Chemical properties of commercially available honey species and the functional properties of caramelization and Maillard reaction products derived from these honey species

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Abstract The chemical parameters and the functionalities of six monofloral honeys of different botanical and geographical origins were investigated. Vitamins B₁, B₂, and C and the protein content of majority of honeys were distinguishable from general honey. Honeys not only were rich in a variety of functional components like flavonoids but also had strong anti-oxidant activities, scavenging activities against ROS, and anti-hypertensive and anti-allergic activities. Honeys were heated at 100 °C for 24 h and their browning intensity during heating process was observed to vary with botanical origin. The functional properties of caramelization and maillard reaction (MR) products derived from honeys during heating were evaluated. The browning of honeys progressed regardless of honey species. Anti-oxidant activities and scavenging activities against superoxide and DPPH radicals of products drastically increased, but ACE and hyaluronidase activities gradually decreased with passage of heating time. It concluded that the products, mainly melanoidins, produced simultaneously to browning process in

caramelization and MR contributed to the expression of its useful function.

Keywords Honey species · Chemical property · Caramelization · Maillard reaction products · Functionality

Introduction

Heating (thermal treatment) cause significant losses that occur in color, enzymatic activity, texture (thickness), and nutritive values (blocking or destruction of lysine) of many kinds of foods. On the contrary, the pleasant flavor such as aldehydes and ketones and the functional substances such as melanoidins are produced during the heating process. Especially, this non-enzymatic browning can be divided in three different reactions, so-called ascorbic acid degradation, the caramelization, and the Maillard reaction (MR). These are influenced by many factors, including the presence of oxygen and amino groups, initial pH, temperature, heating time, and water activity and so on (Wu et al. 2014). The caramelization is browning obtained when sugars are heated over fusion temperature, resulting the generation of an enol intermediate and final dehydration products. The MR is an interaction between a carbonyl group of reducing sugars, aldehydes, or ketones and an amino group of amino acids, proteins, or any nitrogenous compounds, resulting in a variety of brown products, intermediates, and by-products, so-called MR products (MRPs) (Zeng et al. 2017). The MR composed of three major stages such as the initial stage, the intermediate stage, and the final stage, and the color changes. In the initial stage, the sugars and the amines are condensed, and then the Amadori rearrangement happened. During the intermediate stage, the color weaken or become yellow with strong absorption in near-

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ultraviolet. And in the final stage, the highly colored compounds are formed by aldol condensation and aldehyde-amine polymerization with the formation of heterocyclic nitrogen compounds. The determination of the UV–Vis absorbance at 284 and 420 nm are used as one of the browning indicators in foods.

Honeys are viscous and aromatic products appreciated since ancient Grecian times. The texture, appearance, flavor, and sweetness of honeys, as well as its medicinal properties, have attracted the consumers. Traditionally, the honeys in foods have been used as a sweetening agent. Also, honeys are commonly used in the cooking of foods such as cakes, cookies, breads, and meat and fish dishes. In general, honeys contain a large amount of carbohydrates about 80% (about 32.3% glucose and 38.6% fructose) and more than four hundred different minor substances such as aroma constituents, enzymes and proteins (about 0.2%), minerals (about 0.1%), vitamins, organic acids, pigments, and waxes (Kagawa 2017). Therefore, the caramelization and the MR may occur simultaneously during heat treatment. Blidi et al. (2017) tried to investigate the hydroxymethylfurfural (HMF) content and the diastase activity of Cretan honeys to assess the effect of the thermal treatment on the quality of honeys. HMF, an intermediate product in the MR, is produced from the degradation of hexoses such as glucose and fructose that heated in acid condition (Ramírez-Jiménez et al. 2001). They reported the increase of the HMF contents and the decrease of the diastase activities with the increasing intensity of heat in temperature and duration. Özkök and Silici (2017) reported the antioxidant activities of honeybee products such as honeys, bee pollen, royal jelly, and propolis, and their mixtures, as the health protection through natural foodstuffs has been gaining popularity in recent years. They concluded that honeybee products or their mixtures should be included in the daily diet for a healthy life, for these high bioactive characteristics. However, little information has been found about the functional properties such as anti-oxidant and anti-hypertensive activities and anti-allergenicity on honey species during heating process. Therefore, the main objectives of the present study aims (1) to investigate the chemical parameters such as vitamin B₁, B₂, and C, total phenols and flavonoids, and color parameter of six monofloral honeys with different botanical and geographical origin, and (2) to clarify the browning in honeys by heating and the functional properties as anti-oxidative activity, scavenging activity against reactive oxygen species (ROS), anti-hypertensive activity, and anti-allergic activity of the caramelization and the MR products derived from honeys during heating process.

Materials and methods

Samples

Commercially available honey species [acacia (native to Hungary), orange (the United Mexican States), lavender (Spain), blueberry (Canada), litchi (People's Republic of China), coffee (Guatemala)] were purchased from Bee-Skep Co., Ltd., Akita, Japan. All chemicals were of analytical grade. Each honey was diluted with distilled water as necessary. The solution was heated at 100 °C for heat treatment, then was rapidly cooled in water, and was used suitably in the experiment.

