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KINETIC STUDIES ON ANTI-OXIDANT ACTIVITY VIS-À-VIS COLOUR IN TWO KASHMIR HONEYS

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ABSTRACT

The honey is generally heated to prevent crystallization and microbial growth, however thermal treatment may affect honey qualities like antioxidant activity, colour, etc. In this study the kinetics of antioxidant activity change in correlation with honey colour has been studied. Mustard and mixed floral honey samples were heated at 40, 50, 60, 70 and 80°C for 5 to 25 min. and changes in antioxidant activity measured by DPPH method. The results showed that increase in temperature increased kinetic constants so revealing favoured antioxidant activity. The calculated activation energy using Arrhenius equation for antioxidant activity at 40-80°C was 171.65 and 453.36 k calories mol^{-1} for mustard-based and mixed floral-based honey samples, respectively. Both the samples followed first order kinetics upto 60°C. At 70 and 80°C the kinetic trend for mixed floral sample followed zero order kinetics. The results demonstrated that increase in antioxidant activity was significantly correlated with colour change in honey.

Key words: Antioxidant activity, colour, honey, kinetics

INTRODUCTION

Honey is a mixture of carbohydrates, found naturally, with 70-80% sugars, 10-20% water and rest other minor constituents like organic acids, mineral salts, vitamins, proteins, phenolic compounds, lipids and free amino acids (Gomes *et al*., 2010). Honey contains about 200 substances (complex mixture of various sugars and small amounts of other constituents like minerals, proteins, vitamins, organic acids, flavonoids, phenolics, enzymes and other phytochemicals) and is considered an constituent of traditional medicine (White, 1979). It has been used in ethno-medicine since ages, and has recently been used as treatment for burns, gastrointestinal disorders, asthma, infected and chronic wounds, skin ulcers, cataracts and other eye ailments (Castaldo and Capasso, 2002). This beneficial role is attributed to the antibacterial activity of honey. Further, the presence of hydrogen peroxide and minerals particularly copper and iron in honey often leads to the generation of highly reactive hydroxyl radicals as part of antibacterial system (McCarthy, 1995). Thus, it appears that a mechanism is present in honey which restricts the formation and removal of these reactive oxygen species. The honey, as a source of antioxidants, has proved effective against deteriorative oxidative reactions in food caused by light, heat and some metals (McKibben and Engeseth, 2002). It also inhibits the enzymatic browning of fruit and vegetables (Chen *et al.*, 2000), lipid oxidation in meat (Gheldof and Engeseth, 2002) and growth of food-borne and food-spoilage microorganisms

(Mundo *et al.*, 2004). Overall, honey serves as a source of natural antioxidants (Al-Mamary *et al.*, 2002) and plays a vital role in food preservation and human health by combating the damage caused by oxidizing agents. It reduces the risk of heart disease, cancer, immune-system decline, cataracts, different inflammatory processes, etc. (The National Honey Board, 2003). The antioxidants in honey include both enzymes like catalase, glucose oxidase, peroxidase (Ioyrish, 1974) and non-enzymatic substances like ascorbic acid, α-tocopherol (Crane, 1975), carotenoids, amino acids, proteins, organic acids, Maillard reaction products (Al-Mamary *et al.*, 2002), and polyphenolic compounds like flavonoids, flavonols, phenolic acids, catechins and cinnamic acid derivatives.

Honey processing frequently requires heating to reduce viscosity and prevent crystallization or fermentation (Singh *et al.*, 1988). However, heating affects its quality and causes non-enzymatic browning due to Maillard reaction which occurs when sugars condense with free amino acids to form a variety of brown pigments. It is believed that Maillard reaction products (MRPs) act as antioxidants. The antioxidant properties of MRPs are strongly affected by physico-chemical properties of the system and by processing conditions. Polyphenols, ascorbic acid and other carbonyl compounds even if formed during oxidative reactions participate in Maillard reaction itself (Manzocco *et al.*, 2001). The honey colour is one of its most variable features. Darkening of honey during storage may occur because of Maillard reactions, fructose caramelization and polyphenol reactions. The degree of darkening depends on the temperature and/ or time of storage (Pereyra Gonzales *et al.*, 1999). However, the information on the changes in antioxidant activity of Kashmir honey in correlation with colour as the function of heating time is lacking. Therefore, the present study was aimed to determine the effect of temperature on antioxidant activity in correlation with colour of monofloral (mustard) and mixed honeys of Kashmir.

