Antioxidant, anti-α-glucosidase and anti-glycation activities of coffee brew from Robusta coffee beans roasted at different levels

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Abstract

The aim of the present work was to evaluate the bioactive compound compositions of Robusta coffee brew and the antioxidant, anti-α-glucosidase and anti-glycation activities of these compounds, which are properties important for diabetes management. Robusta coffee beans were roasted at the first and second crack levels, followed by grinding and brewing. The DPPH activities of 5 geq/100 mL coffee brew of the first and second crack coffee beans were 1,694 µmol/L and 1,702 µmol/L, respectively, whereas the H-ORAC activities were 67,551 µmol/L and 51,964 µmol/L, respectively. The coffee brew from first crack coffee beans (12.5 geq/100 mL) inhibited the α-glucosidase activity up to 69% as compared to 60% by the brew from the second crack coffee beans. The IC$_{50}$ values of the samples as anti-glycation agents were approximately 0.5 geq/100 mL and 0.6 geq/100 mL in the brews from the first and second crack coffee beans, respectively. Using NMR and LC-MS approaches, the first crack coffee brew was found to contain abundant phenolic acids, which are likely responsible for the antioxidant, anti-α-glucosidase and anti-glycation activities. Interestingly, the bioactive compounds in the second crack sample responsible for similar activities were neither phenolic acids nor melanoids. Further study is therefore needed to evaluate the effectiveness of both Robusta coffee brews for the management of diabetes.

Keywords

Antioxidant, Anti-α-glucosidase, Anti-glycation, Roasting, Robusta coffee

Article history

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Introduction

Coffee is one of the most popular beverages in the world, with an annual consumption averaging 150 million 60 kg bags worldwide (ICO, 2016). Between the two most important traded coffee beans (Arabica and Robusta), Robusta coffee (Coffea canephora) comprises approximately 40% of the world coffee market (ICO, 2014). This figure also represents the level of Robusta coffee consumption as compared to Arabica (Coffea arabica) consumption.

Robusta coffee produces a coffee brew with a very strong and persistent bitter taste, and thus, blending the two species is common in order to have balanced sensory profile. A bitter taste is perceived in beverages containing high concentration of bioactive compounds, especially compounds with phenolic groups (Hečimović et al., 2011). Previous studies reported that bioactive compounds in Robusta coffee brew exhibit antioxidant activity that may be involved in the prevention of non-communicable diseases (Ludwig et al., 2012; Vignoli et al., 2014). This property is also supported by epidemiological studies that show coffee brew consumption prevents type 2 diabetes mellitus (T2DM) (Zhang et al., 2011; Sarriá et al., 2016). In addition, coffee brew may have other functional activities related to T2DM, such as anti-α-glucosidase and anti-glycation activity. These three activities have different roles pertaining to the management of T2DM. Antioxidant compounds have
significant roles from the early stage of the disease since they prevent oxidative stress, which triggers pancreatic injury that leads to the development of T2DM (Fernandez-Gomez et al., 2016). Antioxidant also acts as a radical scavenger for intermediate Maillard reaction products, which usually increase in the blood of T2DM patients (Sadowska-Bartosz and Bartosz, 2015). An anti-α-glucosidase agent is very useful to suppress carbohydrate digestion in the brush-border surface membrane of intestinal cells, which is a promising approach for the management of non-insulin-dependent DM (Zhang et al. 2016). This activity is also beneficial for the control of blood glucose levels in healthy people. Anti-glycation agents prevent the formation of advanced glycation end products (AGEs), wherein AGEs are responsible for diabetes mellitus complications such as vascular syndrome (Şahpaz and Kılınç, 2016).

