Available online at www.sciencedirect.com
SCIENCE DIRECT®
Food Chemistry 95 (2006) 653–657
Food Chemistry
www.elsevier.com/locate/foodchem

Effects of prolonged heating on antioxidant activity and colour of honey

Nihal Turkmen, Ferda Sari, Ender S. Poyrazoglu, Y. Sedat Velioglu *

Ankara University, Faculty of Engineering, Department of Food Engineering, 06110-Dışkapı, Ankara, Turkey

Received 5 August 2004; received in revised form 18 January 2005; accepted 14 February 2005

Abstract

The kinetics of changes in total antioxidant activity as assessed by DPPH radical and brown pigment formation (BPF) in honey heated at different temperatures (50, 60 and 70 °C) for up to 12 days were studied. Antioxidant activity and BPF increased with treatment temperature and time. BPF increased following zero-order kinetics with the activation energy value of 122 kJ/mol^{-1} at 50–70 °C. However, antioxidant activity variation showed different trends according to heating temperatures following second-order, first-order and zero-order kinetics at 50, 60 and 70 °C, respectively. Heating of honey at 70 °C was found to be more effective than 50 and 60 °C for both two parameters. The results demonstrated that antioxidant activity was correlated with increased browning of the samples.

Keywords: Honey; Maillard reaction; Antioxidant activity; Colour; Kinetics; Heating

1. Introduction

Foods can be subjected to some chemical changes during thermal treatment. One of them is the non-enzymatic browning due to Maillard reaction which occurs when sugars condense with free amino acids and leads to the formation of a variety of brown pigments. It is not well known that these reaction products are mutagenic or antimutagenic. It is believed that the Maillard reaction products (MRPs) are acting as antioxidants. Thus, the losses of natural antioxidants during heating could be minimised or compensated by the formation of non-nutrient antioxidants such as MRPs (Manzocco, Calligaris, Mastrocola, Nicoli, & Lerici, 2001; Nicoli, Anese, Parpinel, Franceschi, & Lerici, 1997).

The antioxidant properties of MRPs have been reported to be strongly affected by the physico-chemical properties of the system and by the processing conditions. Polyphenols, ascorbic acid and other carbonyl compounds even if formed during oxidative reactions can take part in the Maillard reaction itself (Manzocco et al., 2001). The heterocyclic compounds such as pyrroles, unsubstitued furans and thiopenes formed during Maillard reaction inhibit hexanal oxidation in considerable amounts (Yanagimoto, Lee, Ochi, & Shibamoto, 2002). But, browning is not directly related to the free radical scavenging properties of MRPs formed at prolonged heating conditions at 100 °C in model systems (Morales & Jiménez-Pérez, 2001). Anese, Manzocco, Nicoli, and Lerici (1999) indicated that treating of tomato juice up to 30 h at 95 °C caused a progressive increase in overall antioxidant properties of juice.

Honey is submitted to thermal treatments for two different reasons: (1) to modify its tendency to crystallisation or delay its appearance; (2) to destroy the

* Corresponding author. Tel.: +90 3123 17 05 50; fax: +90 312 317 87 11.
E-mail address: velioglu@eng.ankara.edu.tr (Y.S. Velioglu).

0308-8146/$ - see front matter © 2005 Published by Elsevier Ltd.
doi:10.1016/j.foodchem.2005.02.004

microorganisms which contaminate it (Fallico, Zappalá, Arena, & Verzera, 2004; Tosi, Ciappini, Ré, & Lucero, 2002). Honey heating is usually carried out in two different ways: in air-ventilated chambers at 45–50 °C for 4–7 days or in hot water (Fallico et al., 2004). Honey quality can be affected by heating during processing or by ageing during storage (Mendes, Proenca, Ferreira, & Ferreira, 1998). One of the effects of thermal treatments is non-enzymatic browning reactions including Maillard reaction (Ibarz, Pagán, & Garza, 2000; Wong & Stanton, 1989). It is well known that depending on the origin, honey contains several flavonoids such as apigenin, pinocembrin, kaempferol, quercetin, galangin, chrysin, hesperetin and phenolic acids such as ellagic, caffeic, *p*-coumaric and ferulic acids, most of them having antioxidant properties (Aljadi & Kamaruddin, 2004; Hausteen, 2002; Peterson & Dwyer, 1998; Yao, Datta, Tomas-Barberan, Martos, & Singanusong, 2003). However, there are very limited data indicating the relationship between antioxidant activity and brown pigment formation (BPF) as a function of heating time at different temperatures. The main objective of this study was to determine changes in both the antioxidant activity and colour of honey during heat treatment, and to determine the kinetic parameters for these two factors.

