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Optimization of Roasting Temperatures on Acrylamide and Melanoidins Contents and Antioxidant Properties of Roasted Broken-Rice Infusion

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Abstract

The price of broken rice is quite low. A method to add value to this raw material of broken rice is to develop broken rice as a new product such as herbal infusion. Herbal infusion is currently the trend of functional foods and experiencing growth in the food market. Roasted broken-rice infusion (RBRI) is a source of large amounts of maillard reaction products (MRPs) relative to strong antioxidant properties. In this study, we aimed to study the effect of various roasting conditions, i.e. 100, 150, and 200°C for 20 min on the melanoidins, acrylamide (as a carcinogen), copper chelating activity, 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity, ferric reducing antioxidant power (FRAP), and ABTS radical scavenging activity of RBRI as well as the study of relationship among their parameters. The higher roasting temperatures affected the darker color in RBRI. Moreover, the highest levels of melanoidins, copper chelating activity, 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity, ferric reducing antioxidant power (FRAP), and ABTS radical scavenging activity of RBRI were obtained from the roasting rice condition at 200°C for 20 min; whereas, the estimated acrylamide content was a moderate level (120 μg kg^{-1}). Obviously, for the relationship analysis, the L*, a*, b*, melanoidins, and acrylamide presented in RBRI could be indices for the antioxidant activities. Therefore, this roasting condition was suitable for RBRI development with the high levels of melanoidins and antioxidant activities.

Introduction

Tea is a popular beverage that is widely consumed around the world, particularly in Asian and European countries (Grigg, 2002). Tea drinking has increased around the world as reported by the International Tea Committee (Dufrene, 2012). Prior research has indicated that tea drinking reduces the risks of cancer, cardiovascular disease, hyperlipidemia, diabetes, and obesity (Tanabe et al., 2008). Tea contains certain properties that avoid

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the risks such as the polyphenolic compounds (catechins and epicatechins), theaflavins, flavonol glycosides, L-theanine, caffeine, theobromine, and volatile organic substances. Hence, tea consumption promotes human health (Khan & Mukhtar, 2013).

Generally, the price of broken rice is quite low about 8-10 bath kg^{-1} (Kwak, 2010). To add value of broken rice, it should be developed as a new product such as herbal infusion. Tea is usually prepared from the cured or fresh leaves of *Camellia sinensis*. The herbal infusion prepared from cereals and/or their by-products is more interesting due to the current popular trend and previous research that has suggested their strong antioxidant properties.

Echavarria et al. (2013) suggested that the antioxidant activities were obtained from the acrylamide formation from asparagine-fructose (ASN-FRU) and/or asparagine-glucose (ASN-GLC) systems, which were found in the roasted rice products. Since the compositions of broken rice include not only histidine, threonine, valine, methionine, lysine, isoleucine, leucine, asparagine, and phenylalanine but also starch (Bekedam et al. 2008). For example, Kwak (2010) noted that the brown rice tea prepared from roasting temperature at 170°C for 10 min increased the highest amounts of γ-aminobutyric acid (GABA) content and Maillard reaction products (MRPs) (high antioxidant properties); whereas, the estimated reducing sugars, total soluble solid and total polyphenol depended on the roasting temperatures and times (Tian et al., 2020).

MRPs are usually produced in many kinds of foods during thermal processing via the reducing sugars interacting with available amino acids. This has an effect on the important food properties such as color, flavor, and stability (Tehrani et al., 2002). The Maillard reaction affects not only flavor and color, but also positive human health (Tamanna & Mahmood, 2015).

