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Effect of palm pollen grain, bee pollen grain and basil oil addition on hmf during honey storage

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Abstract

Honey is an important natural compound that is taken for food and medicine purposes and some additives can be used to increase its health effects and quality. In this research, we investigated the effect of addition palm pollen, bee pollen and basil oil under different storage temperatures on the formation of hydroxymethyl furfural (HMF) and pH. The treatments consist of adding the pollen grains (from 1 to 5%) and the oil (from 100 to 500 ppm) and storage them at different temperatures (30, 40 and 50°C) for one month, two months and three months. The quality was estimated by HMF determination by HPLC method and pH measurement. The storage temperature and best treatment for honey was at 30°C with addition of 2.5% and 5% palm pollen. Storage for one month is better than storage for two months at 40°C and also at 50°C. On the other hand, the pH increases from 4 to 6 with rising temperature and storage time. When adding pollen to honey, it is best to store at low temperature not exceeding 30°C. Also, the addition of basil oil has a better effect than pollen during storage

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Introduction

Benefits of honey are many in food and in medicine (Sultan A. *et al*., 2017). Bee Pollen, Palm Pollen and basil oil are good additives choice for their health impact on food (Hazem M. M. Hassan, 2011) and (L.B.Almeida-Muradiana, 2005), (Veronika K. *et al,* 2014). (Lipika Basumallick *et al*., Thermo). (White, JW, 1994). HMF (Hydroxy-methyl-furfural), or 5-hydroxymethyl-2-furaldehyde is practically absent in fresh and untreated foods, but its concentration tends to rise as a result of heating processes or long-term storage and under acidic conditions that favor its generation. Therefore, it is an indicator of excessive heat-treatment, spoilage, and possible adulteration with other sugars or syrups.

It is often used as indicator for the quality of honey (Askar, 1984), (Fallico *et al*., 2004) and (Mauro Marotti *et al*., 1996). Due to its potential toxic effects, is essential for assessing the conformity of honey. Current European legislation regulates the concentration of HMF which must not exceed 40 mg/kg (for general types of honey) (Veronica K. *et al*, 2014). Exceptions for honey from countries with tropical temperatures: 80mg/kg. HPLC method for HMF determination, adopted as European Quality Standards, is slow but accurate (Spano *et al*., 2006).

Therefore, in this study we investigate the effect of additives (bee pollen, palm pollen and basil oil) and storage temperature on the pH and the formation of HMF in honey. This study will enable us to contribute to optimize treated honey quality.

Material and methods

Sample Preparation:

Honey, Date Pollen and Bee Pollen was taken from the commerce but Essential oil (basil oil) is extracted in the Laboratory Medina city. A mix of honey and additive (Pollen Grain or Oil) is prepared in the Laboratory Medina city and has presented in (Table 1).

Sample storage and analysis

Each sample is stored at specific temperature (30 °C, 40 °C and 50°C) during a period (30 and 60days). For samples under 30 ° C, we stored it until 90days. After that, pH and HMF had measured for each sample and then data is analyzed.

The pH value is done by a pH meter according the (AOAC, 1990) method after honey dilution (10 times).

HMF is measured by HPLC Method (HPLC method is set up in the laboratory according to standard DIN 10751-3) described below:

Five grams of honey samples ware dilutes up to 50 ml with distilled water filtered on 0.45 µm filter and immediately injected in a HPLC (Thermo Scientific; Accella) equipped with PDA detector (Diode Array Detector). The HPLC Column (RESTEK C18, 5 µm, 150 x 4.6 mm). HPLC conditions were the following: isocratic mobile phase, 90% water at 1% of acetic acid and 10% methanol; flow rate, 1ml/min; injection volume, 20µl.

All the solvents ware HPLC grade, and retention time (RT) is 5 min. The signal peak was detected by diode array detector. The chromatogram was monitored at Wavelength of 285 nm and HMF was identified, recorded and plotted. The area of the standard HMF peak VS the standard concentration is the area standard. HMF was identified by splitting the peak in honey with the standard HMF, and by comparison the spectrum of HMF standard with that of honey samples. The amount of HMF was determined using an external calibration curve, measuring the signal at Wavelength 285 nm.