2. Materials and methods

2.1. Sample preparation

Commercial floral honey (80° Bx) was taken directly from a producer. Honey samples (5 g) were distributed into test tubes in triplicate, closed tightly and held in an incubator at 50 and 60 °C for 12 days, and 70 °C for 10 days. At 24 h intervals three tubes from each temperatures were withdrawn, rapidly cooled on ice and stored at −24 °C until the time of analyses. One gram sample was dissolved in 5 mL of distilled water using a vortex-mixer, the solution was centrifuged for 10 min at 10,000*g* and filtered through Whatman No. 1 and analysed for antioxidant activity and BPF.

2.2. Determination of total antioxidant activity

Antioxidant activity was determined by the 2,2,diphenyl-2-picryl-hydrazyl (DPPH) method of Zhang and Hamauzu (2004). Each extract was precisely diluted to 4° Bx with distilled water (DW) using a Atago-5000α digital refractometer. 0.5 mL of honey extract was mixed with an aliquot of 1.5 mL of 0.1 mM DPPH radical (Sigma) in methanol. DW was used as a control instead of extract. The reaction mixture was vortex-mixed and let to stand at 25 °C in the dark for 60 min. Absorbance at 517 nm was measured using a spectrophotometer (Shimadzu UV–vis 1601) using methanol as blank. Antioxidant activity was expressed as percentage inhibition of the DPPH radical and was determined by the following equation (Yen & Duh, 1994):

$$\mathrm{AA}(\%) = \frac{\mathrm{Abs}_{\mathrm{control}} - \mathrm{Abs}_{\mathrm{sample}}}{\mathrm{Abs}_{\mathrm{control}}} \times 100. \quad (1)$$

2.3. Brown pigment formation

BPF was determined by measuring the absorbance of 4° Bx diluted extracts at 420 nm using an spectrophotometer for each temperature and treatment time.

2.4. Kinetic calculations

Regression analyses (Sigma Plot 41) were performed to determine the rate constants for both antioxidant activity and BPF. Activation energy (E_a) was calculated from rate coefficients at different temperatures by applying the Arrhenius equation (Labuza, 1984).

3. Results and discussion

3.1. Antioxidant activity and BPF

Figs. 1 and 2 show the changes in the antioxidant activity and BPF in honey samples subjected to heat treatment at 50–70 °C for various times. Antioxidant activity and BPF increased regularly from the beginning up to 12 days at 50, 60 °C and 10 days at 70 °C, although at different ratios. As the temperature increased, both antioxidant activity and BPF increased. The increase was more noticeable in heated samples at 70 °C than those at 50 and 60 °C, which indicates a large dependence of the antioxidant activity and BPF on time

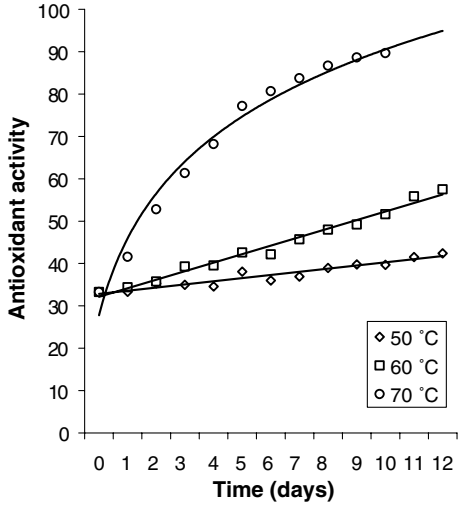

Fig. 1. Antioxidant activity of honey as a function of heating time at various temperatures.

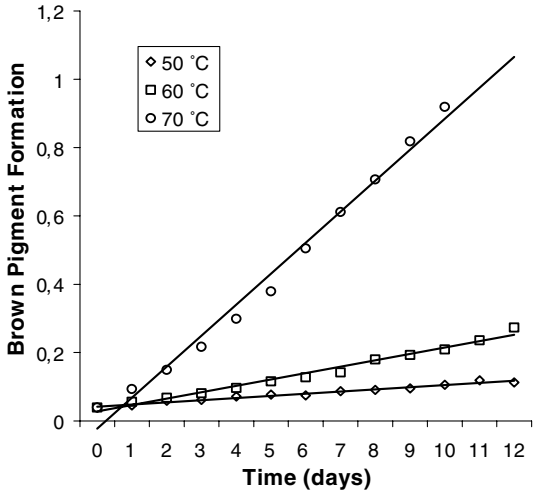

Fig. 2. BPF in honey as a function of heating time at various temperatures.