Melanoidins are one of the MRPs, which are present in general carbohydrate-protein diets and are of interest due to improving the various health promotion activities, e.g. antioxidant, antimicrobial, anti-inflammatory, and antihypertensive activities (Iriondo-dehond et al., 2019).The MRPs are classified into two main groups as follows: (1) the low molecular weight compounds (MW < 1000); (2) the macromolecules, also known as melanoidins (Tehrani et al., 2002). They are found in various kinds of food (e.g. coffee, polished rice, cocoa, bread, malt, and honey), and have a high molecular weight of up to 300 kDa and have more complex molecular structures (Gniechwitz et al., 2008). Besides, acrylamide (one of the MRPs) or acrylic acid amide is a chemical contaminant that is formed during the technological processes of baking, frying, and grilling for certain foods at temperatures > 120°C with low humidity conditions (Soares et al., 2015). It is a contaminant and categorized as a potential carcinogenic (class 2A) by the International Agency of Research on Cancer (IARC) (Soares et al., 2015). The latest regulation from the European Commission (EU) 2017/2158 regulates the presence of acrylamide content in foods (Iriondo-dehond et al., 2019). Although no real legal limit is defined, this regulation introduced benchmark levels with the aim to invite the food industries to implement strategies for reducing acrylamide levels in foods (Tian et al., 2020). Moreover, to assist in the control of acrylamide levels, Food Drink Europe has developed a toolbox indicating possible strategies applicable at different stages of processing of different food categories (Pedreschi et al., 2014). Lasekan and Abbas (2010) further reported that the amount of acrylamide in the food products were as follows: ~450 μg kg^{-1} for roasted coffee;~900 μg kg^{-1} for instant coffee; 272-570 μg kg^{-1} for fried potatoes; 75-1,044 μg kg-1 for bakery products; ≤149 μg kg^{-1} for breakfast cereals; ≤121 μg kg^{-1} for dried food products (Pedreschi et al., 2014).

Therefore, the aim of this research was to estimate how different roasting conditions such as 100, 150, and 200°C for 20 min will affect the acrylamide and melanoidins contents and antixoidant activities in roasted broken-rice infusion (RBRI). Their antioxidant properties were determined by the differences among *in vitro* methods such as copper chelating activity, 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity, ferric reducing antioxidant power (FRAP), copper chelating activity, and ABTS radical scavenging activity.

Materials and methods

1. Chemicals

Carrez I, Carrez II, acrylamide, $^{13}C_3$-acrylamide, 2, 2-Diphenyl-1-picrylhydrazyl (DPPH), 2, 2′-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt, 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ), $FeCl_3.6H_2O$, and copper(II) sulfate were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA).

2. Preparation of roasted broken-rice infusion (RBRI)

Broken rice (*Oryza sativa* L.) was obtained from a local market in Chainat, Thailand. The seeds of broken Thai jasmine rice were separated into 4 treatments and 300 g for each teatment. The seeds were roasted by an hot air oven using the roasting temperatures as follows: 100, 150, and 200°C for 20 min except the control. Then cooled at ambient temperature (32±2°C), the roasted broken-rice infusion (RBRI) samples were obtained and kept in PET/Al/LLDPE (10x15 cm) bags at ambient temperature (32±2°C) prior to the analysis.

3. Color analysis

The sample's color was evaluated with a colorimeter (Minolta CR-300, Minolta Co Ltd., Japan). The colorimeter was calibrated using a standard white plate. Minolta L indicates brightness, (0 = black, 100 = white), a redness (+value = red, -value = green), and b yellowness (+value = yellow, -value = blue). Five readings indicated the surface of the samples for color measurement. An average of five readings for L*, a*, and b* values was reported.

4. Melanoidins assay

Prior to the determination of melanoidins in the RBRI samples, a standard calibration curve of melanoidins was measured at the wavelength at 420 nm (Del Castillo et al., 2002). Because the molecular structure of melanoidins has never been determined yet, hence a melanoidin standard is not available. Therefore, the standard calibration curve was generated using the RBRI extract as a source of melanoidins. A stock solution was obtained from a ratio of the RBRI extract to distilled water, 2:1. This stock solution was diluted sequentially 5 times. For every dilution, the content of melanoidins in each dilution was evaluated using the Lambert-Beer modified formula:

$$C = \frac{A}{ba}$$

Where C = melanoidins content
A = the absorbance of extract solution
b = length of the spectrophotomer's cell (cm)
a = the specific extraction coefficient ($L\ g^{-1}cm^{-1}$)

The value for "a" was 1.1289 $L\ g^{-1}\ cm^{-1}$. The standard calibration curve was generated by the absorbance values as a function of the melanoidins content. For each sample, a 1:9 dilution was measured. Melanoidins were spectrophotometrically evaluated at 420 nm. The melanoidins content was expressed as 100 g^{-1} sample.