All data are statistic analyzed by (SAS 2000 software by SAS Institute Inc.).

Results and discussion

HMF data during storage period for each temperature

Tables 2, 3 and 4 report results of HMF measured for prepared and stored honey samples. At 30° C, HMF

is determined for each sample and for 1, 2and 3 months duration of storage. Data and statistics analysis are presented in Table 2. At 40° C, HMF is determined for each sample and for 1 and 2 months duration of storage. Data and statistics analysis are presented in Table 3. At 50° C, HMF is determined for each sample and for 1and 2 months duration of storage. Data and statistics analysis are presented in Table 4.

Table 1. Sample Preparation.

Sample number	Treatment and quantity		
1	Honey 100g	Control (no additive)	
2	Honey 100g	Addition of Palm Pollen Grain	1.0 g
3	Honey 100g	Addition of Palm Pollen Grain	2.5 g
4	Honey 100g	Addition of Palm Pollen Grain	5.0 g
5	Honey 100g	Addition of Mix Bee Pollen Grain	1.0 g
6	Honey 100g	Addition of Mix Bee Pollen Grain	2.5 g
7	Honey 100g	Addition of Mix Bee Pollen Grain	5.0 g
8	Honey 100g	Addition of Basil oil	50 µg
9	Honey 100g	Addition of Basil oil	25 µg
10	Honey 100g	Addition of Basil oil	10 g

Table 2. HMF value after 1, 2 and 3 months at 30°C storage.

	Storage	Sample number										Mean
	Duration	1	2	3	4	5	6	7	8	9	10	
HMF at 30 °C	0 M	50	50	50	50	50	50	50	50	50	50	50
	1 M	62 f	59d	53.5b	48.9a	56.3c	58.4d	59.5d	62.1f	60.0e	59.2d	57.9A
	2 M	87e	85d	90f	94g	87e	90f	95h	80c	75b	72a	85.5B
	3 M	210 a	295j	246h	218g	213c	279i	308k	209a	211b	215d	240.4C

LSD between treatments at 5% is equal to 1.20 and LSD between Months at 5% is equal to 0.76.

Results, in Table 2, 3 and 4, show that, for 30°C, 40°C and 50°C, there are significant difference resulting from treatments for different storage duration and there are significant difference resulting from concentration inside treatments. Best result (lowest HMF) was, after one month, for 30°C and 40°C, 5% Palm Pollen Grain additive Honey and for 50°C was 50 and 25 µg Basil oil additive Honey and worst result (highest HMF) result was for 5% Mix Bee Pollen Grain additive Honey after two months for 40°C, after 3 months for 30°C and for 5% Palm Pollen Grain additive Honey after two months for 50°C after two months. Best average HMF treatment was for one month and worst average HMF treatment was for two months for 40°C and 50°C and for 3 months for 30°C.

Table 3. and HMF value after 1 and 2 months at 40°C storage.

	Storage	Sample number										Mean
HMF at 40 °C	Duration	1	2	3	4	5	6	7	8	9	10	
	0 M	50	50	50	50	50	50	50	50	50	50	50
	1 M	70e	64b	64b	62a	69.7d	67.8c	70e	68.5c	68c	68.9d	67.2A
	2 M	98d	92c	100e	101f	98d	102g	113h	92c	90b	88a	97.4B

LSD between treatments at 5% is equal to 0.83 and LSD between Months at 5% is equal to 0.53.

