Journal of Food Measurement and Characterization (2018) 12:2358–2365
https://doi.org/10.1007/s11694-018-9852-y

ORIGINAL PAPER

Investigating 5-hydroxymethylfurfural formation kinetic and antioxidant activity in heat treated honey from different floral sources

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Received: 14 February 2018 / Accepted: 8 June 2018 / Published online: 13 June 2018
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Abstract

The quality and biochemical properties of honey are affected by heating or during storage period. The most important biochemical reaction that occurs in this process is the Maillard reaction. HMF (5-hydroxymethylfurfural) is one of the major intermediate products in the Maillard reaction that can lead to quality reduction in heated honey. In this study, the effect of heating on the antioxidant activity, and colour values as Maillard reaction indicators of three different botanical honeys were investigated; the HMF formation was also determined. Temperatures of 50, 70 and 80 °C were applied on the honeys between 0 and 48 h. Total phenolic content, ferric reducing/antioxidant power and scavenging of 2,2-diphenyl-1-picrylhydrazyl free radical assays are used to determine the antioxidant capacity. Results showed that the formation of HMF and the antioxidant properties of honeys were significantly increased during the heating process. Pure HMF compound also showed lower antioxidant activity. The formation of HMF has higher degree of linearity in the fit of the zero order reaction and also it was the highest in the chestnut honey. Furthermore, it was found that as the biochemical value of the honeys increased, the HMF formation decelerated.

Keywords Hydroxymethylfurfural · Kinetics · Honey · Antioxidant activity · Colour

Abbreviations

MRP	Maillard reaction product
HMF	5-hydroxymethylfurfural
TPC	Total phenolic content
FRAP	Ferric reducing/antioxidant power
Trolox®	6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid
TPTZ	2,4,6-tripyridyl-s-triazine
DPPH 2	2-diphenyl-1-picrylhydrazyl
GAE	Gallic acid equivalent
RP-HPLC	Reverse phase high performance liquid chromatography
Ea	Activation energy
K_T	Reaction rate constant
K_R	Pre-exponential factor

Introduction

In recent years, much attention has been paid to bee products in connection with intensified focus on diet-health relationships. Honey is one of the most complex natural mixtures. It mainly consists of carbohydrates and fructose and glucose are the main sugars [1]. It also contains minerals, vitamins, volatile compounds and Maillard Reaction Products (MRPs). It is also rich in flavonoids and phenolic acids that are known sources of antioxidants [2]. The antioxidant capacity of the compounds is connected to a number of different mechanisms such as radical scavenger, hydrogen donors, singlet oxygen quencher, electron donor, and metal

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ion chelating, and acting as a substrate for radicals [3]. Phenolic characterization has been proven to be appropriate for the identification of botanical origin of honey, and therefore being closely related with antioxidant capacity of honey [4]. Many measurement assays including TPC, ferric reducing/antioxidant power (FRAP) and DPPH. radical scavenging activities have been developed to evaluate antioxidant capacity of honey samples [3, 5, 6].

The chemical composition, color, aroma and flavor of honey depend mainly on the flowers, geographical regions, climate and honeybee species involved in its production, and are also affected by weather conditions, processing, packaging and storage conditions [7, 8]. It is natural for some of honey types tend to crystallize especially when temperature decreased. Crystallized honey is not desired for many consumers who prefer liquid honey. Some consumers and beekeepers heat honey to 60 °C or above for delaying and/or overcoming crystallization, and facilitating packaging [9, 10]. It is known that lower temperatures below 40 °C do not affect honey quality, but higher temperatures lead to Maillard reaction. Maillard reaction can be defined as interaction between a carbonyl group of reducing sugars, aldehydes, or ketones and an amino group of aminoacids, proteins, or any nitrogenous compounds [11]. The sugars and the amines are condensed in the first stage of the Maillard reaction, and then the Amadori rearrangement occured. The color weakens or become yellow in the second stage. And in the final stage, the highly colored compounds are formed by aldol condensation and aldehyde-amine polymerization [12]. This reaction causes the formation, degradation or transformation of products such as furfural, 5-hydroxymethylfurfural, melanoidins, acrylamide [13–15]. There are many studies on Maillard reaction products, that have harmful effects including mutagenic and carcinogenic effects [13, 16–19], while other studies have indicated its therapeutic properties such as antioxidant, antiallergenic, antimicrobial and cytotoxic [20–24]. However, the exact source of these effects from MRP's products is still unknown. One of those reaction products, HMF, is naturally formed as an intermediate of the Maillard reaction and from dehydration of hexoses during heat treatment in food industry [25–27]. HMF is a cyclic aldehyde produced by sugar degradation through the Maillard reaction [28]. HMF has been found to be an indicator of the heating process in foods as well as the extent of the Maillard reaction [29, 30]. When honey is heated or stored for a prolonged time, Maillard reaction substances are increased. Therefore, HMF concentration reflects freshness and heating treatment of honey, and in Codex Alimentarius [31] limit for HMF content is 40 mg/kg honey [32]. Many different parameters not only temperature but also pH, metal ions, amino: carbonyl ratio, relative humidity are important in occurring Maillard reactions. However, international standards do not permit any food additives in honey so applied heating to prevent crystallization can be considered as the only parameter to cause Maillard products. Higher levels of HMF may result of severe health problems.

