microwave radiation excites the water molecules and it causes heating and evaporation while the other components are only slightly affected by this excitation. In results variation in Acidity due to bioactivity which it may induce formation of HMF. It's also produced as a result of Maillard reaction and/ or hexose dehydration, practically, HMF is found in fresh honey in low levels, and increases due to heat treatment [30]. Three similar studies were conducted in Czech and Romania [31, 32, 33]

It can be seen that the recovery rates were quantitatively varied in the range of 0.3–100.3% for 4 honey samples with pooled standard deviation with experimental of 0.4–3.1 . As can be seen from Tables 3a and 3b, the Student's t-test for comparison of the mean values and their relative standard deviations demonstrated that there was significant difference between the mean values obtained by two melting procedures Because the experimental t-values ranging from 0.4 to 3.1 are lower than the tabulated t-value of 4.303, it can be concluded that the mean values obtained by two heating approaches contain a significant difference for 2 degree of freedom at 95% confidence. Albeit, It is clear that microwave heating didn't cause a mitigate increase in the concentration of HMF in 4 analyzed honey samples, whereas the application of t-student test showed that the honey melting in the temperature of (50, 80 or 100 °C) has led to a significant increase in the concentration of HMF compound in all studied honey samples

Table 3: HMF content of honey in function of microwave and conventional heating and intervals
HMF levels present prior to and post melting of previously crystalized honey samples under time intervals

Honey type	HMF content prior to melting (mg/kg)	Heat treatment							
		Microwave-based melting		WB- based melting					
		Mean value ±Sd	Melting time/ (sec)	Mean value ±Sd	Melting time/ (sec)	Mean value ±Sd	Melting time/ (sec)	Mean value ±Sd	Melting time/ (sec)
citrus	29.9± 1.3	30±1.4	30	41.7±0.4	150	49.4±0.6	90	59.9±1.1	30
Mountain	36.2±0.7	37.1±2.5	60	44.2±1.3	180	51.7±3.1	120	55.6±0.7	75
Anise	31 ± 1.5	33.4±1.7	45	39.8±0.7	150	45.7±1.2	75	50.3±1	60
black seed (Nigella sativa)	40 ± 1.1	44.3±3.7	90	48.5±1.2	190	54.6±1.2	110	58.7±0.79	30

Table 4: HMF content of honey in function of microwave and conventional heating at time intervals:
- HMF levels present prior to and post melting of previously crystalized honey samples under time intervals
- HMF levels present in thermally treated honey samples under time intervals, and percent recoveries of spiked samples

Honey Type	HMF content prior to heating (mg/kg)	Heat treatment							
		microwave	(HMF) Recovery (%)	50 °C	(HMF) Recovery%	80 °C	(HMF) Recovery (%)	100 °C	(HMF) Recovery (%)
				Mean value ±Sd		Mean value ±Sd			
citrus	29.9± 1.3	30±1.4	0.3	41.7±0.4	39.5	49.4±0.6	65.2	59.9±1.1	100.3
Mountain	36.2±0.7	37.1±2.5	2.5	44.2±1.3	22.1	51.7±3.1	42.8	55.6±0.7	53.6
Anise	31 ± 1.5	33.4±1.7	7.7	39.8±0.7	28.4	45.7±1.2	47.6	50.3±1	62.3
black seed (Nigella sativa)	40 ± 1.1	44.3±3.7	10.8	48.5±1.2	21.3	54.6±1.2	36.5	58.7±0.79	46.8

Table 5: HMF content of honey in function of microwave and conventional heating with the values of the calculated statistical T

Honey type	HMF concentration before heating (mg/kg)	Thermal treatment							
		microwave	T	50 °C	T	80 °C	T	100 °C	T
citrus	29.9± 1.3	30±1.4	0.12	41.7±0.4	43.7	49.4±0.6	53	59.9±1.1	44.9
mountain	36.2±0.7	37.1±2.5	0.6	44.2±1.3	9.7	51.7±3.1	8.3	55.6±0.7	47.1
Anise	31 ± 1.5	33.4±1.7	2.3	39.8±0.7	18.8	45.7±1.2	20.01	50.3±1	32.81
black seed (Nigella sativa)	40 ± 1.1	44.3±3.7	1.96	48.5±1.2	11.9	54.6±1.2	19.4	58.7±0.79	40

