INFLUENCE OF HEAT TREATMENT ON FORMATION OF HYDROXYMETHYLFURFURAL AND HYDROGEN PEROXIDE AS HEATING INDICATORS OF HONEY

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ABSTRACT

The physico-chemical characteristics of fresh cotton (*Gossypium vitifolium*) honey were determined. The indices obtained were within their standard ranges of Egyptian, European and Codex Alimentarius honey Standards. Influences of heating at different temperatures: 60ºC, 70ºC, 80ºC and 90ºC for 1, 3 & 5 min. on development of hydroxymethylfurfural (HMF) and formation of hydrogen peroxide (H_2O_2), as indicator for glucose oxidase activity were measured. The HMF content increased significantly with the prolonged of heating and time especially when honey exposed to temperatures above 70ºC. HMF values for heated samples were lower than the limits allowed by Egyptian, European and Codex Standards suggesting that HMF alone may be insufficient indicator for heating honey as a case in the system of high-temperature short-time. Heat treatments caused a gradual reduction in H_2O_2 formation which was completely inhibited by heating honey above 70ºC for 1 min. According to these results, it is suggested that formation of H_2O_2 could be used simultaneously with HMF as criteria for freshness and/or heating of honey, since glucose oxidase, which produces H_2O_2 in honey is heat sensitive enzyme. No obvious changes in organoleptic characteristics occurred due to such heat treatments of honey.

Key words: Bee honey, HMF, H_2O_2 and heating.

INTRODUCTION

Distinctive characteristics of bee honey are not, primarily, due to its major components which can be found in many sweet products, but to its multitude of minor components originated from the nectar and bees themselves. Many of these substances, which give its specific aroma, flavor and some of its biological activity, are unstable over time and thermolabile. Heating has a negative effect on honey due to the loss of those substances. Some honeys tend to crystallize especially when temperature is decreased. Crystallized honey has an opaque, waxy appearance and less visual impact than liquid honey. These features are not accepted for many consumers who prefer liquid honey. In commercial processing plants, honey is usually heated to 60°C or above for inhibiting microorganisms, facilitating packing and delaying crystallization **(Tosi, *et al.* 2004)**. Official honey standards approve major nine parameters have to be determined including reducing sugars, sucrose, fructose / glucose ratio, moisture, ash, water insoluble solids, acidity, diastase activity and HMF. These tests are laborious and time consuming. HMF or 5-hydroxymethyl-2-furaldehyde, is a cyclic aldehyde formed from fructose and glucose during dehydration. High HMF content indicates deterioration of honey which mainly due unsuitable conditions during storage and / or heating of honey **(Feather, *et al.*, 1982** and **Hoseney, 1984)**. Minor components of

honey *i.e.* enzymes make it different from other sweeteners, but some treatments *e.g.* processing and prolonged storage usually reduce its enzymatic activity **(Huidobro, *et al.* 1995**). On the other hand, honey enzymes, which participate in its ripening and biological value, serve as sensitive indicators of honey treatment. These enzymes which include amylase, invertase and glucose oxidase are, ascending, heat-sensitive **(Crane, 1990)**. The most parameters used as indicators of freshness and overheating of honey are HMF, diastase and invertase. HMF and diastase are included as international quality standards for honey but invertase is considered better than diastase as a freshness index because it is more sensitive to heating **(White, *et al.* 1964; Gonnet, 1965; Dustmann, 1985 and Sancho, *et al.* 1992)**. Besides, some questions in the literatures need answers about the effect of high temperature-short time treatment on honey **(Tosi *et al*, 2004).**

The main of this piece of work is to study the formation of HMF and estimation of glucose oxidase activity (responsible for H_2O_2 production) during heat treatments of honey. Besides, suggesting a simple, rapid and cheap test which can enable consumers to distinguish between natural and adulterated honeys and to find out if honey was exposed to heating or not.