This study evaluates the effect of heating on the HMF formation, antioxidant capacity, total phenolic content and color of honeydew, chestnut, and multifloral honeys at 50, 70, and 80 °C for 0, 1, 2, 5, 12, 24, and 48 h. determine the kinetic parameters of HMF formation and to clarify the antioxidant properties of HMF during the heating process of honey samples. A kinetic model for HMF formation in three honey types after heat treatment was also investigated.

Materials and methods

Samples

Pine (*Pinus brutia* Ten.) honeydew honey, chestnut (*Chestnut sativa* L.) honey and multifloral honey samples were supplied from professional beekeepers from Turkey. Botanical origins of the samples were determined according to palynological analyses [33, 34]. Pollen spectrum in chestnut honey contained > 45% of the corresponding chestnut pollen. Honeydew honey does not contain pollen and the studied third honey, mixed blossom honey, was multifloral honey.

Chemicals and reagents

All chemicals and solvents were of analytical grade. Trolox® (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), 2,4,6-tripyridyl-s-triazine (TPTZ), Folin–Ciocalteu's phenol reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH), and 5-hydroxymethlfurfural were purchased from Sigma-Aldrich (St. Louis, MO).

Spectrophotometric analyses were performed using Spectro UV–Vis spectrophotometer Double PC-8 Autocell Labomed. Inc. (USA). Polytetrafluoroethylene membranes (porosity 0.2 µm) for the filtration were obtained from Sartorius (Goettingen, Germany).

Heating treatment

About 100 g of honey was transferred into a flask (500 ml) and heated at 50, 70, and 80 °C for four different incubation periods (0, 1, 2, 5, 12, 24, and 48 h) in an incubator (Binder, GmbH Bergstr, 14D-78532, Tuttingen, Germany). The samples were mixed with a glass stirrer every 30 min. Following the heating process, the flask was immediately transferred to a refrigerator then analyzed. All experiments were repeated in triplicate.

Determination of antioxidant capacity

TPC of the honeys were determined using Folin–Ciocalteu's reagent and expressed as a reference phenolic acid, gallic acid equivalent (GAE) [35, 36].

Total antioxidant capacity of the samples was measured by FRAP assay. This method is based on the measurement of Fe^{3+} reducing capacity. FRAP reagent was prepared by mixing 300 mM acetate buffer (pH 3.6), 20 mM ferric chloride, and 10 mM 4,6-tripyridlys-triazine (TPTZ) solution in 40 mM HCl. Freshly prepared FRAP reagent was heated at 37 °C, and 100 µl of sample was mixed with 3 ml of FRAP reagent. Absorbance was read at 595 nm against reagent and sample blank containing distilled water which was also incubated at 37 °C for 4 min. The results were expressed in micromole Trolox equivalents per gram of honey.

DPPH radical scavenging activities of the samples was determined based on the method described by Chen et al. [37]. In the presence of antioxidants, the purple colour of DPPH decays, and the change of absorbance is monitored at 517 nm against reagent and sample blank. Briefly, 0.75 ml of a 0.1 mM DPPH in methanol was mixed with equal volume of methanolic honey extracts, and incubated in dark for 50 min, and absorbance was measured at 517 nm in the presence of different concentration of samples (0.1–10 mg/ml). Radical scavenging activity of DPPH was expressed as SC_{50}, which represents the concentration of the extract (mg/ml) required to inhibit 50% of the free radical-scavenging activity; higher SC_{50} value indicates lower radical scavenging activity.

Determination of HMF content

HMF content of samples was performed with RP-HPLC with minor modifications. Five grams of honey was dissolved in 25 ml of distilled water, and mixed with 0.5 ml Carrez-I (potassium hexacyanoferrate) and 0.5 ml Carrez-II (zinc acetate dehydrate) solutions. The mixture was centrifuged at 10,000 rpm for 10 min and the supernatant was filtered on 0.45 mµ membrane and 100 µl from the filtrate was injected to HPLC (Hitachi VWR Elite LaChrom, Japan). HPLC system equipped with an auto sampler, an UV-detector (280 nm) and Zorbax Eclipse XDB-C18 column (4.6 × 150 mm, 5 µm particle size). Gradient program with two solvent system methanol/water (10/90, v/v) at a flow rate of 1.5 ml/min at ambient temperature. HMF was identified by splitting the peak in honey with a standard HMF (Sigma-Aldrich, St. Louis-MO), and by comparison the spectrum of HMF standard with that of honey samples.

Determination of kinetic parameters

The coefficients of determination (R^2) and reaction rate constant (K_T) were accounted from each relation for zero-and first order reactions for all honey types (Eqs. 1, 2) [38].

$$\text{Zero-order reaction} : C = C_0 + K_T t \quad (1)$$

$$\text{First-order reaction} : C = C_0 \exp\left(-K_T t\right) \quad (2)$$

where C_0 is the total initial concentration of HMF (mg/kg), K_T is the reaction rate constant (h^{-1}), C is HMF concentration after t minute heating at a given temperature (mg/kg), t is the isothermal heating time.