5. Acrylamide assay

Acrylamide was measured using a slightly modified method of Rufián-Henares et al. (2007). 450 mg of the RBRI samples was transferred to 5 mL milli-Q water. Afterwards, 100 µL of 10 mg L^{-1} [$^{13}C_3$]-labelled acrylamide methanol solution was loaded into the sample suspension and the mixture was vortexed for 1 min. Then, 750 µL Carrez I and 750 mL Carrez II were loaded into the mixture and were mixed using a vortex for 10 s. Then the mixture was left at 35°C for 10 min and was then centrifuged at 4°C for 15 min at 2400 *g*. The supernatants were individually separated into the microtubes and were frozen. Prior to acrylamide analysis, the supernatants were thawed and centrifuged at 10000 *g* at room temperature for 10 min. 1 mL of the supernatants was clarified on a pre-conditioned Oasis HLB cartridge (Waters, Milford, USA). The first seven drops were removed out and the rest was transferred into the glass vials for further LC-MS analysis. LC-MS was performed on an Agilent 1100 liquid chromatograph coupled to an Agilent Quadrupole MS detector (Agilent Technologies, Palo Alto, USA). The separation was carried out on an Inertsil ODS-3V analytical column (250 x 4.6 mm, 5 µm; GLC Sciences, Tokyo, Japan) at 32°C with isocratic elution. The injection volume was 60 µL, the mobile phase at a ratio of water to formic acid, 99.8 to 0.2 and a flow rate was 0.6 mL min^{-1}. The results were obtained from a selected ion monitoring mode (SIM). Acrylamide and $^{13}C_3$-acrylamide were monitored at *m/z* 72.1 and 75.1, respectively. The calibration curve of acrylamide was carried out by the external standard solutions of acrylamide ranged between 1 µg L^{-1} and 100 µg L^{-1}. The results were expressed as µg kg^{-1} sample.

6. Preparation of RBRI extract for antioxidant activities

The sample extraction was performed using a slightly modified method of Budryn et al. (2009). The RBRI samples were extracted at a ratio of RBRI to distilled water, 1 : 100. Then, the mixture was homogenized by a sonicator (Branson sonicator 1510, NIST, UK) for 5 min and then centrifuged at 7900 *g* (IEC L31 Thermo electron) for 15 min. The supernatant were later filtered through Whatman No. 4 filter paper. The extraction was repeated twice using the method described above. Total supernatatants were then evaporated at 40°C to dryness. The RBRI extract powders were kept at -20 °C prior to

the analysis of antioxidant activities.

7. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

The DPPH assay was applied to evaluate DPPH free radical scavenging activity of the RBRI extract powders, using a slightly modified method of Kraboun et al. (2018). 100 µL of 0.02% DPPH solution in methanol was transferred into a 96-well plate containing 50 µL of the RBRI extract at 4 mg mL^{-1}. Then incubation for 30 min in the dark, the absorbance at 510 nm was determined using a microplate spectrophotometer (Epoch, BioTek, Winooski, VT, USA). The results were calculated as compared with the standard curve of ascorbic acid and expressed as an ascorbic acid equivalent value (mg AA mL^{-1}).

8. ABTS radical scavenging activity

The ability of the RBRI extract powders to scavenge ABTS radical cation was measured using the ABTS assay by a slightly modified method of Re et al. (1999). Briefly, ABTS solution contained the equal quantities of 7 mM ABTS stock solution and 2.6 mM potassium persulfate and incubation for 16 h at room temperature in the dark. Afterwards, ABTS solution was diluted to get an absorbance of 0.700±0.025 at 734 nm using a spectrophotometer. 5 µL of the RBRI extract at 4 mg mL^{-1} was transferred to react with 295 µL of ABTS solution for 6 min in the dark. The results were done at 734 nm using a microplate spectrophotometer (Epoch, BioTek) and converted to Trolox equivalents (TE) values as compared with the standard curve of Trolox. The standard curve of Trolox was plotted from 0 mM to 1 mM. Results are presented in mg $TEmL^{-1}$.