MATERIALS AND METHODS

This work is a completion of a previous one concerned glucose oxidase activity as a quality criterion of freshness or storage of honey **(Mahmoud and Owayss, 2006)**.

The sampling and procedures in the present work were carried out as the following:

Sampling:

A pure cotton (*Gossypium vitifolium* L.) honey was harvested, by ordinarily beekeeping practices, in Aug, 2006 from the apiary of the Faculty of Agriculture, Fayoum University at Fayoum governorate, Egypt. Honeybee colonies were situated in wooden Langstroth's standard hives and headed with local 1st hybrid Carniolan, *Apis mellifera carnica*, queen bees. No supplementary feeding or chemical treatments were applied to these colonies during cotton flow season.

For analysis, 5 kg fresh honey sample were randomly taken and transferred to the laboratory in PVC jars and the analysis samples were prepared for subsequent determinations.

Physico-chemical analyses:

Moisture content, total soluble solids were estimated by measuring refractive index (RI) using Abbe's refractometer, ash was determined by ashing at 550ºC. For acidity (as formic acid), 10 g honey was dissolved in 75 ml distilled water and titrated with 0.1N NaOH, pH values were measured and reducing and non-reducing sugars (as sucrose) were determined according to official methods of analysis reported in **AOAC (1990)** and **Bogadanov, *et al.*(1997)**. The HMF content was determined by the UV spectrophotometric method given by **White (1979)**. Absorbance of honey diluted with distilled water (1:1w/v) was measured at 420 nm as indication of honey color.

Heat treatments:

1- To determine HMF progress during heat treatment, honey samples (25g each) were placed in screw test tubes, then subjected to heating in a thermostatic-shaking water bath at 60ºC, 70 ºC, 80ºC, and 90ºC for 1, 3

and 5 min. for each degree. Time was calculated when temperature reached the required degree, then tubes were cooled rapidly to room temperature (25±2ºC) using current tap water.

2-Another honey samples (25g each) were subjected to the same treatments and thereafter, H_2O_2 concentration (ppm) was determined every 5 min. from 0 min. till 190 min. H_2O_2, as an indicator of glucose oxidase activity was measured according to the method reported by **Lerke *et al,* (1983)** and modified by **López-Sabater *et al,* (1993)**. Unheated samples were compared as control**.**

Sensory evaluation:

Sensory evaluation of heated and unheated honey was carried out using the method described by **Sudha *et al.* (2007)**. The panelists were asked to evaluate the honey for color, odor and taste.

Application test:

For consumer, the following test is proposed to detect if the honey was heated or not: 10g honey should be diluted with 10 ml distilled water (1:1 w/v) and left at room temperature (25ºC) for 10 min. A crushed piece of fresh vegetables (source of catalase and peroxidase) such as peas, potatoes…*etc* should be added to diluted honey. In case of natural or unheated honey, air bubbles will appear and *vice versa* in case of adulterated or heated honey. This method could be explained according to the following equations:

1) $C_6H_{12}O_6 + H_2O$ + Glucose oxidase (in natural and unheated honey) $\rightarrow$ $C_5H_{11}COOH + H_2O_2$

2) $2H_2O_2$ + Catalase or peroxidase (in fresh vegetables) $\rightarrow 2\ H_2O + O_2\uparrow$ (bubbles in diluted honey).

Statistical analysis:

Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS 14.0 for Windows®, ver. 2, SPSS Inc. Chicago, IL, USA). Significance of differences was defined at $p < 0.05$ following **Snedecor and Cochran (1967)**.

RESULTS AND DISCUSSION

Physico-chemical characteristics:

Results in Table 1 summarizes the physico-chemical characteristics of the freshly tested cotton honey compared to those reported in **Egyptian Organization Standards (EOS) (1990)**, **European Union Commission (EUC) (1996)** and **Codex Alimentarius Commission (CAC) (1998)**. The tested honey samples have: moisture content (15.20%); total soluble solids (82.0%); reducing sugars (74.83%); sucrose (4.58 %); total acidity (37.71 meq/kg); HMF (6.10 ppm); ash (0.43 %) and pH (4.66). Absorbance of diluted honey filtrate at 420 nm was 0.418. These results are near from those reported by **Sahinler and Gul (2004)**. **Tosi, *et al*. (2004)**.