and temperature of heating. High correlation coefficients calculated after plotting the data for antioxidant activity versus BPF at different temperatures indicated that there was a strong relationship between both criteria (Table 1). As seen in Fig. 3, increase in antioxidant activity was accompanied by an increase in browning of samples due to MRPs, whose antioxidant properties

Table 1
Regression equations and correlation coefficients (R^2) of antioxidant activity as a function of BPF values for heated samples at 50, 60 and 70 °C

Treatment temperature (°C)	Regression equations[a]	R^2
50	$y = 116.25x + 28.022$	0.955
60	$y = 106.9x + 29.271$	0.987
70	$y = 19.614 \ln(x) + 92.672$	0.993

[a] y: antioxidant activity; x: BPF.

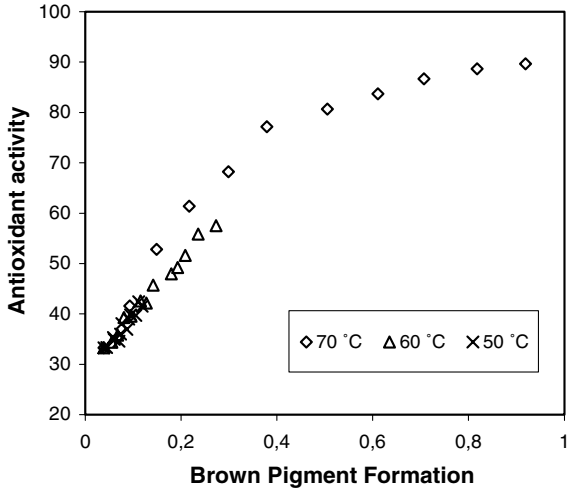

Fig. 3. Antioxidant activity-BPF relation in heated honey samples at 50, 60, 70 °C.

have been reported previously for food and model systems (Giovanelli & Lavelli, 2002; Mastrocola, Munari, Cioroi, & Lerici, 2000; Wagner, Derkits, Herr, Schuh, & Elmadfa, 2002). While antioxidant activity increased linearly with increasing heating time and BPF at 50 and 60 °C, logarithmic increase in antioxidant activity at 70 °C was observed (Figs. 1 and 3). This can be attributed to complexity of non-enzymatic browning reactions because they involve different compounds and proceed through different chemical pathways depending on composition of product and processing conditions (Manzocco et al., 2001; Van Boekel, 2001). This behaviour could be also consequence of the formation of compounds with different antioxidant activity at various stages of Maillard reactions depending on treatment temperatures. Wagner et al. (2002) reported that there were obvious differences in the radical suppressing ability between different molecular weight melanoidin fractions. Also, melanoidins isolated from some food showed different antioxidant activity (Morales & Jiménez-Pérez, 2004). Similar study carried out by Yanagimoto et al. (2002) demonstrated that the effect of various functional groups on heterocyclic compounds found in Maillard reaction with regard to antioxidant activity was different. No data have been found on the antioxidant activity and colour changes during heating of honey. However, the study by Anese et al. (1999) carried out in tomato juice reported that heating at 95 °C up to 30 h caused a progressive increase in overall antioxidant potential of the tomato juice with increasing in optical density. Morales and Jiménez-Pérez (2001) found that in sugar–amino mixtures heated at 100 °C, free radical scavenging activity increased drastically from the first minutes of heating until 12 h, reaching a plateau after that and browning increased regularly during heating time. At high temperatures the formation of brown melanoidins increased the antioxidant properties of milk (Calligaris, Manzocco, Anese, & Nicoli, 2004).

3.2. Reaction kinetics of antioxidant activity and BPF

In order to explain the phenomena of colour and antioxidant activity changes, the data were fitted using kinetic models. The values of the parameters obtained from these fittings are given in Table 2. Zero-order kinetic model described adequately BPF at all temperatures, which is in agreement with previous studies. Zero- or first-order kinetic models have been used to evaluate the appearance of non-enzymatic browning (Bozkurt, Göğüş, & Eren, 1999; Carabasa-Giribet & Ibarz-Ribas, 2000; Davies, Wedzicha, & Gillard, 1997; Garza, Ibarz, & Giner, 1999; Ibarz et al., 2000; Kwok, MacDougall, & Niranjan, 1999; Maskan, Kaya, & Maskan, 2002; Toribio & Lozano, 1984). First- and second-order models also tested but they gave lower correlation coefficients. However, first-order kinetics was also