9. Ferric reducing antioxidant power (FRAP)

The FRAP was evaluated by reduction of Fe^{III+} to Fe^{II+} (Benzie & Strain, 1996). The stock solutions were separately prepared as follows: (1) 300 mM acetate buffer (pH 3.6); (2) 10 mM 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ) solution in 40 mM HCl; (3) 20 mM $FeCl_3 \bullet 6H_2O$ solution. The working solution was incubated at 37°C prior to use, which was composed of 25 mL of acetate buffer, 2.5 mL of TPTZ solution, and 2.5 mL of $FeCl_3 \bullet 6H_2O$ solution. 10 µL of the RBRI extract at 4 mg mL^{-1} was transfered to react with 100 µL of FRAP solution for 4 min in the dark. The colored product, ferrous tripyridyltriazine complex, was measued at 593 nm using a microplate spectrophotometer. Ferrous sulfate was used as a positive control.

10. Copper chelating activity

The copper (II) ion chelating activity was measured using a slightly modified method of Megías et al. (2009). 30 µL of the RBRI extract at 4 mg mL^{-1}, 290 µL of 50 mM sodium acetate buffer (pH 6.0), 10 µL of copper sulfate (2 mg mL^{-1}), and 4 mM PV were transferred into a 96-well plate. The absorbance was evaluated at 632 nm using a microplate spectrophotometer. Copper chelating activity was calculated by the following the equation:

$$\text{Copper chelating activity (\%)} = \frac{(1-\text{Asample})}{\text{Acontrol}} \times 100$$

11. Statistical analysis

The analysis was performed in triplicate. The values are expressed as mean±standard deviation (SD) as shown in the tables of this paper. Statistical analysis was performed using SPSS 16.0 (SPSS Inc., Chicago, IL, USA) for Windows. Data were analyzed by one-way ANOVA, followed by Duncan's multiple range tests. Differences were considered significant at $p \leq 0.05$. Pearson's correlation coefficient (r) was appiled to evaluate correlations between the parameters.

Results and discussion

1. Effect of roasting treatments on colors and melanoidins in RBRI

To explain the effect of the various roasting treatments for the preparation of roasted broken-rice infusion (RBRI) on the levels of colors and melanoidins, the levels of colors (L*, a*, and b*) and melanoidins in the RBRI samples are presented in Table 1. The higher roasting temperatures affected darker color in the RBRI samples. The highest L*, a* and b* values of RBRI from 200°C for 20 min were 30.27, 3.52, and 5.28, respectively. Obviously, these color values of RBRI samples increased with melanoidins content as shown in Table 1. The Maillard reaction is a chemical process without enzyme activity leading to a brown color presented in foods, which the factor significantly affecting this reaction is a high temperature (Garza et al., 1999). Hence, the development of darker color in RBRI resulted from the higher roasting temperatures (Fig. 1). Higher temperatures could accelerate the reaction between the reactive compositions presented in broken rice, including the amino acids (histidine, threonine, valine, methionine, lysine, isoleucine, leucine, asparagine and phenylalanine), and the reducing sugars (obtained from the high-temperature hydrolysis of starch) (Bekedam

et al., 2008) to generate the Maillard reaction products (MRPs), i.e. the polymerised proteins and brown pigment melanoidins (Del Castillo et al., 2002). Bekedam et al. (2008) suggested that higher temperatures can be related to the formation of heterocyclic derivatives in carbohydrate-protein materials. This is in agreement with Daramola (2015), who reported that the amount of melanoidins in *C. albidum* pulp through heating process at 30-70°C for 10-30 min ranged from 0.0364 to 3.6580 g 100 g^{-1}. Furthermore, the amount of coffee melanoidins was between 24.74 and 67.61 g 100 g^{-1} (Bekedam et al., 2008), which seemed to be related to higher roasting temperatures. Del Castillo et al. (2002) suggested that available water-soluble melanoidins corresponded with antioxidant and antimicrobial functions.