MATERIALS AND METHODS

Two types of honey *viz.*, mixed floral honey and mustard honey were procured from a local market in Anantnag (Kashmir) and studied in the Department of Food Engineering and Technology, SLIET, Longowal, Sangrur, Punjab (India) from July 2012 to July 2013.

Determination of total antioxidant activity

The antioxidant activity (AA) of honey was determined by 2,2, diphenyl-2-picryl-hydrazyl (DPPH) method (Zhang and Hamauzu, 2004). Antioxidant activity was expressed as per cent inhibition of DPPH radical and determined by using the following equation of Yen and Duh (1994):

$$\text{AA (\%)} = \frac{\lambda\text{control} - \lambda\text{sample}}{\lambda\text{control}} \times 100 \quad \text{where } \lambda = \text{absorbance at 517 nm}$$

Heat-treatment of honeys

The thermal kinetics of honey, replicated three times, was studied by isothermal heating at selected temperatures (40, 50, 60, 70 and 80°C) for a residence time of 5 to 25 min. The samples were sealed in vials and immersed in a thermostatic water bath for 5, 10, 15, 20 and 25 min. as per the method of Weemaes *et al.* (1999) and Ahmed *et al.* (2002). The desired temperature was considered to have been achieved when the temperature of water bath reached that level. The samples were then transferred to cold-water container immediately after thermal treatment.

Measurement of visual colour

Visual colour was measured by a Hunter colorimeter in terms of L (lightness), 'a' (redness and greenness) and 'b' (yellowness and blueness). The instrument was calibrated with a standard white tile (L = 90.55, a = – 0.71, b = 0.39). A glass petridish containing heat-treated honey samples was

placed above the light source and covered with a white plate and post process Hunter L, a, b values were recorded as per Bozkurt *et al.* (1999).

Kinetic calculations

The order of reaction (K) for the reaction of antioxidant activity change was calculated as per Labuza and Riboh (1982) using the equation: $C = C_0 \exp(-k_T t)$; where, 'C_0' is the total initial concentration of antioxidant activity, 'k_T' is reaction rate constant (min^{-1}), 'C' is antioxidant concentration after t minutes heating at a given temperature and 't' the isothermal heating time. Activation energies (Ea kcal mol^{-1}) were calculated from rate coefficients at various temperatures by applying Arrhenius equation. The other empirical models applied were:

Name of the model	Equation	Reference
Lewis	$C_r = \exp(-kt)$	Krokida *et al.* (2002; Kabganian *et al.* (2002)
Page	$C_r = \exp(-kt^n)$	Midilli *et al.* (2002); Kabganian *et al.* (2002)
Henderson and Pabis	$C_r = a \exp(-kt)$	Kabganian *et al.* (2002); Togrul and Pehlivan,
Logarithmic	$C_r = a \exp(-kt)+c$	(2002)

[Where, C_r = final concentration; k = degradation rate constant; t = heating time; n = order of reaction, & a, c = constant]

RESULTS AND DISCUSSION

It was difficult to assess the change in antioxidant activity at 40°C during the first 10 min. in both honey types. At 40°C the change in antioxidant activity was insignificant for both the types and the antioxidant activity showed a slight increase (Tables 1 and 2). Both the honey types followed 1st

Table 1: Antioxidant activity of mustard honey at different isothermal temperatures

Temperature	Time (min.)	Antioxidant activity (% inhibition)	ln concentration	Ea (kcal mol^{-1})
40°C	5	26.31 ± 1.56	3.269949095	171.65
	10	26.32 ± 1.84	3.270329106	
	15	26.33 ± 0.69	3.270708974	
	20	27.32 ± 0.59	3.307619035	
	25	28.21 ± 0.00	3.339676525	
50°C	5	26.23 ± 0.88	3.266903794	
	10	26.34 ± 0.64	3.271088696	
	15	27.12 ± 0.92	3.300271463	
	20	27.80 ± 1.27	3.325036021	
	25	28.34 ± 1.27	3.344274234	
60°C	5	26.32 ± 0.00	3.270329106	
	10	26.90 ± 1.24	3.292126287	
	15	26.80 ± 1.14	3.288401888	
	20	27.50 ± 0.98	3.314186005	
	25	28.41 ± 0.97	3.346741196	
70°C	5	26.36 ± 0.95	3.271847710	
	10	26.71 ± 1.27	3.285038027	
	15	26.61 ± 0.35	3.281287085	
	20	27.01 ± 0.48	3.296207168	
	25	29.10 ± 1.40	3.370738174	
80°C	5	26.37 ± 0.65	3.272227000	
	10	27.10 ± 0.45	3.299533728	
	15	27.80 ± 0.90	3.325036021	
	20	27.60 ± 1.10	3.317815773	
	25	29.10 ± 1.07	3.370738174	