Chemical parameters

The pH of these honeys was determined using a pH meter (PHL-40; DKK Co., Ltd., Tokyo, Japan). Sugar content was measured using a refractometer (PAL-Pâtissier; Atago Co., Ltd., Tokyo, Japan). Total vitamin B₁ content was investigated by the *p*-aminoacetophenone method, as described by Nakamura et al. (1998). Total vitamin B₂ content was determined by the lumiflavin fluorescence method, as described by Nakamura et al. (1998). Total vitamin C content was measured by the α,α' -dipyridyl method (The Vitamin Society of Japan 1990). Total phenols and total flavonoids were investigated by using the Folin–Ciocalteu method. A 0.2 ml of the honey solution [50% (v/v)] was added 0.2 ml of the Folin reagent in an Eppendorf tube. After 3 min, the mixture was added 0.2 ml of 10% sodium carbonate. After 1 h, the absorbance of the mixture was measured at 760 nm (Slinkard and Singleton 1977) for total phenols. A 0.06 ml of the honey solution [50% (v/v)], 0.24 ml of distilled water, and 0.018 ml of 5% sodium nitrite were mixed in an Eppendorf tube. After 5 min, the mixture was added 0.018 ml of 10% aluminium(III) chloride. After 1 min, the mixture was added 0.12 ml of 1 M NaOH and 0.144 ml of distilled water. The absorbance of the mixture was measured at 510 nm (Kim et al. 2003) for total flavonoids. These contents were expressed as (+)-catechin equivalent. Total protein content was measured by the method of Lowry et al. (1951).

Color measurement

Color analysis was performed using a colorimeter (NR-11A; Nippon Denshoku Industries Co., Ltd., Tokyo, Japan) with illuminant D65 calibrated to black and white standards. The tristimulus $L^*a^*b^*$ measurement mode was used as the relation to human eye response to color. The results were shown as the mean of ten measurements.

Free amino group content

The concentration of the free amino groups on honey species during the heating was measured according to Kuchroo et al. (1983) with some modifications. Briefly, a 0.0125 ml of the sample solution [50% (v/v)], 0.2 ml of 0.21 M sodium phosphate buffer (pH 8.2), and 0.1 ml of 0.01% 2,4,6-trinitrobenzenesulfonic acid were mixed in an Eppendorf tube, and were incubated at 50 °C for 30 min in the dark. The mixture was added 0.2 ml of 0.1 M sodium sulfate, and then was incubated at room temperature for 15 min. The absorbance of the mixture was measured at 420 nm. L-Lysine was used as the standard.

Reducing sugar content

The concentration of the reducing sugars on honey species during the heating in different periods was determined by using the Somogyi–Nelson method, as described by Nakamura et al. (1998). The contents were expressed as D-glucose equivalent.

Browning index

The browning index of honey species during the heating was evaluated over time as described by Fernández-Artigas et al. (1997).

Anti-oxidative activity

The anti-oxidative activities of the samples were investigated using a linoleic acid oxidation system, as described by Nagai et al. (2014). A 0.083 ml of the sample solution [50% (v/v)] and 0.208 ml of 0.2 M sodium phosphate buffer (pH 7.0) were mixed with 0.208 ml of 2.5% (w/v) linoleic acid in ethanol. The oxidation was initiated by the addition of 20.8 µl of 0.1 M AAPH and carried out at 37 °C for 200 min in the dark. The degree of oxidation was measured according to the thiocyanate method for measuring peroxides by reading the absorbance at 500 nm after coloring with FeCl₂ and ammonium thiocyanate. Ascorbic acid, *tert*-butyl-4-hydroxyanisole (BHA), 2,6-di-*t*-butyl-4-methylphenol (BHT), α -tocopherol, and trolox were used as positive control, and distilled water was used as negative one.

Superoxide anion radical scavenging activity

The superoxide anion radical scavenging activities of the samples were measured using a xanthine/xanthine oxidase system, as described by Nagai et al. (2014). A 0.02 ml of the sample solution [50% (v/v)], 0.48 ml of 0.05 M sodium carbonate buffer (pH 10.5), 0.02 ml of 0.15% BSA,

0.02 ml of 3 mM EDTA, 0.02 ml of 0.75 mM NBT, and 0.02 ml of 3 mM xanthine were mixed in an Eppendorf tube. After pre-incubation at 25 °C for 10 min, the mixture was started by adding 6 mU XOD and carried out at 25 °C for 20 min. The reaction was stopped to add 0.02 ml of 6 mM CuCl. The absorbance of the mixture was measured at 560 nm and the inhibition rate was calculated by measuring the amount of formazan that was reduced from NBT by superoxide. Ascorbic acid, BHA, BHT, α -tocopherol, and trolox were used as positive control, and distilled water was used as negative one.

Hydroxyl radical scavenging activity

The hydroxyl radical scavenging activities of the samples were performed using a Fenton reaction system, as described by Nagai et al. (2014). A 0.075 ml of the sample solution [50% (v/v)], 0.45 ml of 0.2 M sodium phosphate buffer (pH 7.0), 0.15 ml of 10 mM 2-deoxy-D-ribose, 0.15 ml of 10 mM FeSO₄-EDTA, 0.525 ml of distilled water were mixed in an Eppendorf tube. The reaction was started by the addition of 0.15 ml of 10 mM H₂O₂. After incubation at 37 °C for 4 h, the reaction was stopped to add 0.75 ml of 1.0% (w/v) 2-thiobarbituric acid in 50 mM NaOH and 0.75 ml of 2.8% (w/v) trichloroacetic acid. The solution was boiled for 10 min, and then cooled in water. The absorbance of the mixture was measured at 520 nm. The activity evaluated as the inhibition rate of 2-deoxy-D-ribose oxidation by hydroxyl radicals. Ascorbic acid, BHA, BHT, α -tocopherol, and trolox were used as positive control, and distilled water was used as negative one.

1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity

The DPPH radical scavenging activities of the samples were determined as described by Nagai et al. (2014). A 0.03 ml of the sample solution [50% (v/v)], 0.24 ml of 99% ethanol, and 0.03 ml of 1.0 mM DPPH-ethanol were rapidly mixed in an Eppendorf tube. After 30 min in the dark, the absorbance of the mixture was measured at 517 nm. Ascorbic acid, BHA, BHT, α -tocopherol, and trolox were used as positive control, and distilled water was used as negative one.