Table (1). Physico-chemical parameters of freshly cotton honey.

Parameter	Tested Honey	CAC	EUC	EOS
Refractive index (at 25ºC)	1.4989	--	--	--
Moisture (%)	15.20	≤ 21	≤ 21	≤ 20
Total soluble solids (%)	82.00	--	--	--
Reducing sugars (%)	74.83	≥ 65	≥ 65	≥70
Sucrose (%)	4.58	≤ 5	≤ 5	≤ 5
Total acidity (meq. formic acid / kg)	37.71	≤ 50	≤ 40	≤ 50
Ash (%)	0.43	≤ 0.6	≤ 0.6	≤ 0.4
HMF (ppm)	6.10	≤ 60	≤ 40	≤ 40
pH	4.66	--	--	--
Absorbance (at 420 nm)	0.418	--	--	--

CAC = Codex Alimentarius Commission EUC = European Union Commission
EOS = Egyptian Organization Standards

Influence of heat treatments on HMF development:

Data in Table 2 and Figure 1 summarize the influence of heat treatments on the HMF of tested cotton honey. The HMF content increased obviously from 6.10 ppm for unheated sample to 6.22, 6.88, 9.70, and 16.90 ppm by heating for 5 min. at 60ºC, 70 ºC, 80ºC, and 90ºC, respectively. It was noticed that HMF increased slowly until 70ºC, then a sharp increase was observed at higher temperatures.

Heating at these temperatures for 1, 3 & 5 min. exhibited that rate of HMF formation was affected by heating time, since this rate was higher in the 1st stage of heating than in the 2nd stage especially at 90ºC, then at 80ºC. These observations agree with those given by **Tosi *et al*, (2001)** who recorded that HMF increased from 10.1 ppm to 32.8 ppm by heating of honey for 1 min. at 100ºC & 140ºC, respectively. Also, **Karabournioti** and **Zervalaki (2001)** found that heating cotton honey for 24 h at 35 ºC, 45ºC, 55ºC, 65ºC & 75ºC increased HMF from 9.7 ppm (unheated) to 9.9, 11.40, 16.50, 52.70 & 173.40 ppm, respectively.

Table (2). Effect of heat treatment on HMF formation in cotton honey.

Heating Temperature (ºC)	Time of heating (min.)	HMF formed (ppm)	Rate of Increase in HMF (%)	Mean ± Sd
Control	**0**	6.10	0.00	6.00±0.01
60	**1**	6.12	0.33	6.12±0.01
	3	6.16	0.98	6.16±0.01
	5	6.22	1.97	6.22±0.01
70	**1**	6.64	8.85	6.64±0.00
	3	6.75	10.65	6.75±0.02
	5	6.88	12.79	6.88±0.03
80	**1**	8.65	41.80	8.65±0.01
	3	9.12	49.51	9.12±0.02
	5	9.70	59.02	9.70±0.02
90	**1**	14.28	134.10	14.28±0.01
	3	15.67	156.88	15.67±0.01
	5	16.90	177.05	16.90±0.01