Table 1a: Relation between R^2 (zero and 1st order) and k (zero and 1st order) at different isothermal temperatures for mustard based honey samples

Temperature (°C)	R^2 zero order	R^2 1st order	Slope	k 1st order	k zero order
40	0.793968048	0.999932595	0.036778862	0.036778862	0.0962
50	0.965731938	0.966327623	0.004173764	0.004173764	0.0996
60	0.886448990	0.890353939	0.003497678	0.003497678	0.0956
70	0.716384069	0.723205132	0.004037236	0.004037236	0.1092
80	0.876487890	0.880839939	0.004306088	0.004306088	0.1362

order kinetics. There was an increase in antioxidant activity at 50°C in both honey types but the increase was low in comparison to other temperatures *viz.*, 60, 70 or 80°C. Both the types followed 1st order kinetics at 50°C. It was difficult to distinguish between 1st and 2nd order kinetics at 60°C but we preferred the latter because of a better fit. At 60°C both the types followed 1st order kinetics but antioxidant activity increased much faster in comparison to 40 and 50°C. At 70 and 80°C kinetic trend changed for mixed floral type and showed zero order kinetics as is indicated by the

Table 2: Antioxidant activity of mixed floral honey at different isothermal temperatures

Temperature	Time (min)	Antioxidant activity (% inhibition)	ln concentration	Ea (kcal mol^{-1})
40°C	5	28.20 ± 1.16	3.339321978	453.35
	10	28.44 ± 1.19	3.347796605	
	15	28.46 ± 1.17	3.348499593	
	20	29.31 ± 0.47	3.377928755	
	25	30.83 ± 0.31	3.428488242	
50°C	5	28.31 ± 1.05	3.343215099	
	10	28.46 ± 0.92	3.348499593	
	15	28.91 ± 0.87	3.364187556	
	20	29.80 ± 0.17	3.394508394	
	25	31.11 ± 0.29	3.437529311	
60°C	5	28.63 ± 1.10	3.354455119	
	10	29.10 ± 1.10	3.370738174	
	15	30.10 ± 0.78	3.404525172	
	20	30.80 ± 0.71	3.427514690	
	25	32.20 ± 0.16	3.471966453	
70°C	5	28.83 ± 0.76	3.361416512	
	10	29.42 ± 0.35	3.381674715	
	15	29.30 ± 0.34	3.377587516	
	20	30.68 ± 0.71	3.423610976	
	25	33.60 ± 0.62	3.514526067	
80°C	5	28.99 ± 0.66	3.366950943	
	10	30.20 ± 0.32	3.407841924	
	15	30.00 ± 0.79	3.401197382	
	20	33.60 ± 0.81	3.514526067	
	25	35.10 ± 0.78	3.558201130	

Table 2a: Relation between R^2 (zero and 1st order) and k (zero and 1st order) at different isothermal temperatures for mixed floral honey sample.

Temperature (°C)	R^2zero order	R^2 1st order	Slope	k 1st order	k zero order
40	0.80315174	0.99988306	0.03386858	0.03386858	0.1226
50	0.89819256	0.90454204	0.00469274	0.00469274	0.1388
60	0.97206659	0.97681521	0.00583598	0.00583598	0.1768
70	0.78030923	0.79178819	0.00696311	0.00696310	0.2116
80	0.88465276	0.88870746	0.00978369	0.00978369	0.3124