Angiotensin I-converting enzyme (ACE) inhibitory activity

The ACE inhibitory activities of the samples were investigated as described by Nagai et al. (2014). A 0.025 ml of the sample solution [50% (v/v)] and 0.095 ml of 0.1 M sodium borate buffer (pH 8.3) containing 4.61 mM hippuryl-L-histidyl-L-leucine (as substrate) and 1.0 M NaCl were pre-

incubated at 37 °C for 5 min in an Eppendorf tube. The mixture was incubated with 0.005 ml of 0.1 M sodium borate buffer (pH 8.3) containing 1 mU ACE and 1.0 M NaCl at 37 °C for 60 min. The enzymatic reaction was stopped to add 0.125 ml of 1.0 M HCl. The resulting hippuric acid was extracted with 0.75 ml of ethyl acetate by violently mixing for 15 s. After centrifugation at 6000 rpm for 3 min, 0.5 ml of the upper layer was transported into the other tube, and then evaporated at 80 °C for 2 h. The hippuric acid was dissolved in 0.5 ml of distilled water, and then the absorbance of the solution was measured at 228 nm.

Hyaluronidase inhibitory activity

The hyaluronidase inhibitory activities of the samples were measured by the Morgan–Elson method (Elson and Morgan 1933) with some modifications. A 0.02 ml of the sample solution and 0.01 ml of hyaluronidase from Ovine testes (1000 U/ml; Wako Pure Chemical Industries, Ltd., Osaka, Japan) were mixed in an Eppendorf tube, and were pre-incubated at 37 °C for 20 min. The mixture was added 0.02 ml of compound 48/80 solution [1 mg of compound 48/80 and 7.5 mg of calcium chloride dihydrate were dissolved in 2.0 ml of 0.1 M sodium acetate buffer (pH 4.0)], and was incubated at the same condition. The enzyme reaction was started by the addition of 0.05 ml of hyaluronic acid solution. After incubation at 37 °C for 40 min, the reaction was stopped by addition of 0.02 ml of 1.77 M NaOH and 0.02 ml of 0.8 M boric acid–0.4 M NaOH. The mixture was boiled for 3 min, and then cooled in water. The mixture was added 0.6 ml of *p*-dimethylaminobenzaldehyde solution (1 g of *p*-dimethylaminobenzaldehyde was dissolved in 1.2 ml of 10 M HCl and 8.8 ml of acetic acid. The solution was diluted ten times by acetic acid before use). After incubation at 37 °C for 20 min, the absorbance of the mixture was measured at 585 nm. The inhibition rate was calculated by the measuring the amount of N-acetyl glucosamine released. Tested sample solution was replaced by the buffer solution for the control. The enzyme solution was replaced by the buffer solution for the blank. Percent inhibition was calculated as the following equation.

$$\text{Inhibition (\%)} = \frac{[(A - B) - (C - D)]}{(A - B)} \times 100$$

A: control OD₅₈₅, B: control blank OD₅₈₅, C: sample OD₅₈₅, D: sample blank OD₅₈₅

Statistical analysis

Except for color analysis, each assay was repeated 3 times independently and the results were reported as mean ± standard deviation (SD).

Results and discussion

Chemical parameters

Chemical parameters of commercially available six honey species with different botanical and geographical origin were measured. The pH at 20 °C of these honeys were as follows: 3.065 (acacia), 3.188 (orange), 2.928 (lavender), 3.452 (blueberry), 3.525 (litchi), and 3.528 (coffee), respectively (Table 1). The pH of these honeys was low compared from those of general honeys (about pH 3.3–4.9). The Brix% at 20 °C of these honeys ranged about 79.7–82.2% (Table 1). Especially, the Brix% of lavender and blueberry honeys was high among these honey. The total vitamin B₁ content of different honey was investigated. As a result, acacia, orange, and litchi honeys did not contain any vitamin B₁. On the contrary, the content of lavender, blueberry, and coffee honey was remarkably high vitamin B₁ about 0.20–1.02 mg/100 g (Table 1). It was reported that the content of common honeys are in minute trace (Kagawa 2017). Vitamin B₂ content on honeys were determined. The content of acacia honey was about three times as those of common honey (Kagawa 2017). Other five honey species showed significantly higher content about 0.11–0.50 mg/100 g (Table 1). Total vitamin C contents of honeys were measured. Surprisingly, the highest vitamin C contents were observed in all honey species. In particular, lavender, blueberry, litchi, and coffee honeys contained high amount of vitamin C compared with acacia and orange ones (Table 1). On the other hand, the common honeys did not contain vitamin C at all (Kagawa 2017).

Total phenols content of different honey was investigated. There were major differences in the content among these honey (Table 1). Lavender and coffee honeys contained the phenols about four time of acacia one, and orange and litchi honeys contained about two times. The total flavonoid content on honey ranged from about 1.48 to 11.2 mg/100 g (Table 1). The ratio of total flavonoid content to total phenol content was as follows: 6.1 (acacia), 10.2 (orange), 13.1 (lavender), 10.6 (blueberry), 11.7 (litchi), and 9.7% (coffee), respectively. The correlation between the total phenols content and the total flavonoid was investigated. As a result, high correlation was demonstrated between these, with $R^2 = 0.913$. It suggested that these honeys possessed large quantities of phenolic compounds except flavonoids. The ingredients in honey vary according to botanical and the geographical origin, processing procedure, and others. Especially, the botanical origin of different monofloral honey has great importance in terms of the vitamin and phenol contents and these compositions.