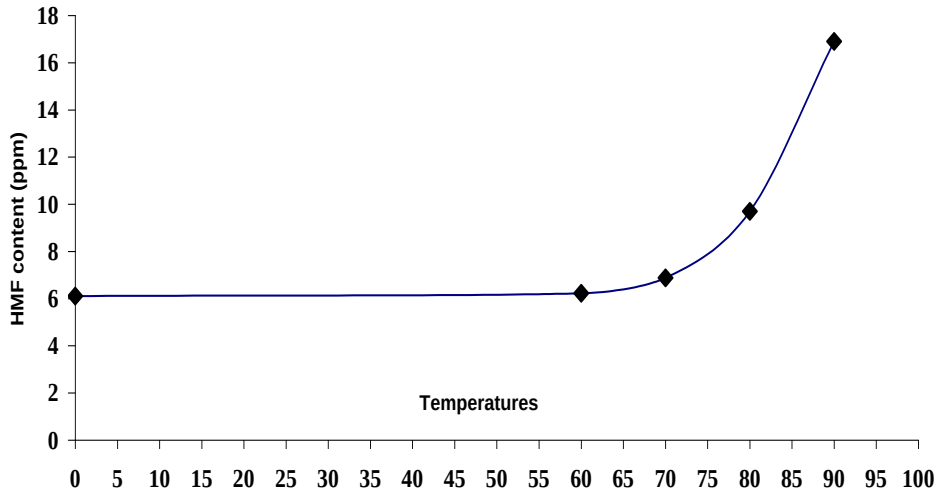

Fig. (1). Effect of heating temperatures for 5 min on HMF formation in cotton honey

Influence of heat treatments on H_2O_2 formation:

Data in Table 3 and Figures 2 & 3 showed that H_2O_2 was affected by heat treatments which caused a gradual decline in its formation which was completely inhibited by heating of honey above 70ºC.

Also, formation of H_2O_2 was affected by both temperature and heating time. However, heating of honey at 60ºC caused an increase of induction period (the period required to begin H_2O_2 formation) which increased from 5 min. for control to 15, 20 & 20 min. by heating for 1, 3 & 5 min., respectively. But, heating of honey at 70ºC extended the induction period from 5 min. for control to 105, 115 & 115 min. by heating for 1, 3 and 5 min., respectively. Besides, H_2O_2 content was very low compared to control or even honey samples heated at 60ºC.

Meanwhile, by heating of honey at 80ºC for 1, 3 and 5 min., glucose oxidase activity was completely inhibited. Also, it was noticed that the fluctuating rate of H_2O_2 formation was affected by both of temperature and time. Some other reasons reported by **Postmes (1995)** and **Kerkivliet (1996**) who explained that decline in H_2O_2 may be attributed to the presence of certain reducing components in honey.

Table (3). Effect of heat treatment on hydrogen peroxide formation (ppm) in cotton honey.

