Effects of prolonged heating on antioxidant activity and colour of honey

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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 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, unsubstituted 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
microorganisms which contaminate it (Fallico, Zappalà, 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, Proença, 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 temperature were withdrawn, rapidly cooled on ice and stored at 4 °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 10,000g 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-1-picryl-hydrazyl (DPPH) method of Zhang and Hamauzu (2004).

Each extract was precisely diluted to 4 Bx with distilled water (DW) using a Atago-5000a 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):

\[
\text{AA} \text{%}_\text{BPF} = \frac{\text{Abs}_{\text{Control}} - \text{Abs}_{\text{Sample}}}{\text{Abs}_{\text{Control}}} \times 100
\]

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 (Ea) was calculated from rate coefficients at different temperatures by applying the Arrhenius equation (Labuza, 1984).

3. Results and discussion

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.](image-url)
have been reported previously for food and model systems (Giyanelli & Lavelli, 2002; Mastrocola, Munari, Cioroi, & Leric, 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 melanoids 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, Göğüs, & 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

![Fig. 2. BPF in honey as a function of heating time at various temperatures.](image)

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>
<thead>
<tr>
<th>Table 1</th>
<th>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</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>R</strong></td>
<td><strong>Treatment temperature (°C)</strong></td>
</tr>
<tr>
<td>50</td>
<td>$y = 116.25x + 28.022$</td>
</tr>
<tr>
<td>60</td>
<td>$y = 106.9x + 29.271$</td>
</tr>
<tr>
<td>70</td>
<td>$y = 19.614 \ln (x) + 92.672$</td>
</tr>
</tbody>
</table>

*a: antioxidant activity, x: BPF.*

![Fig. 3. Antioxidant activity-BPF relation in heated honey samples at 50, 60, 70 °C.](image)
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


Maskan, A., Kaya, S., & Maskan, M. (2002). Effect of concentration and drying processes on color change of grape juice and leather

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References

Table 2

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Temperature ( °C)</th>
<th>Zero-order</th>
<th>First-order</th>
<th>Second-order</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>k (day⁻¹)</td>
<td>k (day⁻¹)</td>
<td>k (day⁻¹)</td>
</tr>
<tr>
<td>Brown pigment formation</td>
<td>50</td>
<td>0.0063</td>
<td>0.0849</td>
<td>1.2346</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>0.0187</td>
<td>0.1507</td>
<td>1.4973</td>
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<td></td>
<td>70</td>
<td>0.0906</td>
<td>0.2887</td>
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<td>Total antioxidant activity</td>
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<td>0.746</td>
<td>0.2020</td>
<td>0.0005</td>
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<td></td>
<td>60</td>
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<td>70</td>
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<td>0.0933</td>
<td>0.0016</td>
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