Table 1 Chemical parameters of commercially available honeys

Parameters	Species					
	Acacia	Orange	Lavender	Blueberry	Litchi	Coffee
pH at 20 °C	3.065	3.188	2.928	3.452	3.525	3.528
Brix% at 20 °C	80.9 ± 0.1	79.7 ± 0.1	82.1 ± 0.1	82.2 ± 0.1	79.2 ± 0.1	79.7 ± 0.1
Total vitamin B ₁ (mg/100 g)	N.D.	0.07	1.02 ± 0.08	0.26 ± 0.02	0.01	0.20 ± 0.02
Total vitamin B ₂ (mg/100 g)	0.03	0.20 ± 0.02	0.24 ± 0.03	0.50 ± 0.05	0.15 ± 0.02	0.11 ± 0.02
Total vitamin C (mg/100 g)	0.51 ± 0.06	1.90 ± 0.12	2.94 ± 0.25	2.93 ± 0.20	2.32 ± 0.17	2.94 ± 0.27
Total phenols (mg/100 g)**	24.3 ± 0.06	49.6 ± 0.08	85.6 ± 0.11	79.2 ± 0.08	54.6 ± 0.10	85.2 ± 0.13
Total flavonoids (mg/100 g)**	1.48 ± 0.01	5.05 ± 0.03	11.2 ± 0.03	8.40 ± 0.03	6.38 ± 0.03	8.30 ± 0.03
Total proteins (g/100 g)	1.39 ± 0.01	1.60 ± 0.01	1.99 ± 0.01	1.78 ± 0.01	1.72 ± 0.01	2.10 ± 0.01
Colour parameter						
<i>L</i> *	11.97 ± 1.03	3.58 ± 0.19	4.83 ± 0.37	5.65 ± 0.51	4.20 ± 0.22	3.32 ± 0.09
<i>a</i> *	5.19 ± 0.32	4.24 ± 0.25	5.96 ± 0.49	10.57 ± 0.84	3.85 ± 0.17	1.55 ± 0.04
<i>b</i> *	24.18 ± 2.11	3.09 ± 0.14	4.91 ± 0.33	6.30 ± 0.61	3.08 ± 0.19	1.77 ± 0.06

** (+)-catechin equivalent

Color parameter

The color of acacia honey was glossy and bright and clear yellow–orange: that was the amber color. Orange, lavender, and blueberry honey showed same color: these were glossy and bright and clear reddish yellow. The color of litchi honey was glossy, bright and clear, and dark brown red like the color of the feathers of the bird of prey, black kite. Coffee honey had also glossy, bright and clear, and dark brown tinged blackness color. The parameters of honey were as follows: $L^* = 11.97$, $a^* = 5.19$, $b^* = 24.18$ (acacia), $L^* = 3.58$, $a^* = 4.24$, $b^* = 3.09$ (orange), $L^* = 4.83$, $a^* = 5.96$, $b^* = 4.91$ (lavender), $L^* = 5.65$, $a^* = 10.57$, $b^* = 6.30$ (blueberry), $L^* = 4.20$, $a^* = 3.85$, $b^* = 3.08$ (litchi), and $L^* = 3.32$, $a^* = 1.55$, $b^* = 1.77$ (coffee), respectively (Table 1). Among these honey, the mean L^* and b^* values of acacia honey were the highest, suggesting with the strong whiteness and yellowness. The redness was strong in blueberry honey, followed by in lavender one, although the color of overall appearance looked exactly the same in orange, lavender, and blueberry honeys. On the other hand, coffee honey showed the lowest mean L^* , a^* , and b^* values, suggesting with the strong blackness and the weak redness and yellowness colors (Table 1). It seemed that the color of honey species were affected by the botanical origins of different monofloral honeys, resulting in the different color.

Effect of heating time on the free amino group content

First, the free amino group content of honey species were determined as follows: 0.87 (acacia), 1.51 (orange),

3.11 (lavender), 2.52 (blueberry), 1.48 (litchi), and 3.67 mM (coffee), respectively (Fig. 1a). The contents on lavender, blueberry, and coffee honey was high compared with those on other honeys. Different honey types were heated at 100 °C till 24 h, and free amino group content of the caramelization and MR products derived from honeys were investigated. As a result, all of the products linearly increased with the extended heating time; high correlation was shown between these contents and heating time with $R^2 = 0.93$ – 0.99 . The content on these products derived from coffee honey was highest after 24 h among these honeys tested, followed by those derived from lavender, blueberry, litchi, orange, and acacia honeys (Fig. 1a).

Effect of heating time on the reducing sugar content

The reducing sugar content on honey species was as follows: 96.2 (acacia), 109.4 (orange), 103.4 (lavender), 127.1 (blueberry), 108.5 (litchi), and 110.5 mg/ml (coffee), respectively (Fig. 1b). Different honey were heated at 100 °C till 24 h, and reducing sugar contents of the caramelization and the MR products derived from honeys were determined. As a result, the contents on the products derived from lavender, litchi, and coffee honey increased with the passage of the heating time. On the other hand, the content of the products derived from acacia and blueberry honey increased till 6 h after heating, and then decreased (Fig. 1b). Orange honey showed an increase 12 h. Especially, it indicated a pronounced increase in reducing sugars derived from litchi and coffee honey from 12 to 24 h after heating.

Fig. 1 Effect of heating time on the free amino group contents and the reducing sugar contents of honey species. **a** free amino group content (mM), **b** reducing sugar content (mg/ml)

Effect of heating time on the browning index

The honey species were heated at 100 °C for 24 h, and the UV absorbance (as the initial browning) and the browning intensity (as the latter browning) was measured over time at 284 and 420 nm, respectively. As a result, the UV absorbance at 284 nm gradually increased in each honey, suggesting the progression of the initial browning (Fig. 2a). There is no significant variation with the degree of the initial browning on honey species, although the UV absorbance at 284 nm after 24 h was high in this order: blueberry > lavender > litchi > orange honey. That is, it suggested that the increase in the UV absorbance at 284 nm form an uncolored compound, which can be the precursor of brown pigment formation in the caramelization and the MR.