In this study, K_T and the activation energy (Ea) values on temperature were also calculated from Arrhenius equation (Eq. 3):

$$K_T = K_R \exp\left(-Ea/RT\right) \quad (3)$$

where K_R is the pre-exponential factor (h^{-1}), Ea is the activation energy (kcal/mol) and R is the universal gas constant (= 8314 J/mol K = 1985 cal/mol K).

Color analysis

Color measurement of the honeys was carried out using colorimeter (CR-10, Minolta, Osaka, Japan) and the measurements were recorded as Hunter *L**, *a** and *b** color values with minor modifications [39, 40]. Honey samples were heated to 50 °C to dissolve sugar crystals and then placed in a 3 ml cell and incubated at 50 °C for 30 min. The color values were expressed as L* for darkness/lightness (0 black, 100 white), a* (− a greenness, + a redness), and b* (− b blueness, + b yellowness).

Statistical analysis

The statistical analyses were performed with SPSS software 16.0 (Chicago, IL). Results are presented as means ± standard deviations of three parallel measurements. Statistical analysis of the results was based on Kruskal–Wallis test and Pearson correlation analysis. Significance was set at $p < 0.05$ and $p < 0.01$ levels.

Results and discussion

Hydroxymethylfurfural (HMF) content

The initial HMF contents of studied samples were lower than the allowed maximum limit of 40 mg/kg as recommend by Codex Alimentarius [31]. HMF content of honey samples are shown in Table 1. The initial HMF concentration

Table 1 HMF contents of honeys (mg/kg)

T (°C)	Time (h)	Samples		
		Multifloral honey	Honeydew honey	Chestnut honey
50	0	2.03 ± 0.12^{a}	2.08 ± 0.20^{a}	17.07 ± 1.88^{ab}
	1	2.03 ± 0.14^{a}	2.12 ± 0.21^{a}	17.23 ± 1.71^{ab}
	2	2.05 ± 0.10^{a}	2.34 ± 0.11^{a}	17.30 ± 2.10^{ab}
	5	2.05 ± 0.27^{a}	2.79 ± 0.28^{a}	18.83 ± 2.82^{ab}
	12	2.31 ± 0.13^{a}	3.03 ± 0.12^{a}	19.83 ± 2.26^{ab}
	24	2.68 ± 0.54^{a}	3.92 ± 0.63^{a}	21.80 ± 2.50^{ab}
	48	3.47 ± 0.38^{a}	5.57 ± 0.64^{a}	27.73 ± 3.05^{ab}
70	0	2.03 ± 0.12^{a}	2.08 ± 0.31^{a}	17.07 ± 1.89^{ab}
	1	2.12 ± 0.14^{a}	2.13 ± 0.42^{a}	17.43 ± 2.02^{ab}
	2	3.22 ± 0.22^{a}	3.01 ± 0.34^{a}	19.67 ± 2.09^{ab}
	5	3.85 ± 0.38^{a}	4.29 ± 0.69^{a}	21.88 ± 4.38^{ab}
	12	29.45 ± 4.32^{ab}	35.12 ± 3.12^{ab}	47.92 ± 4.44^{abc}
	24	86.65 ± 10.39^{bcd}	62.25 ± 6.22^{abc}	94.96 ± 13.29^{cd}
	48	143.98 ± 20.16^{d}	147.57 ± 22.14^{d}	139.58 ± 18.14^{d}
80	0	2.03 ± 0.12^{a}	2.08 ± 0.31^{a}	17.07 ± 1.87^{ab}
	1	2.20 ± 0.10^{a}	2.49 ± 0.28^{a}	18.57 ± 2.06^{ab}
	2	4.34 ± 0.41^{a}	4.53 ± 0.33^{a}	25.65 ± 3.12^{ab}
	5	6.07 ± 0.73^{a}	6.70 ± 0.80^{a}	27.15 ± 4.39^{ab}
	12	178.96 ± 15.20^{d}	$220,45 \pm 25.40^{de}$	207.50 ± 32.20^{de}
	24	382.67 ± 38.27^{e}	$331.84 \pm 66,37^{e}$	374.36 ± 43.05^{e}
	48	881.75 ± 132.26^{fg}	839.07 ± 83.90^{f}	935.46 ± 93.54^{g}

Lowercase letters (a–g): Means with different letters indicate significant differences ($p < 0.05$). Average values are expressed as mean ± S.D. of three replicate determinations

for multifloral, honeydew and chestnut were found as 2.03, 2.08 and 17.07 mg/kg, respectively. HMF values were relatively lower at 50 °C during 48 h incubation for all the honey samples; however, a great increase in HMF was observed above 50 °C ($p < 0.05$). HMF content of all samples were not higher than 40 mg/kg for 50, 70 and 85 °C during to first 5 h. Dramatic increase in HMF formations was observed after 5 h of incubation period for 70 and 80 °C.

Determination coefficients at different process temperatures (R^2) were higher in zero-order rate than in first-order in our study (Table 2). Samples have high degree of linearity in the fit of the zero order reaction. Honeydew honey has the highest degree of linearity in the fit model. The HMF formation in honey has been reported as zero-order by Boonchiangma et al. [41] for Thai honey; pseudo-first-order by Fallico et al. [42] for unifloral honey; first-order by Bulut and Kilic [43] for mixed flower honey, and by Turhan et al. [44] for multifloral honey.