Time (min)	Control	Heating for 1 min			Heating for 3 min			Heating for 5 min		
		60ºC	70ºC	80ºC	60ºC	70ºC	80ºC	60ºC	70ºC	80ºC
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
5	0.11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
10	0.81	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
15	1.15	0.06	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
20	1.14	0.19	0.00	0.00	0.08	0.00	0.00	0.03	0.00	0.00
25	1.12	0.49	0.00	0.00	0.21	0.00	0.00	0.13	0.00	0.00
30	1.09	0.85	0.00	0.00	0.42	0.00	0.00	0.27	0.00	0.00
35	1.08	1.13	0.00	0.00	0.65	0.00	0.00	0.45	0.00	0.00
40	1.06	1.33	0.00	0.00	0.87	0.00	0.00	0.65	0.00	0.00
45	1.05	1.46	0.00	0.00	1.03	0.00	0.00	0.82	0.00	0.00
50	1.02	1.52	0.00	0.00	1.15	0.00	0.00	0.96	0.00	0.00
55	0.99	1.53	0.00	0.00	1.22	0.00	0.00	1.06	0.00	0.00
60	0.96	1.52	0.00	0.00	1.25	0.00	0.00	1.14	0.00	0.00
65	0.94	1.50	0.00	0.00	1.28	0.00	0.00	1.19	0.00	0.00
70	0.91	1.48	0.00	0.00	1.28	0.00	0.00	1.22	0.00	0.00
75	0.88	1.44	0.00	0.00	1.26	0.00	0.00	1.23	0.00	0.00
80	0.84	1.40	0.00	0.00	1.25	0.00	0.00	1.24	0.00	0.00
85	0.81	1.36	0.00	0.00	1.23	0.00	0.00	1.24	0.00	0.00
90	0.78	1.31	0.00	0.00	1.23	0.00	0.00	1.24	0.00	0.00
95	0.71	1.26	0.00	0.00	1.20	0.00	0.00	1.23	0.00	0.00
100	0.67	1.21	0.00	0.00	1.17	0.00	0.00	1.23	0.00	0.00
105	0.65	1.17	0.01	0.00	1.15	0.00	0.00	1.21	0.00	0.00
110	0.61	1.12	0.03	0.00	1.12	0.00	0.00	1.20	0.00	0.00
115	0.57	1.06	0.05	0.00	1.09	0.01	0.00	1.19	0.01	0.00
120	0.55	1.01	0.08	0.00	1.07	0.01	0.00	1.18	0.02	0.00
125	0.51	0.96	0.10	0.00	1.04	0.02	0.00	1.17	0.03	0.00
130	0.48	0.91	0.13	0.00	1.01	0.03	0.00	1.15	0.04	0.00
135	0.45	0.86	0.14	0.00	0.98	0.03	0.00	1.14	0.05	0.00
140	0.45	0.82	0.16	0.00	0.95	0.05	0.00	1.13	0.05	0.00
145	0.43	0.78	0.18	0.00	0.92	0.05	0.00	1.11	0.07	0.00
150	0.42	0.74	0.20	0.00	0.91	0.06	0.00	1.10	0.08	0.00
155	0.42	0.70	0.22	0.00	0.87	0.06	0.00	1.09	0.09	0.00
160	0.42	0.67	0.24	0.00	0.83	0.08	0.00	1.07	0.11	0.00
165	0.42	0.64	0.26	0.00	0.81	0.08	0.00	1.06	0.13	0.00
170	0.42	0.61	0.29	0.00	0.78	0.10	0.00	1.05	0.15	0.00
175	0.42	0.58	0.32	0.00	0.76	0.10	0.00	1.04	0.17	0.00
180	0.42	0.57	0.34	0.00	0.74	0.12	0.00	1.03	0.19	0.00
185	0.42	0.54	0.36	0.00	0.73	0.13	0.00	1.02	0.20	0.00
190	0.42	0.53	0.36	0.00	0.73	0.13	0.00	1.02	0.20	0.00

Sensory evaluation:

Sensory evaluation of heated honey at 60ºC, 70ºC, 80ºC and 90ºC for 5 min. compared to unheated honey (control) was evaluated and the results are

summarized in Table 4. Data shown in this table revealed no obvious differences in color, odor and taste of honey samples as a result of heat treatments.