On the contrary, large difference among honeys was observed in the degree of the latter browning, and the browning intensity rapidly increased with the heating time. Particularly, the absorbance at 420 nm of coffee, lavender, and blueberry honeys drastically increased

Fig. 2 Effect of heating time on the browning index of honey species. **a** UV absorbance as the initial browning (relative value at 284 nm), **b** browning intensity as the latter browning (relative value at 420 nm), **c** polymerization extent (284/420 nm ratio)

approximately linearly from 3 h after heating (Fig. 2b). The absorbance at 420 nm of litchi honey hardly changed till 12 h after heating, but rose precipitously between 12 and 24 h. The absorbance at 420 nm on coffee honey after 24 h heating was approximately two and a half times compared with those on acacia, orange, and litchi honeys. It suggested that great quantities of free amino groups and reducing sugars contributed to the browning of coffee honey after heating for 24 h compared with other honeys (Fig. 1).

The 284/420 nm (absorbance ratio) is used as indicator of the polymerization extent. The absorbance ratio of

honeys during each heating time was calculated. As a result, the ratio on blueberry honey linearly increased till 6 h after heating, and then decreased till 12 h, and became constant (Fig. 2c). Acacia, orange, and lavender honey showed as increase in ratio till 6 or 12 h after heating, and then became constant. On the contrary, the polymerization reaction of litchi honey did not occur till 6 h after heating, but subsequently the ratio suddenly increased to about 3.6, and then drastically decreased to about 1.0. The pH of litchi honey was high and the Brix% was lower among these honeys. Moreover, the anti-oxidative activity and DPPH radical scavenging activity on litchi honey after 24 h heating were high among these honeys. It was observed that polymerization in litchi honey during heating differed to that for other honey types tested.

Anti-oxidative activity

First, the anti-oxidative activities of honey species were measured to evaluate the inhibition effects at the initiation stage of linoleic acid peroxidation. As a result, the activities on lavender and blueberry honeys after peroxidation for 200 min were remarkably high as well as those on 5 mM ascorbic acid, 1.0 mM BHA and BHT, and 0.1 mM trolox (Table 2). Orange, litchi, and coffee honeys had relatively higher activities as 0.1 mM BHA and BHT. The activity on acacia honey did not slightly reach those on 0.01 mM BHA, BHT, and trolox. The activities of the caramelization and MR products derived from these honeys during heating at 100 °C for 24 h were investigated. Each sample showed a noticeably large increase of activity with the passage of the heating time (Table 2). Especially, the product derived from lavender honey after 6 h heating exhibited high activity as well as 1.0 mM trolox. Also, the activities on the products derived from acacia, lavender, blueberry, litchi, and coffee honeys after 24 h heating were similar to those on 1.0 mM α -tocopherol and trolox. Surprisingly, the product derived from orange honey on each heating time perfectly inhibited linoleic acid peroxidation (Table 2). High correlation was shown between the activities on the products after 24 h heating and the contents of total phenols and total flavonoids in honeys with $R^2 = 0.8833$ and $R^2 = 0.8928$, respectively. It suggested that the production of melanoidins, one of MRPs contributed to the increase of the activities of these with the passage of the heating time.

Superoxide anion radical scavenging activity

First, the superoxide anion radical scavenging activities of honey species were investigated. High activities was exhibited by orange and coffee honey about 63.0 and

84.5%, respectively (Table 3). On the other hand, the activity of acacia honey was similar to that of 0.01 mM BHA, about 27.5%; this was higher than 1.0 mM ascorbic acid, but was lower than 0.1 mM BHA. The lavender, blueberry, and litchi honeys perfectly scavenged the radicals. The activities of the caramelization and MR products derived from these honeys during heating at 100 °C for 24 h were determined over time. The activity on the product derived from orange honey linearly increased with increasing the concentration of the sample. The products derived from other honey species showed the highest activities after 3 h heating; these completely scavenged the radicals.

Hydroxyl radical scavenging activity

First, the hydroxyl radical scavenging activities of honey species were evaluated. The coffee honey possessed the highest and the same activity as 0.1 mM trolox, although the activity was not up to those of 0.1 mM BHA and BHT and 1.0 mM trolox (Table 3). The activities on lavender and blueberry honeys were similar to that of 1.0 mM α -tocopherol. On the contrary, acacia and orange honeys had the activities about 57.6 and 58.2% inhibition, respectively. The correlation coefficients between the activities and total phenols, total flavonoids, and total vitamin C contents were calculated as follows: $R^2 = 0.5288$ (total phenols), $R^2 = 0.2908$ (total flavonoids), and $R^2 = 0.3300$ (total vitamin C), suggesting no correlation among these factors. Next, the activities on the caramelization and the MR products derived from these honeys during heating at 100 °C for 24 h were measured. The products derived from acacia, orange, lavender, and litchi honeys showed the increase of the activities with passage of the heating time, whereas the product derived from coffee honey was observed the decrease of the activity. There was no change in the activity of the product derived from blueberry honey regardless of the heating time.