This result shows clearly that, for the control, during storage, HMF formation increases with rising of temperature which is conforming to the (Technical Sheet, 2016) and (Michael J. *et al.* 1990). Whereas, Pollen Grain addition make HMF Higher than control at High temperature (40 and 50°C) and during storage (more than on month) and effectively in literature we know that Pollen grain contains nutrients that could contribute to HMF formation. Pollen Grain content like amino acid and phenols (Hazem M. M. Hassan, 2011) and (L. B. Almeida Muradiana, 2005) reacts with sugar in Maillard reaction and HMF formation. Plant phenolic is generally degraded or undergoes undesirable reactions such as enzymatic oxidation by extended extraction times and high temperatures (Biesaga, M. *et al.* 2013), (Davidov-Pardo, G. *et al.* 2011).

Table 4. HMF value after 1 and 2 months at 50°C storage.

HMF at 50 °C	Storage Duration	Sample number										Mean
		1	2	3	4	5	6	7	8	9	10	
	0 M	50	50	50	50	50	50	50	50	50	50	50
	1 M	200b	223e	246g	249f	210d	285h	370i	203c	190a	190a	254.6A
	2 M	1800e	2216g	2357h	3517k	900a	923b	1409c	2719g	1815f	1751d	1940.7B

LSD between treatments at 5% is equal to 0.89 and LSD between Months at 5% is equal to 0.56.

Table 5. HMF value after 1 month at 30, 40 and 50°C storage temperature.

After 1 Month	Storage Temperature	Sample number										
		1	2	3	4	5	6	7	8	9	10	Mean
	30°C	62c	59b	53.5a	48.9a	56.3b	58.49b	59.58b	62.15c	60.06c	59.26b	56.9A
	40°C	70b	64a	64a	62a	69.7b	67.8b	70b	68.5b	68b	68.9b	67.3B
	50°C	200b	223d	246e	249h	210c	285f	370g	203b	190a	190a	260.6C

LSD between treatments at 5% is equal to 4.19 and LSD between temperatures at 5% is equal 2.29.

HMF temperature data for each storage period

The HMF results are grouped in month 1 and month 2 in tables 5 and 6 for 30°C, 40°C and 50°C, while, table 7 deals with month 3 for 30 °C. Results in tables 5, 6 and 7 show that after 1 and 2 months storage, for 30, 40 and 50°C there are significant difference resulting from treatments for different storage duration and there are significant difference resulting from concentration inside treatments. On the other hand, results, after 3 months storage show for 30°C there are significant difference resulting from treatments.

Table 6. HMF value after 2 months at 30, 40 and 50°C storage temperature.

After 2 Month	Storage Temperature	Sample number									
		1	2	3	4	5	6	7	8	9	10
	30°C	87 e	85 d	90 f	94 g	87 e	90 f	95 g	80 c	75 b	72 a
	40°C	98 d	92 c	100 e	101 e	98 d	102 f	113 g	92 c	90 b	88 a
	50°C	1800e	2216g	2357h	3517k	900a	923b	1409c	2719i	1815f	1751d

LSD between treatments at 5% is equal to 1.0 and LSD between temperatures at 5% is equal 1.9.

At 30°C, for the 1st month, best HMF treatment was for 2.5 and 5% Palm Pollen Grain additive Honey and worst HMF treatment was for 50 µl Basil Oil additive Honey, for the 2nd month, best HMF treatment was for 1µl Basil Oil additive Honey and worst HMF treatment was for 5% of Palm and Bee Pollen Grain additive Honey and at the 3rd month, best result was for 50µg Basil oil additive Honey and worst result was for 5% Bee Pollen Grain additive Honey. At 40°C, for the 1st month, best HMF treatment was for all Palm

Pollen Grain additive Honey treatments and worst HMF treatment was for all other treatments and for the 2nd month, best HMF treatment was for all 1µl Basil Oil additive Honey and worst HMF treatment was for 5% Bee Pollen Grain additive Honey. At 50 °C, for the 1st month, best HMF treatment was for 25 and 10 µl Basil Oil additive Honey treatments and worst HMF treatment was for 5% Palm Pollen Grain additive Honey and for the 2nd month, best HMF treatment was for 1% Bee Pollen Grain additive Honey and worst HMF treatment was for 5% Palm Pollen Grain additive Honey.