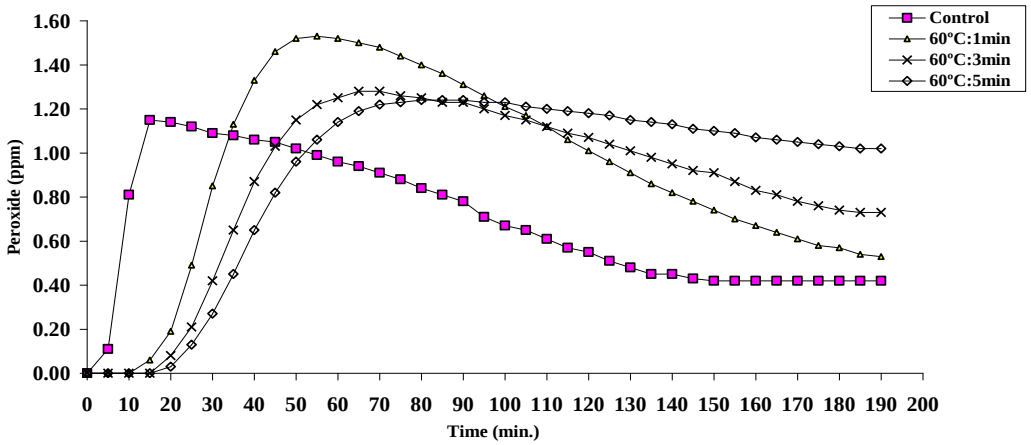

Fig. (2). Effect of heating time at 60°C on peroxide formation

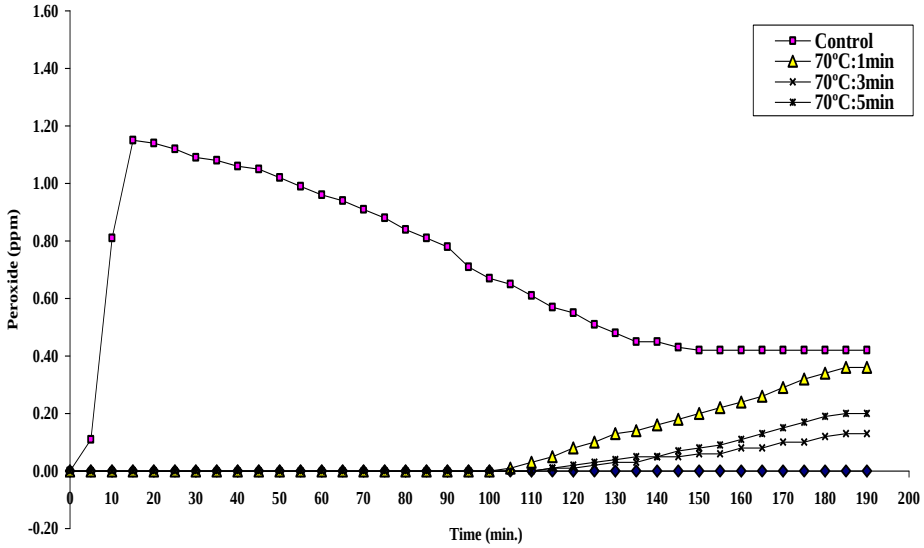

Fig. (3). Effect of heating time at 70°C on peroxide formation

Table (4). Sensory evaluation of heated honey samples at different temperatures for 5 min compared to non heated (control).

Parameter	Control honey	Heated honey			
		60°C	70°C	80°C	90°C
Color (20)	18.00±0.20	17.97±0.25	18.10±0.30	18.17±0.06	18.50±0.60
Odor (40)	37.97±0.06	37.50±0.17	38.53±0.21	38.70±0.10	39.00±0.17
Taste (40)	37.03±0.06	36.80±0.10	37.53±0.06	37.63±0.15	38.03±0.15

Application test:

The results of that test revealed that heating at 80ºC and more for 1 min. inhibited the glucose oxidase activity.

CONCLUSION

According to the present findings, it could be concluded that HMF determination is not sufficient for the detection of short time-heating of honey. Exposing honey in a thermal treatment till 90ºC for 5 min. caused a significant increase in HMF content. But, this increase was accepted by Egyptian, European and Codex Standards. Meanwhile, utilization of H_2O_2, as indicator for glucose oxidase activity, may be useful as an index of heating. Also, it could be suggested that, utilization of this procedure in the standard methods can help detection of the genuineness of honey and /or heating.

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تأثير المعاملات الحرارية على تكون كل من الهيدروكسي ميثايل فورفورال وفوق أكسيد الهيدروجين كدليلين على تسخين عسل النحل

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يعد عسل زهرة القطن من أكثر الأعسال المصرية ميلا للتبلور خاصة عند انخفاض درجة الحرارة شتاءً ،مما يتطلب تسخينه لإسالته حتى يسهل تداوله وتعبئته. كذلك يتعرض العسل على النطاق التجاري لعمليات التسخين بغرض البسترة وإطالة الفترة اللازمة للتبلور، حيث يعد العسل المتبلور غير مقبول لدى كثير من المستهلكين.