DPPH radical scavenging activity

The DPPH radical scavenging activities of honey species were investigated. Orange, lavender, and blueberry honeys exhibited the same activities as 1.0 mM BHT. Coffee honey showed slightly higher activity than 0.1 mM BHA and trolox (Table 3). The activity of litchi honey was the lowest among these honey, about 12.7%. It was not shown the correlation between the activities and total phenols, total flavonoids, and total vitamin C contents (data not shown). Next, the activities on the caramelization and the MR products derived from honeys during heating at 100 °C for 24 h were determined over time. As a result, each product showed the increase of the activity with passage of

Table 2 Antioxidative activities of the caramelization and the MR products derived from commercially available honeys

Sample species (heat time)	Absorbance (500 nm)		
	Time (min)		
	50	100	200
Acacia (0 h)	0.067 ± 0.003	0.132 ± 0.011	0.271 ± 0.015
(3 h)	0.073 ± 0.005	0.113 ± 0.008	0.241 ± 0.011
(6 h)	0.050 ± 0.003	0.078 ± 0.004	0.168 ± 0.009
(12 h)	0.033 ± 0.002	0.044 ± 0.003	0.105 ± 0.007
(24 h)	0.022 ± 0.002	0.022 ± 0.001	0.033 ± 0.002
Orange (0 h)	0.030 ± 0.003	0.072 ± 0.004	0.177 ± 0.012
(3 h)	0.031 ± 0.003	0.057 ± 0.003	0.130 ± 0.006
(6 h)	0.018 ± 0.002	0.042 ± 0.004	0.096 ± 0.007
(12 h)	0.012	0.032 ± 0.003	0.060 ± 0.004
(24 h)	0.005	0.006 ± 0.001	0.000
Lavender (0 h)	0.038 ± 0.004	0.040 ± 0.005	0.099 ± 0.010
(3 h)	0.025 ± 0.002	0.025 ± 0.003	0.058 ± 0.003
(6 h)	0.024 ± 0.002	0.022 ± 0.001	0.038 ± 0.002
(12 h)	0.021 ± 0.002	0.022 ± 0.002	0.035 ± 0.002
(24 h)	0.016 ± 0.001	0.018 ± 0.001	0.033 ± 0.002
Blueberry (0 h)	0.020 ± 0.001	0.037 ± 0.002	0.122 ± 0.008
(3 h)	0.023 ± 0.002	0.037 ± 0.002	0.085 ± 0.006
(6 h)	0.035 ± 0.002	0.039 ± 0.002	0.083 ± 0.005
(12 h)	0.029 ± 0.001	0.027 ± 0.001	0.054 ± 0.003
(24 h)	0.027 ± 0.001	0.024 ± 0.001	0.043 ± 0.002
Litchi (0 h)	0.059 ± 0.003	0.110 ± 0.011	0.210 ± 0.011
(3 h)	0.039 ± 0.002	0.074 ± 0.008	0.145 ± 0.009
(6 h)	0.028 ± 0.002	0.057 ± 0.003	0.118 ± 0.008
(12 h)	0.026 ± 0.002	0.046 ± 0.002	0.063 ± 0.003
(24 h)	0.016 ± 0.001	0.032 ± 0.001	0.030 ± 0.001
Coffee (0 h)	0.021 ± 0.001	0.055 ± 0.003	0.146 ± 0.010
(3 h)	0.015 ± 0.001	0.031 ± 0.002	0.077 ± 0.005
(6 h)	0.012 ± 0.001	0.029 ± 0.002	0.050 ± 0.003
(12 h)	0.012 ± 0.001	0.028 ± 0.002	0.031 ± 0.002
(24 h)	0.012	0.026 ± 0.001	0.024 ± 0.001
Ascorbic acid (1.0 mM)	0.022 ± 0.001	0.135 ± 0.006	0.469 ± 0.027
(5.0 mM)	0.016 ± 0.001	0.032 ± 0.003	0.090 ± 0.008
BHA (0.01 mM)	0.084 ± 0.005	0.120 ± 0.008	0.245 ± 0.012
(0.1 mM)	0.056 ± 0.003	0.090 ± 0.006	0.165 ± 0.010
(1.0 mM)	0.054 ± 0.002	0.057 ± 0.003	0.100 ± 0.006
BHT (0.01 mM)	0.082 ± 0.003	0.112 ± 0.009	0.248 ± 0.011
(0.1 mM)	0.058 ± 0.004	0.108 ± 0.005	0.173 ± 0.008
(1.0 mM)	0.044 ± 0.002	0.051 ± 0.003	0.093 ± 0.005
α-Tocopherol (1.0 mM)	0.006	0.025 ± 0.001	0.028 ± 0.002
Trolox (0.01 mM)	0.084 ± 0.005	0.094 ± 0.006	0.262 ± 0.013
(0.1 mM)	0.038 ± 0.002	0.051 ± 0.003	0.123 ± 0.008
(1.0 mM)	0.011 ± 0.001	0.031 ± 0.002	0.032 ± 0.002
Control	0.379 ± 0.008	0.715 ± 0.025	1.406 ± 0.041

Table 3 Superoxide anion radical, hydroxyl radical, and DPPH radical scavenging activities of the caramelization and the MR products derived from commercially available honeys