The rate of the reaction in the zero order reactions is equal to the K_T constant of the reaction. K_T values (that includes all parameters that affect the reaction rate, except for concentration) tended to increase with temperature process. This result indicates higher HMF formation at high temperature in honeys. Chestnut honey showed higher rate constant than honeydew honey and multifloral honey at 50 and 80 °C but lower rate constant than honeydew honey and multifloral honey at 70 °C.

The inverse exponential relationship between Ea and K_T in the Eq. (3) concludes that reactions with very high activation energy have very low reaction rate. As activation energy decreases, the reaction rate increases. The activation energy obtained was found as 33.03 kcal mol^{-1} for chestnut honeys, and 41.61 kcal mol^{-1} for honeydew honeys, and 48.88 kcal mol^{-1} for multifloral honeys. These results showed that chestnut honeys were the least sensitive to temperature. Formation rate of HMF in multifloral honeys showed that the lowest antioxidant activity was the most dependent on the process of temperature comparing to other honeys which showed higher antioxidant activity. Turhan et al. [44] studied the quality of honeydew and floral honeys influenced by thermal treatment and found that thermal formation kinetics of HMF fitted to a first-order reaction. They calculated Ea values as 11.61 kcal mol^{-1} for honeydew honeys and 22.32 kcal mol^{-1} for floral honeys, during the process time ranging between 15 and 90 min, and process temperature ranging between 75 and 100 °C. Tosi et al. [9] found the Ea value in honey to be 53.9 kcal mol^{-1} between 100 and 160 °C.

K_R, pre-exponential factor (h^{-1}), is an empirical relationship between temperature and rate coefficient and represents

Table 2 The rate constants (k_T) at different temperatures, activation energy (Ea) values and the pre-exponential factor (K_R) for HMF formations in honeys for zero order reactions and first order reactions

	Samples	T (°C)	k_t (h^{-1})	R^2	*Ea* (kcal mol^{-1})	R^2	K_R (h^{-1})
Zero order reaction	Multifloral honey	50	0.0307	0.99	48.88	0.999	2.68×10^{-32}
		70	3.1472	0.98			
		80	18.719	0.99			
	Honeydew honey	50	0.0712	0.99	41.61	0.999	1.01×10^{-27}
		70	3.0668	0.99			
		80	17.582	0.98			
	Chestnut honey	50	0.2173	0.99	33.03	0.980	2.11×10^{-22}
		70	2.7148	0.98			
		80	19.65	0.98			
First order reaction	Multifloral honey	50	0.0116	0.99	19.20	0.966	8.10×10^{-12}
		70	0.0951	0.83			
		80	0.1329	0.79			
	Honeydew honey	50	0.0200	0.95	14.57	0.978	6.62×10^{-9}
		70	0.0932	0.84			
		80	0.1293	0.77			
	Chestnut honey	50	0.0099	0.99	16.56	0.998	6.22×10^{-10}
		70	0.0471	0.90			
		80	0.0872	0.86			

the frequency of collisions between reactant molecules. Multifloral honeys with value of K_R (2.68×10^{-32} h^{-1}) are much sensitive to the process of temperature than honeydew (1.01×10^{-28} h^{-1}) and chestnut honeys (2.11×10^{-32} h^{-1}). This may be explained by the fact that chestnut and honeydew honeys have higher phenolic compounds than multifloral honey as well as antioxidant capacity.

Antioxidant properties

It is known that antioxidant activity of honey originates from phenolic compounds [3, 5]. Total phenolic content and antioxidant properties of honeys are presented in Tables 3, 4 and 5. Initially, the total phenolic compounds of multifloral, honeydew and chestnut honeys were found to be 4.20 ± 0.50, 11.10 ± 1.70, 65.20 ± 1.50 mg GAE/100 g, respectively. However, the FRAP values of the honeys are measured as 827.8 ± 19.97, 2334.467 ± 55.21, 3728.444 ± 137.00 µM Trolox/g, respectively.

Chestnut honey showed the highest antioxidant capacity. Similar results have been reported in the literature that chestnut honey has the highest total phenolic compounds and antioxidant capacity [3–5, 45]. Interestingly, indicator parameters of antioxidant capacity such as total phenolic,