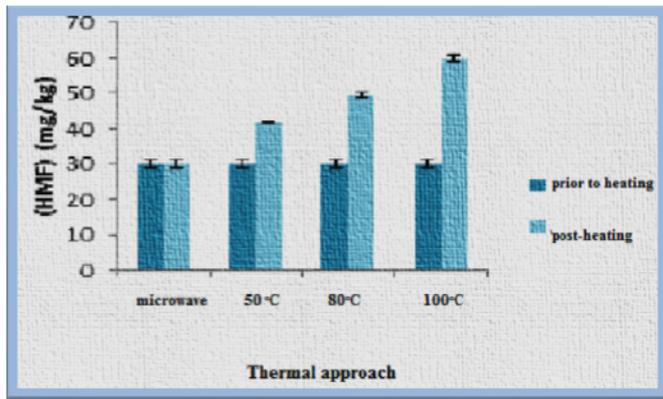


Fig 7: HMF content of citrus honey in function of microwave and conventional heating

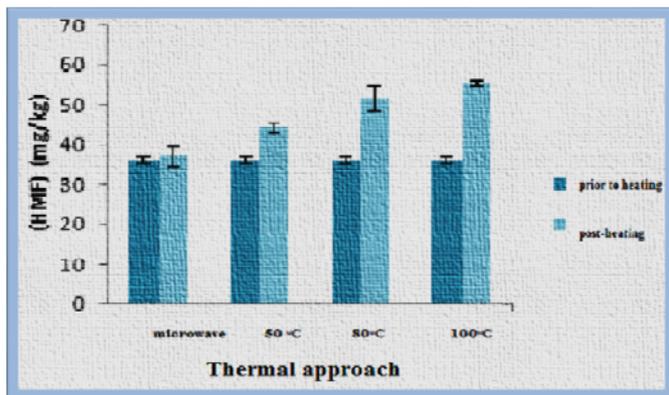


Fig 8: HMF content of mountain honey in function of microwave and conventional heating

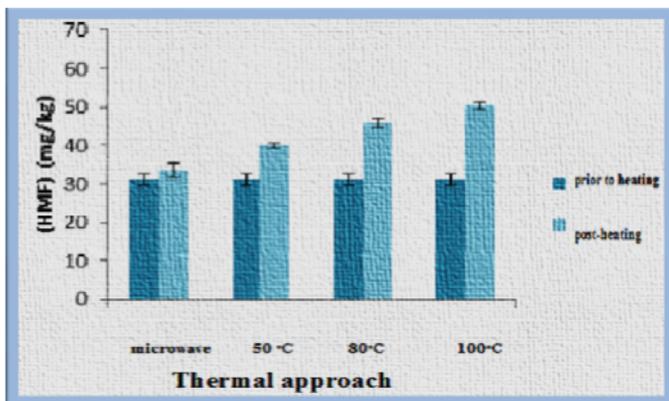


Fig 9: HMF content of Anise honey in function of microwave and conventional heating

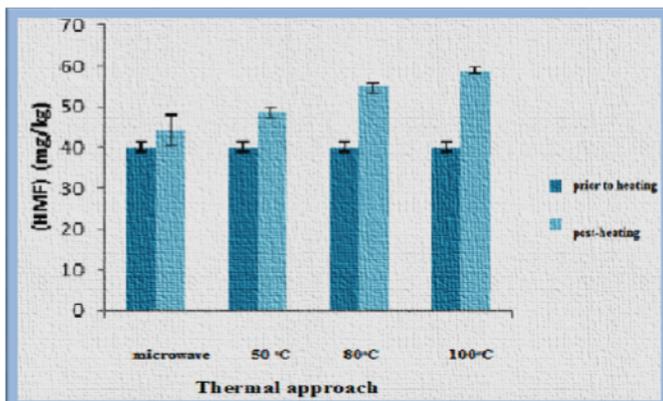


Fig 10: HMF content of black seed (Nigella sativa) honey in function of microwave and conventional heating

4 Conclusions

In this research a spectrophotometric (white) for determining HMF content in honey samples has been developed. The high resolution spectrometry proved to be a powerful tool for HMF quantification allowing minimizing standard deviations

Elevated concentrations of HMF are an indicator of conventional overheating, storage in poor conditions and age of honey. While, HMF content of honey samples affected significantly from storage time and heating, HMF content turned out to be a reliable indicator of microwave heating. It is important to note that not all the energy supplied from microwave irradiation was used to mitigate the process of honey heating, which could not absorb all the microwaves propagated to the cavity and hence some energy was lost to the other parts of the cavity and the surrounding. Hence, safety of honey constituents can be preserved

HMF content also increased for all honey samples, but not too far from the level measured in the unheated sample. Therefore, strong heating and long storage increase HMF content as a result of damage enzyme activity and due to honey composition. Currently it can be assumed that during proper storage conditions of large amounts of honey it is realistic to observe the stricter internal standard for honey which should be stored in a cool, dark place and should be consumed when fresh.

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