Roasting is one of the most important processes that affect coffee brew properties. During the initial roasting process, drying occurs, and water evaporates from the coffee beans. Then, the water content reduces from 12% to 2% (Fadai et al., 2017). Evaporation of water and organic compounds produces gas, which creates an internal pressure in the coffee bean matrices, thus causing expansion. The higher internal pressure will trigger cellulose structure damage in the coffee bean cell wall (pyrolysis). This damage causes cracking with a special popping sound, namely, the first (1st) crack (Gloess et al., 2014). Further roasting will create a second sound similar to a snapping sound, namely, the second (2nd) crack (Gloess et al., 2014). The first and second cracks are two critical points for the development of the colour, flavour and aroma of coffee beans (Fadai et al., 2017).

Despite this fact, to our knowledge, the association between critical roasting levels, bioactive compound composition and antioxidant, anti-α-glucosidase, and anti-glycation activities of coffee brews is unknown, especially for Robusta coffee. Therefore, the present work was designed to evaluate the effect of two critical roasting levels (i.e., first and second) on the bioactive compound composition and antioxidant, anti-α-glucosidase and anti-glycation activities of coffee brews. The present work will be useful for predicting the potential use of the Robusta coffee brew to prevent or manage T2DM.

Materials and methods

Sample preparation

Green Robusta coffee beans from the EK1 type were procured from PT Lintang Visikusuma, Jakarta, Indonesia. Green beans (1 kg) were roasted in a 5 kg gas powered roaster machine (TOPER TKMX5, Turkey) with two roasting levels (first crack and second crack). The inlet temperature was adjusted to 200°C. The roasting process was stopped after the first or second crack, depending on the desired roasting level. The profile of the roasting process is presented in Table 1. Then, the roasted beans were ground. The ground coffee (5 g) was brewed by pouring 100 mL hot water (5 geq/100 mL), boiling for 1 min, cooling in an ice bath for 2 min, and filtering with filter paper. The filtrate was used for further analysis.

Chemicals

All chemicals used in the present work were of analytical grade.

Evaluation of antioxidant activity (AA) by DPPH method

The DPPH assay protocol developed by Suda et al. (2005) was followed to measure the antioxidant activity of the sample based on the colour intensity reduction of radical DPPH. A series of sample and standard (Trolox) dilutions were used for the DPPH value calculation. The DPPH value was obtained by dividing the sample curve slope by the Trolox curve slope, and it was stated as the Trolox equivalent (µmol TE/L).

Evaluation of AA by hydrophilic oxygen radical absorbance capacity (H-ORAC) method

The antioxidant activity of the coffee sample
was also determined using the H-ORAC value, a method validated by Watanabe et al. (2012). This method is used to measure the scavenging capacity of hydrophilic compound solutions against peroxyl radicals induced by 2,2′-azobis(2-aminopropane) dihydrochloride (AAPH). In this method, the effect of an antioxidant on the radical fluorescence intensity was observed using a fluorescence probe over a time course. The area under the fluorescein decay curve for the sample was compared to the blank to evaluate the samples’ antioxidant activity. The antioxidant activity was stated as the Trolox equivalent (µmolTE/L).

Anti-glycation assay

Anti-glycation activity was analysed using the procedure described by Lavelli et al. (2011) with modifications. Briefly, 50 µL sample (2.5, 5, 7.5, and 10 mg eq/mL) and aminoguanidine sulphate hydrate as a positive control (2.5, 5, 7.5, and 10 mg/mL) were placed in a 24-well plate. This was followed by the addition of 900 µL 0.1 M phosphate buffer (sodium phosphate buffer pH 7.4 treated by chelex resin), 300 µL BSA solution (50 mg/mL in water), and 300 µL fructose solution (0.22 g/mL in water). The control sample reaction was also determined by replacing the sample or the positive control with water. The sample mixtures were incubated at 37°C for 72 h. The BSA glycation induced by fructose was monitored using a luminescent spectrophotometer (Tecan Infinite® 200, Switzerland) every 24 h at an excitation/emission wavelength pair λ = 370/440. The inhibition of BSA glycation by the sample was stated as the IC₅₀ (the concentration for 50% inhibition). The % inhibition = 100 × (fluorescence intensity of the sample after incubation – intensity before incubation – intensity of the control sample) / intensity of the control sample.