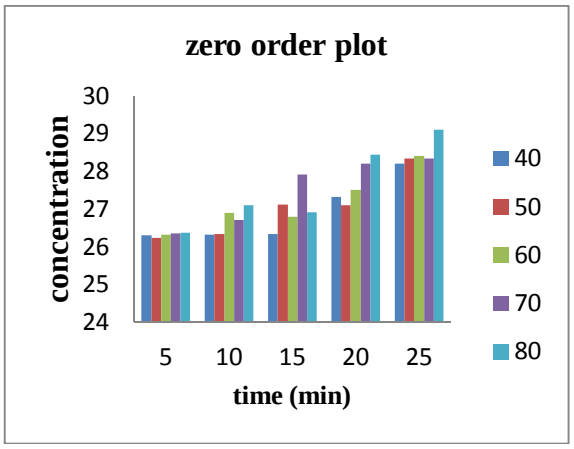

Fig. 1: Zero order antioxidant activity at different isothermal temperatures (mustard honey)

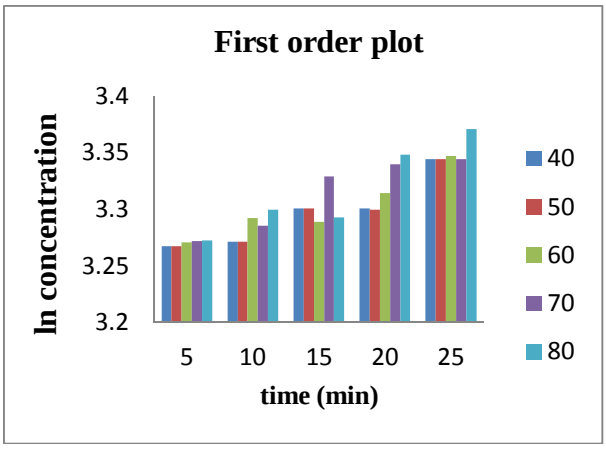

Fig. 2: First order antioxidant activity at different isothermal temperatures (mustard honey)

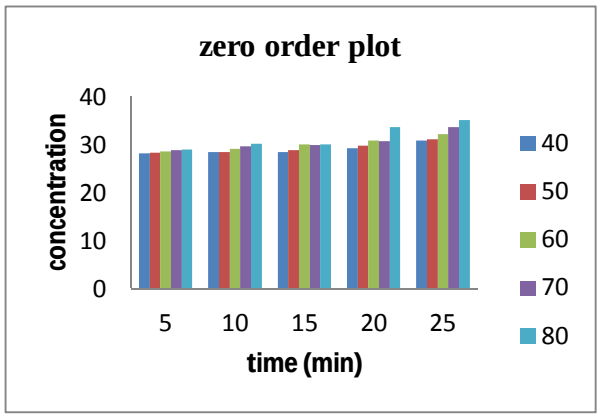

Fig. 3: Zero order antioxidant activity at different isothermal temperatures (mixed floral honey)

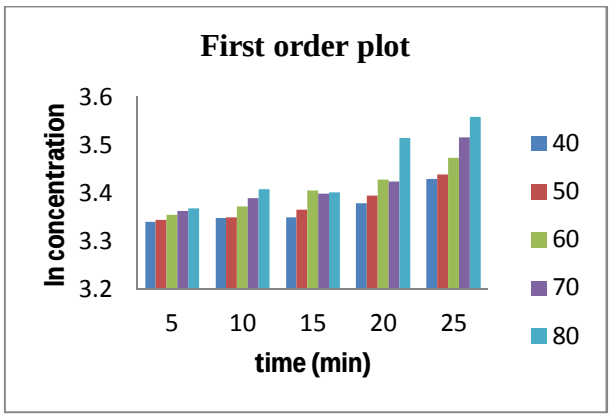

Fig. 4: First order antioxidant activity at different isothermal temperatures (mixed floral honey)

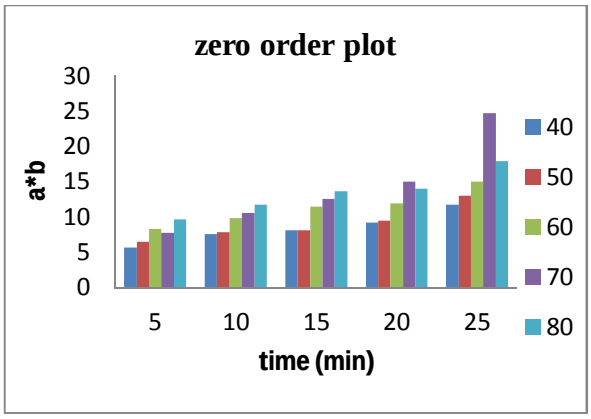

Fig. 5: Zero order graphical representation of L, a, b values of color at different isothermal temperatures (mustard honey sample)