وفي هذه الدراسة تم تقدير بعض الصفات الطبيعية والكيماوية لعسل القطن، وتتبع تأثير المعاملات الحرارية عليه ، وقد تميز العسل المختبر بانخفاض محتواه الرطوبي (١٥.٢%) وارتفاع محتواه من السكريات المحولة (٧٤.٠٣%) بينما كان السكروز (٤.٥٨%) كذلك وجد أن قيم الخصائص الطبيعية والكيماوية مثل الرماد (٠.٤٣%) والحموضة الكلية (٣٧.٧١ ملليمكافيء/كجم)، والهيدروكسي ميثايل فورفورال (٦.١ جزء في المليون)، ورقم الأس الهيدروجيني (٤.٦٦) قد جاءت جميعها ضمن الحدود المقبولة للمواصفات القياسية المصرية ودستور الأغذية الأوروبى والدولى.

وبدراسة تأثير المعاملات الحرارية، لعسل القطن المختبر، على درجات حرارة مختلفة: ٦٠، ٧٠، ٨٠، ٩٠ °م لمدة ١، ٣،٥ دقائق على تطور تكون كل من الهيدروكسي ميثايل فورفورال وفوق أكسيد الهيدروجين (الذى هو نتيجة نشاط إنزيم جلوكوز أكسيديز الموجود أصلا فى العسل) فقد لوحظت زيادة معنوية في قيم الهيدروكسي ميثايل فورفورال، والتى تأثرت بكل من درجة الحرارة وزمن التسخين وكان معدل الزيادة مرتفعاً بالتسخين فوق ٧٠°م لمدة ١دقيقة. وهذه القيم كانت أقل من تلك المسموح بها من قبل المواصفات المصرية ودستور الأغذية الأوروبى مما يعني عدم كفاية استخدام الهيدروكسي ميثيل فورفورل بمفرده للحكم على مدى تعرض العسل للمعاملات الحرارية خاصة كما يتبع عند إجراء نظام التسخين على درجات حرارة عالية لمدة قصيرة.

بينما أدت نفس المعاملات الحرارية إلى تناقص ملحوظ في نشاط إنزيم جلوكوز أوكسيديز والذي توقف تماما في العينات التي عوملت على درجات حرارة أعلى من ٧٠°م لمدة دقيقة واحدة. بينما أدى التسخين على ٦٠°م لمدة دقيقة لإطالة فترة التحضين اللازمة لنشاط إنزيم جلوكوز أكسيديز وتكوين فوق أكسيد الهيدروجين من ١٠ دقائق فى حالة العينة الغير مسخنة إلى ٣٠ دقيقة فى العينة المسخنة على ٦٠°م.

لذا تقترح هذه الدراسة استخدام تقدير فوق أكسيد الهيدروجين في العسل كدليل على نشاط الإنزيم المسئول عن تكوينه (جلوكوز أوكسيديز)، وبالتالى كدليل على تعرض العسل لمعاملات حرارية من عدمه.

ويمكن للمستهلك أن يجرى هذا الاختبار بطريقة مبسطة كالتالى: تخفف ١٠جم من العسل بـ ١٠ مل ماء، وتقلب جيدا وتترك على درجة حرارة الغرفة لمدة ١٠ دقائق ثم يضاف للمحلول قطع صغيرة من مجروش البطاطس أو البسلة أو الفاصوليا الطازجة. يلاحظ أنه في حالة العسل غير المسخن تصاعد فقاقيع غاز في المحلول وتكون رغاوي على السطح بينما لا تتكون أية فقاقيع في حالة العسل الذي تعرض لمعاملات حرارية. وتفسير ذلك أن العسل يحتوى على إنزيم جلوكوز آكسيديز، والذي ينشط فقط عند تخفيف العسل بالماء ويقوم هذا الإنزيم بتحويل جزء من الجلوكوز إلى حامض جلوكونيك + فوق أكسيد هيدروجين، والذى يتحول بدوره إلى ماء وأكسجين يتصاعد في صورة فقاقيع في وجود إنزيمات كتاليز وبيرأكسيديز، والموجودة بالفعل ضمن مكونات الخضر الطازجة.