Sample species (heat time)	Scavenging activity (%)		
	Superoxide anion radical	Hydroxyl radical	DPPH radical
Acacia (0 h)	27.5 ± 0.83	57.6 ± 2.11	16.8 ± 0.23
(3 h)	> 100	62.0 ± 2.15	19.3 ± 0.34
(6 h)	> 100	74.6 ± 3.28	23.3 ± 0.49
(12 h)	> 100	73.6 ± 3.01	31.3 ± 0.61
(24 h)	> 100	76.6 ± 3.64	42.8 ± 0.88
Orange (0 h)	63.0 ± 2.97	58.2 ± 3.04	26.5 ± 0.71
(3 h)	68.5 ± 3.10	60.2 ± 3.20	29.6 ± 0.82
(6 h)	73.3 ± 3.46	59.6 ± 2.99	35.0 ± 0.96
(12 h)	99.7 ± 0.21	60.5 ± 3.17	39.6 ± 1.20
(24 h)	> 100	70.3 ± 3.44	52.1 ± 2.19
Lavender (0 h)	> 100	70.5 ± 3.22	30.3 ± 0.89
(3 h)	> 100	72.6 ± 3.51	34.9 ± 0.97
(6 h)	> 100	77.6 ± 4.01	42.6 ± 1.14
(12 h)	> 100	83.0 ± 4.12	46.7 ± 1.32
(24 h)	> 100	97.7 ± 4.86	60.5 ± 2.46
Blueberry (0 h)	> 100	69.2 ± 2.99	26.6 ± 0.82
(3 h)	> 100	66.3 ± 2.36	36.2 ± 1.06
(6 h)	> 100	64.5 ± 2.27	40.7 ± 1.19
(12 h)	> 100	70.4 ± 3.15	50.0 ± 1.58
(24 h)	> 100	74.7 ± 3.33	63.1 ± 2.01
Litchi (0 h)	> 100	51.2 ± 1.97	12.7 ± 0.19
(3 h)	> 100	59.9 ± 2.04	21.1 ± 0.23
(6 h)	> 100	62.8 ± 2.38	29.5 ± 0.77
(12 h)	> 100	64.4 ± 2.54	42.0 ± 1.02
(24 h)	> 100	66.0 ± 2.71	57.3 ± 1.82
Coffee (0 h)	84.5 ± 3.81	90.6 ± 4.11	19.5 ± 0.39
(3 h)	> 100	76.5 ± 3.37	30.5 ± 0.88
(6 h)	> 100	73.2 ± 3.30	41.4 ± 1.17
(12 h)	> 100	77.5 ± 3.68	53.5 ± 1.64
(24 h)	> 100	80.3 ± 4.01	64.7 ± 2.25
Ascorbic acid (1.0 mM)	14.7 ± 0.20	13.2 ± 0.21	3.1 ± 0.04*
(5.0 mM)	89.9 ± 5.31	17.6 ± 0.71	34.1 ± 2.01**
BHA (0.01 mM)	29.3 ± 0.52	59.1 ± 0.78	5.5 ± 0.04
(0.1 mM)	36.4 ± 0.91	93.3 ± 1.39	17.5 ± 0.36
(1.0 mM)	51.9 ± 1.36	95.2 ± 1.44	72.7 ± 3.58
BHT (0.01 mM)	11.7 ± 0.19	82.8 ± 0.91	3.9 ± 0.03
(0.1 mM)	46.6 ± 1.02	97.6 ± 1.55	7.9 ± 0.08
(1.0 mM)	48.4 ± 1.17	>100.0	31.7 ± 0.76
α-Tocopherol (1.0 mM)	52.6 ± 4.18	67.6 ± 4.34	87.6 ± 2.75
Trolox (0.01 mM)	46.4 ± 0.98	81.5 ± 0.63	0.1 ± 0.01
(0.1 mM)	58.1 ± 1.12	91.8 ± 1.17	17.9 ± 0.20
(1.0 mM)	76.1 ± 1.89	>100.0	86.3 ± 3.27

* 0.1 mM ascorbic acid; ** 1.0 mM ascorbic acid

the heating time. The increase ratio of the activity during heating was high about 45% in the products derived from litchi and coffee honey, followed by those derived from

lavender and blueberry honeys (about 30.2 and 36.5%, respectively), and those from acacia and orange honey (about 26%).

ACE inhibitory activity

The ACE inhibitory activities of honey species were measured. Acacia honey entirely inhibited ACE activity. Orange and lavender honeys had distinguishing high inhibitory activities about 94.4 and 96.0%, respectively (Table 4). Also, the activities on blueberry, litchi, and coffee honeys possessed remarkable high inhibitory activities against ACE, suggesting honeys tested showed strong ACE inhibition. Next, honeys were heated at 100 °C during 24 h, and then the activities on the caramelization and the MR products derived from honeys were investigated. In spite of honey species, the activity on the product derived from each honey decreased with the passage of heating time. The products derived from acacia and orange honeys retained the activities about 50% against ACE activity, but those derived from lavender and blueberry honeys decreased the activities to about 33–35%. Especially, the products derived from litchi and coffee honeys completely lost the activities after 24 h heating (Table 4). The quality or quantity of the proteins, peptides, or amino acids from different botanical origins may contribute largely to the inhibitory activities on honey species.

Hyaluronidase inhibitory activity

The hyaluronidase inhibitory activities of honey species were determined. As a result, acacia honey perfectly inhibited the activity (Table 4). Litchi and coffee honeys had strong inhibitory activities about 95.4 and 90.3%, respectively. Considerable high activities were observed in orange and blueberry honeys. Lavender honey approximately inhibited the activity by half. Sodium cromoglicate is used as one of commercially available anti-allergic drugs against atopic dermatitis based on food allergies, allergic conjunctivitis, and allergic rhinitis. The inhibitory activities on honeys were calculated as sodium cromoglicate equivalent. As a result, these activities were as follows: 1.12 (acacia), 0.90 (orange), 0.61 (lavender), 0.85 (blueberry), 1.07 (litchi), and 1.02 μ M (coffee), respectively. Next, the activities on the caramelization and the MR products derived from honeys during heating at 100 °C for 24 h were measured over time. The pattern was different among honey species tested. The activities on the products derived from acacia, blueberry, and coffee honeys gradually decreased. The product derived from lavender honey did not change the activity till 3 h, and then suddenly decreased the activity. On the contrary, the activities on the products derived from orange and litchi slightly increased with the passage of heating time (Table 4). The browning intensities as the latter browning were low on orange and litchi honeys among these honeys (Fig. 2b). It was suggested that the differences on the inhibitory activities were

due to the differences on the contents of the caramelization and the MRPs.