Table 1 Color characteristics and melanoindins content in RBRI from different roasting conditions between 100°C and 200°C for 20 min

Temperature (°C) and Time (min)	L*	a*	b*	Melanoidins (g 100g^{-1})
Control (without treatment)	80.75+4.50^{c}	0.54+0.02^{a}	0.99+0.14^{a}	n.d.
100°C and 20 min	55.11+3.85^{b}	0.75+0.06^{ab}	2.35+0.22^{b}	1.27+0.03^{a}
150°C and 20 min	45.58+4.66^{b}	1.23+0.11^{b}	3.85+0.14^{c}	5.98+0.13^{b}
200°C and 20 min	30.27+2.85^{a}	3.52+0.41^{c}	5.28+0.44^{d}	10.35+0.45^{c}

Remark: Different letters behind means within a column are significantly different ($p \leq 0.05$)
n.d.: not detected

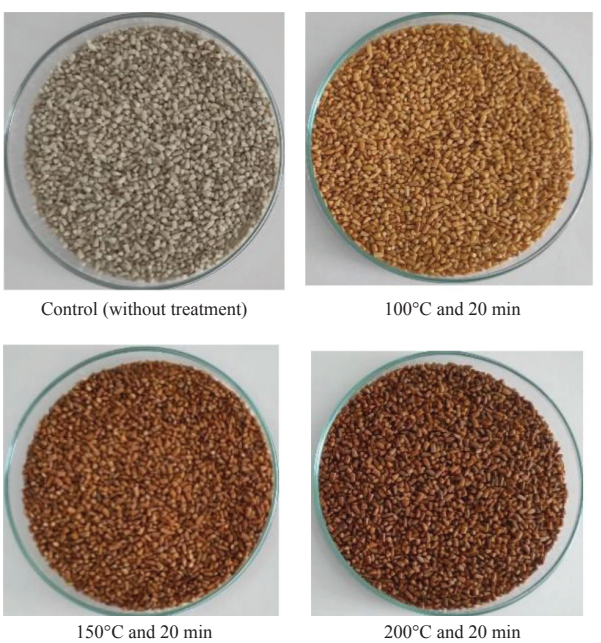

Fig. 1 Appearance of RBRI from different roasting conditions between 100°C and 200°C for 20 min

2. Effect of roasting treatments on acrylamide content in RBRI

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) stated that acrylamide is harmful to human health (Michalak et al., 2017). Acrylamide is generated from the reaction between an α-hydroxycabonyl compound (e.g. the reducing sugars) and asparagine during the high-temperature cooking methods, e.g. frying, roasting, and baking (Gökmen et al., 2006). Acrylamide causes many kinds of cancer in animals when it is consumed at very high concentration (Bent et al., 2012).

As shown in Fig. 2, the acrylamide contents in RBRI ranged from 30 to 120 μg kg^{-1}, which were higher than those previously reported in the literature for rye bread (Fredriksson et al., 2004). The acrylamide content in RBRI prepared from 200°C for 20 min was 4-fold higher than that in the control (without treatment). This indicated that the higher amount of acrylamide in RBRI was dependent on roasting temperature. This may be a result from higher temperatures accelerating the acrylamide formation from the reaction between asparagine and the reducing sugars presented in rice (Claus et al., 2006). This is in agreement with Akkarachaneeyakorn et al. (2010), who reported that the increased acrylamide contents were related to the increased roasting times and temperatures. Lasekan & Abbas (2010) further suggested that the acrylamide formation in the heated carbohydrate-rich foods was 150-4,000 μg kg^{-1}, followed by 5-150 μg kg^{-1} for protein-rich foods (a moderate level of acrylamide), and lastly ≤ 5 μg kg^{-1} for unheated or boiled foods. Hence, the RBRI samples had a moderate level of estimated acrylamide content. Obviously, the formation of acrylamide content in RBRI was related to the melanoidins content (Table 1).

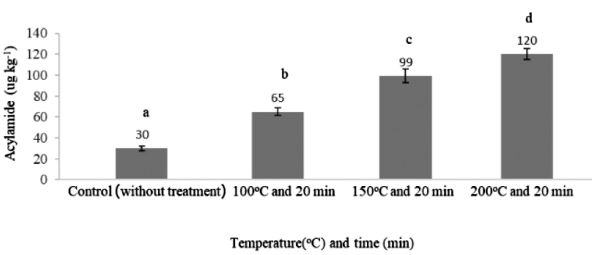

Fig. 2 Acrylamide content in RBRI obtained from roasting at different temperatures between 100°C and 200°C for 20 min