Table 7. HMF value after three months at 30°C storage temperature.

After 3 Month	Storage Temperature	Sample number									
		1	2	3	4	5	6	7	8	9	10
	30°C	210 a	295 k	246 f	218 e	213 c	279 g	308 l	209 a	211 b	215 d

LSD between treatments at 5% is equal to 1.7.

After one month of storage, HMF is determined for each sample and at 30° C, 40° C and 50° C. Data and statistics analysis are presented in Table 5. After two months of storage, HMF is determined for each sample and at 30° C, 40° C and 50° C. Data and statistics analysis are presented in Table 6. After three months of storage, HMF is determined for each sample and at 30° C. Data and statistics analysis are presented in Table 7.

pH Between treatments inside each month results

pH is determined after one and two months of storage and at 30° C, 40° C and 50° C. Data and statistics analysis are presented in Table 8.

Table 8. pH value after one and 1 and 2 months at 30, 40 and 50°C storage.

Storage Temperature	Storage Duration	Sample number										Mean
		1	2	3	4	5	6	7	8	9	10	
30°C	1 M	4.65	4.39	4.34	4.27	4.61	4.56	4.52	4.61	4.62	4.62	4.52
	2 M	4.52	4.47	4.28	4.24	4.27	4.38	4.35	4.39	4.42	4.4	4.37
40°C	1 M	4.48	4.33	4.33	4.54	4.42	4.43	4.84	4.84	4.84	4.84	4.60
	2 M	4.47	4.05	4.29	4.26	4.26	4.29	4.53	4.43	4.28	4.35	4.30
50°C	1 M	4.48	4.47	4.33	4.54	4.42	4.43	4.84	4.84	4.84	4.84	4.60
	2 M	6.03	5.93	5.65	5.69	5.71	5.65	5.55	5.49	5.64	5.62	5.70

LSD between treatments at 5% is equal to 0.95 and LSD between temperatures at 5% is equal to 2.1.

PH data during storage period for each temperature: Result, in Table8, indicates that there is no effect on pH during storage of honey except at 50°C after 2 months were pH rise from 4 to 6.

This result shows clearly that, pH increases with rising of temperature and storage (from 50°C and 2 months) which is conforming to (Samina Q. *et al.* 2013).

Comparison Between treatments inside each month results

For the 1st month, 5% Palm Pollen Grain additive Honey was the best result for 30°C and 40°C bat it was 25 and 50µl Basil Oil additive Honey at 50°C whereas the worst result was addition of 50 µg Basil Oil which is equal to control for 30°C, 5% Mix Bee Pollen Grain additive Honey which is equal to control for 40°C and 50°C; for the second month, addition of 10 µg Basil oil was the best result for 30°C and 40°C and 1 % addition of Mix Bee Pollen Grain for 50°C whereas the worst result was addition of 5% Mix Bee Pollen Grain for 30°C and for 40°C and it was for 5% Palm Pollen Grain additive Honey for 50°C. for the third month, for 30°C, 50 µg Basil oil was the best treatment whereas the worst result was addition of 5% Mix Bee Pollen Grain.

Conclusion

When using Pollen Grain as additives in honey for healthy and quality improvement during storage in the warm regions of the Middle East we expected for good results. Whereas HMF and pH indicates this Kind of additives don't fit this target at 40 and 50 but at 30 it was at limit accepted.

This study has enabled us to identify the best storage treatment. Thus during one month, 30°C was the better than 40°C and 40°C is better than 50°C for 2.5 and 5% Palm Pollen Grain additive Honey. Whereas one month storage is better than two months storage under a temperature of 40°C and 50°C. On the other hand, pH increases from 4 to 6 with rising of temperature and storage duration (from 50°C and 2 months). We suggest that addition of Pollen grain to honey need to be just before consumption or fridge storage but addition of essential oil can be any time. Further work can be done in using ginger, Ginseng and other essential oil as additives to check effect on HMF Formation and pH during storage.

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