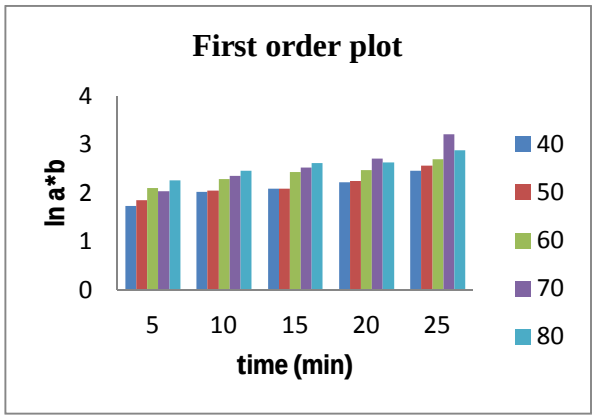

Fig. 6: First order graphical representation of L, a, b values of color at different isothermal temperatures (mustard honey sample).

coefficient of correlation (Table 1 and 2), but for mustard based honey samples the kinetic trend remained the same and the antioxidant activity increase was not regular. A similar phenomenon for antioxidant activity in honey heated at 60, 70 and 80°C has been reported by Turkmen *et al.* (2005).

A similar phenomenon for hydroxymethyl furfural (HMF) formation in honey heated at 50-100°C has been reported by Fallico *et al.* (2004).

From Table 1a and 2a, a clear increase in kinetic constants with temperature was observed. This showed that increase in treatment temperature favoured antioxidant activity. The calculated activation energy using Arrhenius equation for antioxidant activity was 171.65 kcal mol^{-1} at 40-80°C for mustard honey and 453.3565 kcal mol^{-1} for mixed floral honey. These values of activation energies resembles with those reported by Turkmen *et al.* (2005).

The correlation of antioxidant activity with colour was statistically significant as revealed by the R^2 value which ranged from 0.806603 to 0.988284 for HMF, and R^2 valve ranged from 0.917922 to 0.994433 for antioxidant activity. Frankel *et al.* (1998) noticed a significant correlation between antioxidant capacity determined by spectrophotometric DPPH method and honey colour. The antioxidant activity followed 1st order kinetics upto the treatment temperature of 60°C for both the samples; after this temperature the kinetic trend significantly changed for mixed floral samples. The quantifiable changes in antioxidant activity were noticed at higher temperatures (70 and 80°C).

Table 3: L, a, b values of mustard honey sample at different isothermal temperatures

S. No.	Temperature	Time	L	A	B	a*b	ln a*b
1	40°C	5	21.95	1.83	3.11	5.691	1.738938
		10	21.12	1.89	3.99	7.541	2.020368
		15	29.93	1.93	4.20	8.106	2.092604
		20	30.79	2.00	4.60	9.200	2.219203
		25	30.90	2.30	5.10	11.730	2.462149
	$R^2_{(0\ order)}$	$R^2_{(1st\ order)}$	Slope	$k_{(0\ order)}$	$k_{(1st\ order)}$	$t_{1/2}$	Ea
	0.94933	0.960218	0.032905	0.274726	0.03291	21.605	457.9517
2	50°C	5	22.79	1.89	3.40	6.426	1.860352
		10	28.73	1.96	3.99	7.820	2.056735
		15	28.18	1.98	4.10	8.118	2.094083
		20	22.50	2.10	4.50	9.450	2.246014
		25	25.75	2.50	5.20	13.000	2.564949
	$R^2_{(0\ order)}$	$R^2_{(1st\ order)}$	Slope	$k_{(0\ order)}$	$k_{(1st\ order)}$	$t_{1/2}$	
	0.873843	0.925492	0.0319695	0.295552	0.0319695	21.67693	
3	60°C	5	31.60	1.96	3.70	8.256	2.110940
		10	31.96	2.34	4.20	9.828	2.285235
		15	28.43	2.51	4.60	11.456	2.438513
		20	37.79	2.68	5.30	11.887	2.475445
		25	30.79	2.69	5.90	14.956	2.705112
	$R^2_{(0\ order)}$	$R^2_{(1st\ order)}$	Slope	$k_{(0\ order)}$	$k_{(1st\ order)}$	$t_{1/2}$	
	0.949647	0.964927	0.0275711	0.30918	0.0275711	25.13502	
4	70°C	5	29.43	1.98	3.90	7.722	2.044073
		10	28.79	2.30	4.60	10.580	2.358965
		15	30.74	2.56	4.90	12.544	2.529242
		20	31.93	2.58	5.80	14.964	2.705647
		25	32.64	3.80	6.50	24.700	3.206803
	$R^2_{(0\ order)}$	$R^2_{(1st\ order)}$	Slope	$k_{(0\ order)}$	$k_{(1st\ order)}$	$t_{1/2}$	
	0.871876	0.956242	0.0534428	0.7668	0.0534428	12.96713	
5	80°C	5	30.10	2.30	4.80	11.040	2.401525
		10	30.40	3.40	5.70	19.380	2.964241
		15	32.10	4.90	6.78	33.220	3.503152
		20	33.20	5.80	7.73	44.837	3.803033
		25	34.30	6.90	8.36	57.684	4.054979
	$R^2_{(0\ order)}$	$R^2_{(1st\ order)}$	Slope	$k_{(0\ order)}$	$k_{(1st\ order)}$	$t_{1/2}$	
	0.93803	0.9524897	0.0280899	0.37346	0.0280899	24.67074	