3. Effect of roasting treatments on antioxidant activities in RBRI

The DPPH radical scavenging activity, ABTS radical scavenging activity, ferric reducing capacity, and copper chelating activity in RBRI extracts at 4 mg mL^{-1} increased with the roasting temperatures and are shown in Fig. 3. The DPPH radical scavenging activities, ABTS radical scavenging activities, ferric reducing capacities, and copper chelating activities in RBRI extracts were in the range of 5.12-20.25 mg AA mL^{-1}, 6.5-12.32 mg TE mL^{-1}, 2.14-8.33 mmol Fe^{2+} mL^{-1}, and 14.25-96.65%, respectively. All antioxidant activities in RBRI extracts of RBRI from 200°C for 20 min were more than 2 times higher than those from the control (without treatment) (Fig. 3A-D). This is in agreement with Yilmaz & Toledo (2005), who suggested that the Maillard reaction products (MRPs) in a histidine-glucose system at 120°C for 30 min enhanced peroxyl radical scavenging activity. Del Castillo et al. (2002) noted that higher temperatures of roasting process improved antioxidant capacities presented in a lot of the products, e.g. coffee, wheat germ, polished rice, hazelnuts, and sweet almonds. The antioxidant activities of RBRI were related to the amounts of melanoidins and acrylamide as shown in Table 1 and Fig. 2, respectively.

These resulted antioxidant activities might be obtained from the acrylamide formation from asparagine-fructose (ASN-FRU) and/or asparagine-glucose (ASN-GLC) systems, which were found in the roasted rice products (Echavarria et al., 2013), since the compositions of broken rice include not only histidine, threonine, valine, methionine, lysine, isoleucine, leucine, asparagine and phenylalanine but also starch (Bekedam et al. 2008). Wagner et al. (2002) also demonstrated that melanoidins, as a high molecular weight (HMW) product were mostly responsible for antioxidant activities. Oracz & Zyzelewicz (2019) confirmed that the HMW melanoidins formation during heating process period indicated stronger antioxidant activities. Echavarria et al. (2013) revealed that melanoidins and acrylamide had stronger antioxidant properties via FRAP, ABTS, DPPH, and oxygen radical absorbance capacity (ORAC) comparable to those of commonly used food antioxidants (Rufián-Henares et al., 2007). Furthermore, Rufián-Henares et al. (2007) indicated that the presence of some active compounds (comprising more than one active group OH or NH_2) such as phenolic compounds, quinones, and LMW melanoidins might be bound to the structure of HMW melanoidins through non-covalent bonds and improves their biological properties (Rufián-Henares et al., 2007).