Anti-α-glucosidase assay

The anti-α-glucosidase activity was determined by the measurement of the samples’ inhibition of the activity of the enzyme anti-α-glucosidase, as previously described by Kim (2015) with some modifications. A crude extract of the α-glucosidase enzyme was obtained by preparing a suspension of 100 mg rat intestinal acetone powder in 1 mL 0.1 M sodium phosphate buffer (pH 7.0). The extract was separated by centrifugation at 10,000 g for 45 min using a fixed angle rotor at 45° at 4°C in a Himac centrifuge unit (Himac CF16RX; Hitachi Koki Co., Ltd., Tokyo, Japan). Sample solutions were prepared by mixing samples (25 µL) with 0.1 M phosphate buffer (100 µL), 0.25 M maltose in phosphate buffer (100 µL), and 10 mg/mL crude enzyme solution (25 µL) in a deep well plate. The control sample and the positive control were prepared by replacing the sample with DMSO and acarbose, respectively. The plate was incubated at 37°C for 40 min to enable glucose production by the enzymatic hydrolysis of maltose. The hydrolysis was stopped by adding 250 µL 0.2 M Na₂CO₃. The sample solution (16 µL) was placed in a 96-well plate, mixed with glucose colour reagent (240 µL), incubated for 5 min in a micro thermosthaker (PST-60HL-4; Biosoan, Riga, Latvia), and analysed with a microplate reader at 505 nm. A glucose calibration curve was used to quantify the glucose concentration in the sample mixture. The inhibition of the sample or the positive control regarding glucose production was calculated as:

\[ \text{IA} (\%) = 100 - \left( \frac{(A-B)}{(C-D)} \right) \times 100 \]  
(Eq. 1)

where A = sample producing glucose, B = blank sample producing glucose, C = control sample, and D = blank sample for control producing glucose.

NMR analysis

Proton nuclear magnetic resonance (NMR) spectra of the coffee brew samples were recorded on a JEOL ECX 500 (500 MHz) (JEOL USA, Inc.). Deuterium oxide (D₂O) (purity ≥ 99.9%) was used as the solvent. Tetramethylsilane (TMS) was used as the internal standard for calibrating ¹H chemical shifts.

HPLC analysis

Phenolic acids in the coffee brew were analysed using a Shimadzu HPLC system (Shimadzu Corporation, Kyoto, Japan). This device was coupled with a DAD detector. The coffee sample was filtered through a 0.45-µm filter membrane (Sartorius; Germany). Then, the coffee sample (10 µL) was eluted at 40°C in a Cosmosil 5C18-MS-II column, 4.6 × 150 mm, 5 µm (Nacalai Tesque, Inc., Kyoto, Japan). The mobile phase (A: formic acid 0.05% and B: HPLC grade MeOH) was passed through the column at 1 mL/min and in gradient mode: 0 min (95% A and 5% B), 0 - 30 min (10% A and 90% B), 30 - 35 min (10% A and 90% B), 35 - 40 min (95% A and 5% B), and 40 - 50 min (95% A and 5% B). The components were detected in the range between 200 and 400 nm, and recorded at 320 nm. The concentration of all phenolic acids was calculated by a 5-point 5-O-caffeoylquinic acid standard curve with concentrations range from 62.5 to 1,000 mg/L.
LC-MS analysis

The analysis was conducted using a Shimadzu LC-MS system (LC-MS-2020; Shimadzu Corporation, Kyoto, Japan) coupled with a DAD detector. Sample (2 mL) was injected into a COSMOSIL 5C18-MS-II column (2.0 mm i.d. × 150 mm) (Nacalai Tesque, Inc., Kyoto, Japan) at 40°C. The mobile phase and the elution mode were the same as that in the method previously mentioned in the HPLC analysis. The absorbance was scanned across the range of 200-400 nm, and recorded at 320 nm. The effluent from the LC system was further delivered to the MS system using a microprobe. Electrospray ionisation (ESI) for MS analysis was performed using the following conditions: m/z range (150–1,000); desolvation gas (N2) with drying and nebulizer flow rate 15 L/min and 1.5 L/min, respectively; scan speed (88 µ/s); detection mode (positive and negative ion mode); ESI voltage (+4.5 kV, -3.5 kV); capillary temperature (250°C); DL temperature (250°C); heat block temperature (200°C); and detector voltage (-1.1 kV, -1.0 kV).