Table 3 Properties of multifloral honeys

Sample	Temperature °C	Time (h)	Total phenolic content (mg GAE/g)	FRAP (µmol Trolox/g)	DPPH (SC_{50})	*L** (0 black, 100 white)	*a** (−greenness, +redness)	*b** (−blueness, +yellowness)
Multifloral honey	Initial sample		0.042 ± 0.01^{a}	827.800 ± 19.97^{a}	0.083 ± 0.01^{f}	26.775 ± 0.06^{fgh}	2.099 ± 0.04^{e}	0.168 ± 0.07^{f}
	50	5	0.043 ± 0.01^{a}	860.817 ± 16.36^{a}	0.071 ± 0.03^{e}	25.847 ± 0.12^{cde}	2.137 ± 0.03^{e}	0.436 ± 0.15^{h}
		24	0.078 ± 0.08^{ab}	1407.662 ± 14.43^{b}	0.060 ± 0.01^{d}	27.011 ± 0.06^{h}	2.647 ± 0.19^{f}	0.259 ± 1.13^{g}
		48	0.084 ± 0.01^{ab}	1486.386 ± 40.79^{b}	0.046 ± 0.02^{c}	26.261 ± 0.29^{def}	1.618 ± 0.29^{d}	-0.062 ± 0.04^{e}
	70	5	0.098 ± 0.02^{abc}	1812.014 ± 62.31^{b}	0.042 ± 0.03^{c}	25.619 ± 0.23^{abc}	1.540 ± 0.03^{d}	-0.591 ± 0.06^{cd}
		24	0.166 ± 0.03^{bcde}	2505.559 ± 45.65^{c}	0.023 ± 0.01^{a}	25.325 ± 0.12^{abc}	1.181 ± 0.20^{c}	-0.732 ± 0.12^{b}
		48	0.184 ± 0.05^{bcdef}	2674.778 ± 33.68^{c}	0.085 ± 0.01^{f}	25.859 ± 0.29^{cde}	1.109 ± 0.37^{c}	-0.609 ± 0.12^{bc}
	80	5	0.085 ± 0.01^{ab}	1565.055 ± 586.61^{b}	0.033 ± 0.07^{b}	25.006 ± 0.19^{a}	0.186 ± 0.02^{b}	-1.023 ± 0.08^{a}
		24	0.140 ± 0.01^{abcd}	2720.237 ± 33.20^{c}	0.046 ± 0.01^{c}	26.987 ± 0.27^{gh}	-0.195 ± 0.12^{a}	-1.000 ± 0.16^{a}
		48	0.229 ± 0.01^{def}	4544.574 ± 145.26^{d}	0.021 ± 0.03^{a}	25.221 ± 0.03^{b}	-0.147 ± 0.04^{a}	-1.094 ± 0.06^{a}

Means with different letters within the same column are significantly different ($p<0.05$)

Averages values are expressed as mean ± S.D. of three replicate determinations

Table 4 Properties of honeydew honeys

Sample	Temperature °C	Time (h)	Total phenolic content (mgGAE/g)	FRAP (μmol Trolox/g)	DPPH (SC_{50})	*L* (0 black, 100 white)	*a** (− greenness, + redness)	*b** (− blueness, + yellowness)
Honeydew honey	Initial sample		0.111 ± 0.02^{abc}	2334.467 ± 55.21^{a}	0.030 ± 0.003^{jk}	26.598 ± 0.11^{fgh}	0.103 ± 0.04^{cde}	0.632 ± 0.496^{i}
	50	5	0.229 ± 0.15^{def}	2377.405 ± 322.82^{a}	0.020 ± 0.001^{efgh}	26.428 ± 0.06^{efgh}	0.076 ± 0.01^{cde}	0.119 ± 0.01^{gh}
		24	0.240 ± 0.06^{def}	2844.413 ± 333.29^{a}	0.019 ± 0.001^{defg}	26.358 ± 0.20^{defg}	0.100 ± 0.01^{cde}	-0.216 ± 0.06^{ef}
		48	0.264 ± 0.03^{ef}	3613.333 ± 881.09^{bc}	0.014 ± 0.002^{cdef}	26.842 ± 0.11^{fgh}	0.110 ± 0.03^{cde}	-0.545 ± 0.08^{bcd}
	70	5	0.140 ± 0.04^{abcd}	2505.019 ± 22.80^{a}	0.017 ± 0.001^{cdefg}	27.055 ± 0.74^{h}	0.153 ± 0.02^{de}	-0.336 ± 0.04^{de}
		24	0.158 ± 0.02^{bcde}	3609 ± 301.00^{bc}	0.014 ± 0.001^{cdef}	26.909 ± 0.41^{gh}	0.057 ± 0.02^{bcde}	-0.183 ± 0.13^{ef}
		48	0.209 ± 0.06^{cdef}	4119.006 ± 63.07^{cd}	0.012 ± 0.002^{bcd}	25.782 ± 0.12^{bcd}	0.074 ± 0.01^{cde}	0.095 ± 0.09^{gh}
	80	5	0.169 ± 0.02^{bcde}	3446.039 ± 48.48^{b}	0.026 ± 0.001^{hij}	25.006 ± 0.19^{a}	0.186 ± 0.02^{e}	-1.023 ± 0.08^{a}
		24	0.284 ± 0.06^{f}	5864.209 ± 113.04^{e}	0.007 ± 0.001^{ab}	26.987 ± 0.27^{gh}	-0.195 ± 0.12^{a}	-1.000 ± 0.16^{a}
		48	0.485 ± 0.03^{g}	10219.902 ± 128.96^{f}	0.006 ± 0.003^{ab}	25.221 ± 0.03^{ab}	-0.147 ± 0.04^{ab}	-1.094 ± 0.06^{a}

Means with different letters within the same column are significantly different ($p < 0.05$)

Averages values are expressed as mean ± S.D. of three replicate determinations

Table 5 Properties of chestnut honeys and pure HMF and antioxidant properties of compared with reference antioxidant