In the present study, it found that the pH at 20 °C of honey species tested was quite low compared with the general honeys, due to great amounts of organic acids produced by enzymatic action. Total vitamin B₁, B₂, and C contents and the protein content of most honey was distinguishing high in comparison to the general honeys, suggesting the influence of the botanical and the geographical origin. These honeys contained large quantities of phenol compounds except flavonoids. These honeys with different botanical and geographical origin not only were rich in a variety of functional components, such as vitamins and phenols but also had strong anti-oxidant activities, scavenging activities against ROS, and anti-hypertensive and anti-allergic activities. It seemed that the browning intensity of honey types during heating process were affected by the botanical origins of different monofloral honeys, resulting in the different UV absorbance at 284 and 420 nm, and the 284/420 nm (absorbance ratio). Furthermore, the functional properties of the caramelization and the MR products derived from honeys during heating were evaluated over time. The browning of honeys progressed regardless of honey species, and anti-oxidative activities and scavenging activities against superoxide anion radicals and DPPH radicals of the products drastically increased with passage of the heating time. On the contrary, the ACE and the hyaluronidase inhibitory activities of the products gradually decreased with passage of the heating time. The MR is an efficient method to improve the functional properties such as solubility, heat stability, emulsifying and anti-oxidant properties, and anti-allergenicity (Guo and Xiong 2013). In the MR, Amadori products can form cross-links between adjacent proteins or those with amino groups, resulting polymeric aggregates (advanced glycation end products, AGEs). AGEs are related to the deterioration of Alzheimer dementia, atherosclerosis, chronic renal failure, and diabetes. Finally, it suggested that useful properties of the products derived from honeys during heating were due to melanoidins, one of MRPs. On the other hand, it is well known that HMF (an intermediate product in the MR) content is one of quality indicator in the honey international trade (Singh and Bath 1997). The commercial qualities of the honeys decrease with the increase of HMF content. The HMF formation is related to the chemical composition, pH, and botanical origins (Fallico et al. 2004). The storage time and heating also affected the HMF content in honey types (Ajlouni and Sujirapinyokul 2010; Bath and Singh 2001; Kedzierska-Matysek et al. 2016; Singh and Bath 1998). Ajlouni and Sujirapinyokul (2010) reported the maximum increase of HMF content on Australian honey by heating at 85 °C for 2 min. Kedzierska-Matysek et al. (2016) reported the

Table 4 Effect of heat time on angiotensin I-converting enzyme and hyaluronidase inhibitor activities of the caramelization and the MR products derived from commercially available honeys

Sample species (heat time)	Activity (%)	
	ACE inhibition	Hyaluronidase inhibition
Acacia (0 h)	> 100	> 100
(3 h)	79.7 ± 0.85	92.2 ± 1.54
(6 h)	60.4 ± 0.72	81.4 ± 1.17
(12 h)	56.8 ± 0.65	79.1 ± 0.98
(24 h)	54.2 ± 0.49	77.1 ± 0.95
Orange (0 h)	94.4 ± 1.47	79.1 ± 1.20
(3 h)	90.9 ± 1.23	84.5 ± 1.25
(6 h)	58.6 ± 0.73	87.6 ± 1.33
(12 h)	53.1 ± 0.63	88.0 ± 1.28
(24 h)	50.0 ± 0.46	88.8 ± 1.46
Lavender (0 h)	96.0 ± 1.23	50.8 ± 0.97
(3 h)	82.8 ± 0.97	50.5 ± 0.89
(6 h)	49.7 ± 0.55	38.8 ± 0.76
(12 h)	42.5 ± 0.48	38.8 ± 0.75
(24 h)	33.3 ± 0.39	14.0 ± 0.34
Blueberry (0 h)	92.9 ± 1.06	74.0 ± 1.21
(3 h)	74.6 ± 0.83	70.6 ± 1.17
(6 h)	63.3 ± 0.76	67.7 ± 0.93
(12 h)	50.5 ± 0.72	58.3 ± 0.86
(24 h)	35.4 ± 0.51	58.4 ± 0.78
Litchi (0 h)	87.9 ± 1.15	95.4 ± 1.68
(3 h)	84.9 ± 1.00	95.5 ± 1.54
(6 h)	75.2 ± 0.89	97.2 ± 1.76
(12 h)	32.9 ± 0.52	97.6 ± 1.62
(24 h)	0	98.3 ± 1.80
Coffee (0 h)	80.8 ± 0.96	90.3 ± 1.82
(3 h)	79.7 ± 0.91	90.7 ± 1.77
(6 h)	69.2 ± 0.87	87.6 ± 1.49
(12 h)	39.1 ± 0.48	84.1 ± 1.35
(24 h)	0	52.7 ± 0.58

maximum increase of HMF content on Polish rape honey by heating at 80 °C for 15 min. It is not known the relation between the functionalities such as anti-oxidative activity, scavenging activities against ROS, and ACE and hyaluronidase inhibitory activities and the formation of HMF in honey species to our knowledge. Further investigation is planned to elucidate a primary factor to contribute to these functional properties.

Conclusion

The chemical parameters and the functionalities of six monofloral honeys with different botanical and geographical were investigated. Total vitamins B₁, B₂, and C and the protein contents of most honeys were distinguishing high

compared with general honey. Honeys not only were rich in a variety of functional components contained flavonoids but also had strong anti-oxidant activities, scavenging activities against ROS, and anti-hypertensive and anti-allergic activities. Honeys were heated at 100 °C for 24 h. The browning intensity on honeys during heating process was affected by botanical origins of honeys. The functional properties of caramelization and MR products derived from honeys during heating were evaluated. The browning of honeys progressed regardless of honey species. Anti-oxidant activities and scavenging activities against superoxide and DPPH radicals of products drastically increased, but ACE and hyaluronidase activities gradually decreased with passage of heating time. It concluded that the products, mainly melanoidins, produced simultaneously to browning

process in caramelization and MR contributed to the expression of its useful function.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interests.

Human and animal rights The authors declare that this article does not contain any studies with human or animal subjects.

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