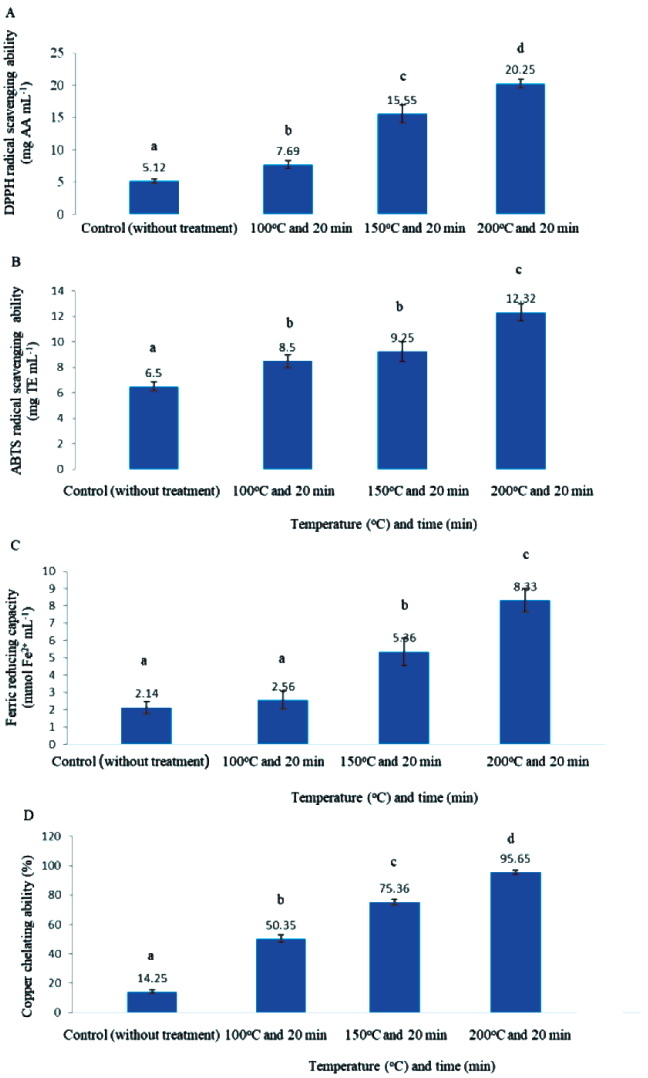

Fig. 3 DPPH radical scavenging activity (A), ABTS radical scavenging activity (B), ferric reducing capacity (C), copper chelating activity (D) of RBRI extracts at 4 mg mL^{-1} of RBRI prepared from 100°C to 200°C for 20 min

4. Relationship between color, melanoidins, acrylamide and antioxidant activities in RBRI

Pearson's correlation coefficients (r) among color, melanoidins, acrylamide, and antioxidant properties in RBRI are shown in Table 2. Positive correlations were found between melanoidins and antioxidant activities, between melanoidins and acrylamide, and between a* or b* and antioxidant activities. Whereas, negative correlations were found between L* and acrylamide, and

Table 2 Pearson's correlation among colors, melanoidins, acrylamide and antioxidant capacities of RBRI

	L*	a*	b*	Melanoidins	Acrylamide	DPPH	ABTS	FRAP	Copper chelating ability
L*	1								
a*	-0.958*	1							
b*	-0.955*	0.875	1						
Melanoidins	-0.878	0.798	0.852	1					
Acrylamide	-0.922*	0.888	0.998**	0.968**	1				
DPPH	-0.879	0.987**	0.774	0.785	0.985**	1			
ABTS	-0.754	0.989**	0.986**	0.928*	0.944*	0.898	1		
FRAP	-0.956*	0.945*	0.985**	0.988**	0.935*	0.899	0.965**	1	
Copper chelating ability	-0.911*	0.985**	0.896	0.978**	0.925*	0.874	0.945*	0.986**	1

Remark: * Correlation is significant at the 0.05 level (2-tailed)
** Correlation is significant at the 0.01 level (2-tailed)

between L* and FRAP or copper chelating ability (Table 2).

Therefore, L*, a*, b*, and melanoidins parameters could be the useful indicators for estimating the antioxidant activities and/or acrylamide (a carcinogen) in RBRI. This is in agreement with Pontis et al. (2014), who reported that a darker color in honey was shown to be associated with higher antioxidant activities and melanoidins level. Furthermore, Woffenden et al. (2001) reported that the higher antioxidant activities available in darker malts increased with the levels of reductones and melanoidins. In roasted coffee, the free radical scavenging activities occurred in the nonphenolic fraction were dependent on the accumulation of darker MRPs (Sacchetti et al., 2009).

Conclusion

The present study established the DPPH radical scavenging activity, ABTS radical scavenging activity, ferric reducing capacity, copper chelating activity, and color and acrylamide and melanoidins contents in RBRI depending on higher roasting temperatures. The highest DPPH radical scavenging activity, ABTS radical scavenging activity, ferric reducing capacity, and copper chelating activity obtained from the RBRI prepared from 200°C for 20 min were 20.25 mg AA mL^{-1}, 12.32 mg TE mL^{-1}, 8.33 mmol Fe^{2+} mL^{-1}, and 96.65%, respectively. To obtain the highest antioxidant potential, the suggested ratio of solid and water at 40°C should be 1:100 according to the preparation method of RBRI extract for the analysis of antioxidant activities. However, the acrylamide contents present between 30 and 120 µg kg^{-1} in the RBRI samples were observed and their amount might not be a problem for the consumption due to no real legal limit of acrylamide defined from the EU. In the relationship study, the L*, a*, b*, melanoidins, and acrylamide presented in RBRI could be the indicators for the antioxidant activities.

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