Sample	Temperature °C	Time (h)	Total phenolic content (mgGAE/g)	FRAP (μmol Trolox/g)	DPPH (SC_{50})	*L** (0 black, 100 white)	*a** (− greenness, + redness)	*b** (− blueness, +yellowness)
Chestnut honey	Initial sample		0.652 ± 0.02^{a}	3728.444 ± 137.01^{ab}	0.027 ± 0.003^{ijk}	26.689 ± 0.31^{fgh}	-0.153 ± 0.03^{a}	-0.407 ± 0.16^{f}
	50	5	0.791 ± 0.09^{b}	3749.658 ± 76.66^{ab}	0.021 ± 0.001^{fghi}	26.746 ± 0.12^{fgh}	-0.140 ± 0.01^{ab}	-1.091 ± 0.06^{d}
		24	0.799 ± 0.08^{b}	5090.858 ± 176.20^{e}	0.018 ± 0.001^{cdefg}	25.207 ± 0.08^{ab}	-0.037 ± 0.02^{d}	-0.625 ± 0.03^{e}
		48	0.806 ± 0.04^{b}	6120.333 ± 872.74^{f}	0.019 ± 0.002^{efgh}	25.841 ± 0.85^{cde}	-0.183 ± 0.01^{a}	-2.385 ± 0.22^{a}
	70	5	0.773 ± 0.06^{b}	4319.708 ± 77.60^{cd}	0.015 ± 0.002^{cdef}	26.923 ± 0.17^{gh}	-0.050 ± 0.01^{d}	-0.193 ± 0.10^{g}
		24	0.769 ± 0.01^{b}	$4666.537 \pm 172.50^{cde}$	0.013 ± 0.001^{cde}	26.933 ± 0.04^{gh}	-0.076 ± 0.02^{bc}	-2.065 ± 0.03^{b}
		48	0.876 ± 0.04^{b}	5764.668 ± 144.91^{f}	0.012 ± 0.001^{bc}	25.882 ± 1.01^{cde}	-0.093 ± 0.01^{bc}	-1.662 ± 0.03^{c}
	80	5	0.808 ± 0.18^{b}	4114.617 ± 58.49^{bc}	0.024 ± 0.001^{ghij}	26.883 ± 0.10^{fgh}	-0.186 ± 0.02^{a}	-1.611 ± 0.10^{c}
		24	1.197 ± 0.08^{c}	4932.193 ± 252.82^{de}	0.007 ± 0.001^{ab}	26.557 ± 0.01^{fgh}	-0.042 ± 0.03^{d}	-1.196 ± 0.06^{d}
		48	1.898 ± 0.05^{d}	6134.373 ± 293.02^{f}	0.005 ± 0.001^{a}	25.579 ± 0.10^{abc}	-0.018 ± 0.02^{de}	-1.097 ± 0.08^{d}
HMF				341 ± 38	114.644 ± 2.70			
BHT					0.114 ± 0.002			

Means with different letters within the same column are significantly different ($p < 0.05$)

Averages values are expressed as mean ± S.D. of three replicate determinations

FRAP and DPPH results were increased with elevated temperature during the process. Heating process enhanced antioxidant capacity of honeys. TPC was slightly increased during the incubation period at 50 °C, then it was substantially increased at 70 and 80 °C ($p < 0.05$), and this increase was linear to incubation times.

Strong positive correlations ($p < 0.01$) between the HMF concentrations and phenolic contents (*r*: 0.412; *p*: 0.00), HMF concentrations and FRAP (*r*: 0.616; *p*: 0.00) were found (Table 6). FRAP values were increased during the heating process of the honeys. The reason behind the increasing of antioxidant activity is that either some MRPs that occurred in the heating process may give colored products with antioxidant test reagents, or these products have actually antioxidant activities. Literature studies were reported that some MPRs have antioxidant properties [13, 19, 46]. A strong positive correlation ($p < 0.01$) between antioxidant capacity, phenolic contents and the FRAP values (*r*: 0.593; *p*: 0.00) during heating process was also determined (Table 6). A strong negative correlation was observed between DPPH (SC_{50}) and the other antioxidant assay ($r_{DPPH-Total\ phenolic\ content}$: −0.507; $r_{DPPH-FRAP}$: −0.666). The lower SC_{50} indicates the higher free radical scavenging activity; the higher antioxidant activity. Strong negative relationship between DPPH radical scavenging

Table 6 Correlations between studied parameters

		HMF	Phenolic content (mgGAE/g)	FRAP (mmol Trolox /g)	DPPH (SC_{50})	*L*	*a*	*b*
HMF	Pearson correlation, *r*	1	0.412(**)	0.616(**)	−0.303(**)	−0.293(**)	−0.300(**)	−0.249(*)
	Sig. (2-tailed), *p*		0.000	0.000	0.004	0.005	0.004	0.018
Phenolic content (mgGAE/g)	Pearson correlation, *r*	0.412(**)	1	0.593(**)	−0.507(**)		−0.463(**)	−0.573(**)
	Sig. (2-tailed), *p*	0.000		0.000	0.000		0.000	0.000
FRAP(mmol Trolox /g)	Pearson correlation, *r*	0.616(**)	0.593(**)	1	−0.666(**)		−0.633(**)	−0.587(**)
	Sig. (2-tailed), *p*	0.000	0.000		0.000		0.000	0.000
DPPH (SC_{50})	Pearson Correlation, *r*	−0.303(**)	−0.507(**)	−0.666(**)	1		0.784(**)	0.447(**)
	Sig. (2-tailed), *p*	0.004	0.000	0.000			0.000	0.000
*L**	Pearson Correlation, *r*	−0.293(**)						
	Sig. (2-tailed), *p*	0.005						
*a**	Pearson Correlation, *r*	−0.300(**)	−0.463(**)	−0.633(**)	0.784(**)		1	0.536(**)
	Sig. (2-tailed), *p*	0.004	0.000	0.000	0.000			0.000
*b**	Pearson Correlation, *r*	−0.249(*)	−0.573(**)	−0.587(**)	0.447(**)		0.536(**)	1
	Sig. (2-tailed), *p*	0.018	0.000	0.000	0.000		0.000	