Table 2
Kinetic parameters for BPF and antioxidant activity in honey at different temperatures

Parameter	Temperature (°C)	Zero-order		First-order		Second-order		
		k (day^{-1})	R^2	k (day^{-1})	R^2	k (day^{-1})	R^2	E_a (kJ/mol^{-1})
Brown pigment formation	50	0.0063	0.972	0.0849	0.941	−1.2346	0.856	122
	60	0.0187	0.981	0.1507	0.976	−1.4973	0.828	
	70	0.0906	0.987	0.2887	0.922	−1.6734	0.581	
Total antioxidant activity	50	0.746	0.917	0.0202	0.920	−0.0005	0.921	
	60	2.0101	0.982	0.0455	0.990	−0.0011	0.985	
	70	0.746	0.924	0.0933	0.858	−0.0016	0.770	

acceptable. On the other hand, changes in antioxidant activity with treatment time were fitted to second-order, first-order and zero-order reaction kinetics at 50, 60 and 70 °C, respectively. Actually it was difficult to distinguish between a first-order and second-order kinetics at 50 °C but we preferred the latter because of a better fit. Different trends in kinetic parameters according to the heating temperatures applied which affect the activities of reactants can be result of differences in temperature sensitivity of various reaction steps in Maillard reaction (Van Boekel, 2001). A similar phenomenon for HMF formation in honey heated at 50–100 °C has been reported (Fallico et al., 2004). No study has been found for the changes in antioxidant activity during thermal processing of honey. On the other hand, Suh, Kim, Lee, Lee, and Choi (2004) found that the antiradical capacity variation of mulberry fruit extract with treatment time at 80–100 °C was fitted to both the first- and zero-order models. But these authors proposed the latter, which is consistent with the result obtained for 70 °C from this study. According to Table 2, a clear increase in kinetic constants with temperature was observed. This indicates that increase in treatment temperature favoured both antioxidant activity and BPF.

The calculated activation energy using Arrhenius equation for BPF was 122 kJ/mol at 50–70 °C. This value was lower than the value of 132 kJ/mol given for boiled grape juice (Bozkurt et al., 1999) and higher than 77.1 kJ/mol for apple puree (Ibarz et al., 2000), indicating that browning reaction in honey is more temperature sensitive compared to boiled grape juice but less sensitive to temperature increase than apple puree. The difference in browning rate of honey could be explained by differences in its amino acid and reducing sugar contents compared to both boiled grape juice and apple puree. Other factors which could possibly affect the kinetics of Maillard browning are the type and thermal stability of amino acids and reducing sugars which participate in the reaction.

In conclusion, increased heat treatment leads to development of 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. Hence, a balance between positive and negative effects should be taken into account before simulating their formation during processing.

References

Aljadi, A. M., & Kamaruddin, M. Y. (2004). Evaluation of the phenolic content and antioxidant capacities of two Malaysian floral honeys. *Food Chemistry, 85*, 513–518.

Anese, M., Manzocco, L., Nicoli, M. C., & Lerici, C. R. (1999). Antioxidant properties of tomato juice as affected by heating. *Journal of the Science of Food and Agriculture, 79*, 750–754.

Bozkurt, H., Göğüş, F., & Eren, S. (1999). Nonezymic browning reactions in boiled grape juice and its models during storage. *Food Chemistry, 64*, 89–93.

Calligaris, S., Manzocco, L., Anese, M., & Nicoli, M. C. (2004). Effect of heat-treatment on the antioxidant activity of milk. *International Dairy Journal, 14*, 421–427.

Carabasa-Giribet, M., & Ibarz-Ribas, A. (2000). Kinetics of colour development in aqueous glucose systems at high temperatures. *Journal of Food Engineering, 44*, 181–189.

Davies, C. G. A., Wedzicha, B. L., & Gillard, C. (1997). Kinetic model of the glucose–glycine reaction. *Food Chemistry, 60*, 323–329.

Fallico, B., Zappalá, M., Arena, E., & Verzera, A. (2004). Effects of conditioning on HMF content in unifloral honeys. *Food Chemistry, 85*, 305–313.

Garza, S., Ibarz, A., & Giner, J. P. J. (1999). Non-enzymatic browning in peach puree during heating. *Food Research International, 32*, 335–343.

Giovanelli, G., & Lavelli, V. (2002). Evaluation of heat and oxidative damage during storage of processed tomato products. I. Study of heat damage indices. *Journal of the Science of Food and Agriculture, 82*, 1263–1267.