Logarithmic model and Handerson and Pabis model showed statistical significance and other models were also tested but they showed no statistical significance.

Colour

First order kinetic model described adequately color changes at all temperatures, which is in agreement with previous studies (Bozkurt, *et al*, 1999). Zero or first-order kinetic models have been used to evaluate the appearance of non-enzymatic browning Bozkurt, *et al*, (1999). Zero and second order models were also tested but they gave lower correlation coefficients.

Table 4: Correlation of antioxidant activity with colour

Temperature	Time (min)	Antioxidant activity (% inhibition)	Colour	R^2
40°C	5	26.31 ± 1.56	5.69 ± 0.09	0.917922
	10	26.32 ± 1.84	7.54 ± 0.79	
	15	26.33 ± 0.69	8.11 ± 0.56	
	20	27.32 ± 0.59	9.20 ± 1.27	
	25	28.21 ± 0.00	11.73 ± 1.46	
50°C	5	26.23 ± 0.88	6.43 ± 0.78	0.930292
	10	26.34 ± 0.64	7.82 ± 0.28	
	15	27.12 ± 0.92	8.12 ± 0.14	
	20	27.80 ± 1.27	9.45 ± 0.78	
	25	28.34 ± 1.27	13.00 ± 0.71	
60°C	5	26.32 ± 0.00	8.27 ± 0.00	0.949671
	10	26.90 ± 1.24	9.83 ± 1.41	
	15	26.80 ± 1.14	11.46 ± 0.65	
	20	27.50 ± 0.98	11.89 ± 2.96	
	25	28.41 ± 0.97	14.96 ± 2.83	
70°C	5	26.36 ± 0.95	7.72 ± 1.41	0.981178
	10	26.71 ± 1.27	10.58 ± 0.96	
	15	26.61 ± 0.35	12.54 ± 2.49	
	20	27.01 ± 0.48	14.96 ± 0.93	
	25	29.10 ± 1.40	24.70 ± 2.26	
80°C	5	26.37 ± 0.65	11.04 ± 1.44	0.994433
	10	27.10 ± 0.45	19.38 ± 0.57	
	15	27.80 ± 0.90	33.22 ± 4.04	
	20	27.60 ± 1.10	44.84 ± 3.20	
	25	29.10 ± 1.07	57.69 ± 1.41	

The correlation of antioxidant activity with color was statistically significant as is indicated in the Table 4. R^2 valve ranges from 0.917922 to 0.994433 for antioxidant activity. Frankel et al. (1998) found a significant correlation between the antioxidant capacity determined by the spectrophotometric DPPH method and honey color.

In conclusion, increased heat treatment (above 60°C) leads to the increase in antioxidant activity which has positive effects on human health-in honey due to formation of Maillard reaction products, but browning that occurred by heating is not desirable for consumers.

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