activity (SC_{50} values) and HMF concentration (*r*: −0.303; *p*: 0.004) were found.

Moreover, radical scavenging activity and ferric reducing antioxidant capacity of HMF were also studied to evaluate its antioxidant properties using various concentrations of pure HMF, and the results were compared with Trolox® (Table 6). Obtained results indicate that HMF has antioxidant activity according to studied antioxidant methods, but the activities are lower than antioxidant activity of Trolox® and honey. There was much information in related studies on the relationship between HMF concentration and antioxidant activity [5, 13, 39, 47]. However, a direct study has not found that pure HMF has antioxidant character. It was reported that roasted coffee beans showed strong DPPH radical scavenging activity as parallel to HMF increasing [48]. Mastrocola et al. [49] studied in a model system the progress of the Maillard reaction and the antioxidant action of Maillard reaction products on lipid oxidation. The authors have demonstrated that antioxidant activity was substantially increased by browning of the samples.

Color parameters

Color is one of the quality parameters of honey. Floral sources, phenolic compounds, mineral content, and amount of Maillard products, particularly HMF content are important factors that affect color of honey. Color values of the studied honeys were monitored during the heating progress with regard to colour measurements based on Hunter L*, a*, and b* scales. L*, a*, and b* values were generally decreased for all studied samples indicating that color of honey was gradually becoming to black, green and blue due to heating process (Table 6). Significantly negative correlation was found between the HMF and color parameters (L*, a*, and b*) during the heating process. FRAP values and color parameters have strong negative correlation ($p < 0.01$) particularly of *a** (r: −0,633) and *b** (r: −0,587) values.

Conclusion

Physicochemical properties including HMF content, antioxidant activity, total phenolic content of honey samples from different botanical sources were strongly affected by the heating process parameters. HMF concentrations were increased especially above 50 °C. Antioxidant capacity and color values have been shown to increase with increasing HMF concentrations. Pure HMF exhibited a weak antioxidant effect. The formation of HMF has higher degree of linearity in the fit of the zero order reaction. The formation rate of HMF was found higher in chestnut honey than multifloral and honeydew honeys. This may be due to its phenolic composition. HMF content can be considered as an index of the shelf life and thermal treatment exposition of honey.

Further studies are needed to illuminate complex relations between all of those variables.

Compliance with ethical standards

Conflict of interest The authors declare to have no potential conflict of interest.