Hausteen, B. H. (2002). The biochemistry and medical significance of flavonoids. *Pharmacology and Therapeutics, 96*, 67–202.

Ibarz, A., Pagán, J., & Garza, S. (2000). Kinetic models of non-enzymatic browning in apple puree. *Journal of the Science of Food and Agriculture, 80*, 1162–1168.

Kwok, K. C., MacDougall, D. B., & Niranjan, K. (1999). Reaction kinetics of heat-induced colour changes in soymilk. *Journal of Food Engineering, 40*, 15–20.

Labuza, T. P. (1984). Application of chemical kinetics to deterioration of foods. *Journal of Chemical Education, 61*, 348–358.

Manzocco, L., Calligaris, S., Mastrocola, D., Nicoli, M. C., & Lerici, C. R. (2001). Review of non-enzymatic browning and antioxidant capacity in processed foods. *Trends in Food Science and Technology, 11*, 340–346.

Mastrocola, D., Munari, M., Cioroi, M., & Lerici, C. R. (2000). Interaction between maillard reaction products and lipid oxidation in starch-based model. *Journal of the Science of Food and Agriculture, 80*, 684–690.

Maskan, A., Kaya, S., & Maskan, M. (2002). Effect of concentration and drying processes on color change of grape juice and leather (pestil). *Journal of Food Engineering, 54*, 75–80.

Mendes, E., Proenca, E. B., Ferreira, I. M. P. L. V. O., & Ferreira, M. A. (1998). Quality evaluation of Portuguese honey. *Carbohydrate Polymers, 37*, 219–223.

Morales, F. J., & Jiménez-Pérez, S. (2001). Free radical scavenging capacity of Maillard reaction products as related to colour and fluorescence. *Food Chemistry, 72*, 119–125.

Morales, F. J., & Jiménez-Pérez, S. (2004). Peroxyl radical scavenging activity of melanoidins in aqueous systems. *European Food Research and Technology, 218*, 515–520.

Nicoli, M. C., Anese, M., Parpinel, M. T., Franceschi, S., & Lerici, C. R. (1997). Loss and/or formation of antioxidants during food processing and storage. *Cancer Letters, 114*, 71–74.

Peterson, J., & Dwyer, J. (1998). Flavonoids: dietary occurrence and biochemical activity. *Nutrition Research, 18*, 1995–2018.

Suh, H. J., Kim, J. M., Lee, H., Lee, S. W., & Choi, Y. M. (2004). Thermal kinetics on antiradical capacity of mulberry fruit extract. *European Food Research and Technology, 219*, 80–83.

Toribio, J. L., & Lozano, J. E. (1984). Non-enzymatic browning in apple juice concentrate during storage. *Journal of Food Science, 49*, 889–892.

Tosi, E., Ciappini, M., Ré, E., & Lucero, H. (2002). Honey thermal treatment effects on hydroxymethylfurfural content. *Food Chemistry, 77*, 71–74.

Van Boekel, M. A. J. S. (2001). Kinetics aspects of the Maillard reaction: a critical review. *Nahrung/Food, 45*, 150–159.

Wong, M., & Stanton, D. W. (1989). Nonenzymic browning in kiwifruit juice concentrate systems during storage. *Journal of Food Science, 54*, 669–673.

Wagner, K.-H., Derkits, S., Herr, M., Schuh, W., & Elmadfa, I. (2002). Antioxidative potential of melanoidins isolated from a roasted glucose–glycine model. *Food Chemistry, 78*, 375–382.

Yanagimoto, K., Lee, K., Ochi, H., & Shibamoto, T. (2002). Antioxidative activities of heterocyclic compounds formed in Maillard reaction products. *International Congress Series, 1245*, 335–340.

Yao, L., Datta, N., Tomas-Barberan, F. A., Martos, I., & Singanusong, R. (2003). Flavonoids, phenolic acids and abscisic acid in Australian and New Zealand *Leptospermum* honeys. *Food Chemistry, 81*, 159–168.

Yen, G. C., & Duh, P. D. (1994). Scavenging effect of methanolic extracts of peanut hulls on free-radical and active oxygen species. *Journal of Agricultural and Food Chemistry, 42*, 629–632.

Zhang, D., & Hamauzu, Y. (2004). Phenolics, ascorbic acid, carotenoids and antioxidant activity of broccoli and their changes during conventional and microwave cooking. *Food Chemistry, 88*, 503–509.