References

1. S. Soares, J.S. Amaral, M.B.P. Oliveira, I. Mafra, Compr. Rev. Food Sci. Food Saf. **16**(5), 1072–1100 (2017)
2. K. Pyrzynska, M. Biesaga, TrAC Trends Anal. Chem. **28**(7), 893–902 (2009)
3. M. Kucuk, S. Kolayli, Ş Karaoglu, E. Ulusoy, C. Baltaci, F. Candan, Food Chem. **100**, 526–534 (2007)
4. J.M. Alvarez-Suarez, M. Gasparrini, T.Y. Forbes-Hernández, L. Mazzoni, F. Giampieri, Foods **3**(3), 420–432 (2014)
5. F. Tezcan, S. Kolayli, H. Sahin, E. Ulusoy, B.F. Erim, J. Food Nutr. Res. **50**, 33–40 (2011)
6. J. Bertoncelj, U. Dobersˇek, M. Jamnık, T. Golob, Food Chem. **105**, 822–828 (2007)
7. O. Escuredo, I. Dobre, M. Fernández-González, M.C. Seijo, Food Chem. **149**, 84–90 (2014)
8. F. Tornuk, S. Karaman, I. Ozturk, O.S. Toker, B. Tastemur, O. Sagdic, A. Kayacier, Ind. Crops Prod. **46**, 124–131 (2013)
9. E. Tosi, M. Ciappini, H. Lucero, Food Chem. **77**(1), 71–74 (2002)
10. B. Fallico, M. Zappala, E. Arena, A. Verzera, Food Chem. **85**, 305–313 (2004)
11. D.A.C. Yáñez, M. Gagneten, G.E. Leiva, L.S. Malec, LWT-Food Sci. Technol. **89**, 344–349 (2018)
12. T. Nagai, N. Kai, Y. Tanoue, N. Suzuki, J Food Sci. Technol. **55**(2), 586–597 (2018)
13. L. Manzocco, S. Calligaris, D. Mastrocola, M.C. Nicoli, C.R. Lerici, Trends Food Sci. Technol. **11**, 340–346 (2001)
14. J.M. Silván, J. van de Lagemaat, A. Olano, M.D. Del Castillo, J. Pharm. Biomed. Anal. **41**, 1543–1551 (2006)
15. O. Yıldız, H. Şahın, M. Kara, R. Alıyazıcıoglu, Ö Tarhan, S. Kolaylı, Acad. Food J. **8**, 44–51 (2010)
16. P. Vagnarelli, A.D. Sario, M.T. Cuzzoni, P. Mazza, L.D. Carli, J. Agric. Food Chem. **39**, 2237–2239 (1991)
17. G.C. Yen, C.F. Chau, D.J. Lii, J. Agric. Food Chem. **41**, 771–776 (1993)
18. G.C. Yen, L.C. Tsai, Food Chem. **47**, 11–15 (1993)
19. N. Turkmen, F. Sarı, E.S. Poyrazoglu, Y.S. Velioglu, Food Chem. **95**, 653–657 (2006)
20. F. Chevalier, J.M. Chobert, C. Genot, T. Haertle, J. Agric. Food Chem. **49**, 5031–5038 (2001)
21. A. Kato, Food Sci. Technol. Res. **8**, 193–199 (2002)
22. R.C. Borrelli, C. Mennella, F. Barba, M. Russo, G.L. Russo, K. Krome, H.F. Erbersdobler, V. Faist, V. Fogliano, Food Chem. Toxicol. **41**, 1367–1374 (2003)
23. M. Lindermeier, T. Hofmann, J. Agric. Food Chem. **52**, 350–354 (2004)
24. Y. Yilmaz, R. Toledo, Food Chem. **93**, 273–278 (2005)
25. J.M. Ames, The Maillard reaction. (B. J. F. Hudson Ed., 1992) pp. 150–154
26. L.W. Kroh, Food Chem. **51**, 373–379 (1994)
27. J.A. Rufıán-Henares, C. Delgado-Andrade, F.J. Morales, *Non-enzymatic browning:the case of the Maillard reaction*. (C. Delgado-Andrade, J. A. Rufıán-Henares, eds. 2009), pp. 9–19
28. D.M. Bastos, E. Monaro, E. Siguemoto, M. Séfora, *Food Industrial Processes-Methods and Equipment*. (InTech, Rijeka, 2012)
29. E. Guerra-Hernandez, B. Garcıa-Vılanova, J. Montılla-Gomez, J. Liq. Chromatogr. **15**, 2551–2559 (1992)
30. S. Albala´-Hurtado, M.T. Veciana-Nogue´S, A. Marine´-Font, M.C. Vidal-Carou, J. Agric. Food Chem. **46**, 2998 (1998)
31. Codex Alimentarius Commission, 1st edn. Revised (Rev. 2001) p. 2
32. S. Ajlouni, P. Sujirapınyokul, Food Chem. **119**, 1000–1005 (2010)
33. J. Loveaux, J. Pourtallıe, G. Vorwohl, Bull. Apic. Inf. Doc. Sci. Technol. **16**, 7 (1973)
34. W. Von Der Ohe, L. Persano-Oddo, M.L. Piana, M. Morlot, P. Martin, Apidologie **35**, 18–25 (2004)
35. K. Slinkard, V.L. Singleton, Am. J. Enol. Vitic. **28**, 49–55 (1977)
36. A. Meda, C.E. Lamıen, M. Romıto, J. Mıllogo, O.G. Nacoulma, Food Chem. **91**, 571–577 (2005)
37. L.A. Chen, M. Mehta, A.R. Berenbaum, I. Zanger, N.J. Engeseth, J. Agric. Food Chem. **48**, 4997–5000 (2000)
38. T.P. Labuza, D. Riboh, Food Technol. **36**, 55–74 (1982)
39. D. Aslanova, E. Bakkalbası, N. Artık, Int. J. Food Prop. **13**, 904–912 (2010)
40. O. Yildiz, M. Alpaslan, Food Technol. Biotechnol. **50**, 98–106 (2012)
41. S. Boonchiangma, S. Chanthai, S. Srijaranai, S. Srijaranai, J. Food Process Eng. **34**(5), 1584–1596 (2011)
42. B. Fallico, M. Zappala, E. Arena, A. Verzera, Food Chem. **85**(2), 305–313 (2004)
43. L. Bulut, M. Kilic, J. Food Process. Preserv. **33**(1), 22–32 (2009)
44. N. Turhan, M. Tetik, F. Karhan, H.R. Gurel, Tavukcuoglu, LWT Food Sci. Technol. **41**, 1396–1399 (2008)
45. A.O. Sarıkaya, E. Ulusoy, N. Öztürk, M. Tuncel, S. Kolayli, J. Food Biochem. **33**, 470–481 (2009)
46. D. Mastrocola, M. Munari, J. Agric. Food Chem. **48**, 3555–3559 (2000)
47. T. Kahraman, S.K. Buyukunal, A. Vural, S.S. Altunatmaz, Food Chem. **123**, 41–44 (2010)
48. E. Nebesny, G. Budryn, Eur. Food Res. Technol. **217**(2), 157–163 (2003)
49. D. Mastrocola, M. Munari, M. Cioroi, C.R. Lerici, J. Sci. Food Agric. **80**, 684–690 (2000)