Fractionation of coffee brews using Sep-Pak C18 open column cartridges

Fractionation was performed using an ODS Sep-Pak C18 open column cartridges (Waters, New York, USA). The coffee brew (2 mL) was sequentially eluted with 10 mL each of five different solvents: water, 20% MeOH, 40% MeOH, MeOH, and EtOH. Each fraction was collected, dried, diluted (in 2 mL water for water and 20% MeOH fractions or in 2 mL MeOH for 40% MeOH, MeOH, and EtOH fractions), and used for the assays.

Fractionation using ultrafiltration

The coffee brew fractions separated on the ODS Sep-Pak were further separated using an Amicon® ultra-0.5 centrifugal filter device with regenerated cellulose, 10 kDa (Millipore; Ireland Ltd, Tullagreen, Carrigtwohill Co. Corfirl Ireland Rep), according to Bartel et al. (2015) with some modification. The coffee fraction (0.5 mL) was centrifuged at 10,000 g for 22 min at room temperature (20°C) using a Himac centrifuge unit (Himac CF16RX; Hitachi Koki Co., Ltd., Tokyo, Japan). The filtrate containing low molecular weight compounds (LMW) (MW < 10 kDa) was evaporated, diluted in 0.5 mL water, and used for the assays. The retentate containing high molecular weight compounds (HMW) (MW > 10 kDa) was recovered by inverting the Amicon® device using centrifugation at 1,000 g for 10 min. The HMW samples were evaporated, diluted in 0.5 mL water, and used for the H-ORAC and anti-α-glucosidase assays.

Statistical analysis

Analyses were performed using a t-test (p < 0.05) to compare the activities of coffee brews from two different roasting levels. Activities of the fractions separated from the second crack sample were analysed using one-way analysis of variance (ANOVA) followed by Duncan’s test for multiple comparisons.

Results and discussion

Functional properties of coffee brews from the first and second crack roasted beans

The functional properties of the coffee brews from Robusta coffee beans roasted at two levels (first and second crack levels) are presented in Table 2. The observed functional properties were antioxidant activity by DPPH and H-ORAC values, anti-α-glucosidase and anti-glycation activities.
Based on the DPPH value, there was no significant difference between the two samples if the value was calculated based on the value per mL sample solution. However, if the value was calculated based on the soluble solid in the coffee brew, the second crack sample had a slightly lower DPPH value than the first crack sample ($p < 0.05$).

The H-ORAC value of the second crack sample was lower than the H-ORAC value of the first crack sample ($p < 0.05$), at 77% of the H-ORAC value of the first crack sample. Even though the calculation of the H-ORAC value was based on only the value per litre sample solution, it was able to differentiate the samples’ activity. Since the compounds extracted into the coffee brew samples were mostly polar compounds, the H-ORAC method was likely more sensitive for determining their antioxidant activity than the DPPH method (Watanabe et al., 2012; Grajeda-Iglesias et al., 2016).

The $\alpha$-glucosidase inhibition of the second crack sample was lower than that of the first crack sample ($p < 0.05$). The anti-glycation activity of the samples also showed similar results; whereby the anti-glycation activity of the second crack sample at all incubation durations was 0.8 times weaker than that of the first crack sample ($p < 0.05$).

Overall, it was found that the antioxidant, anti-$\alpha$-glucosidase, and anti-glycation activities of the second crack sample were slightly lower than those of the first crack sample. The changes found in the functional properties of the sample with different roasting levels were mainly due to alteration of the bioactive composition during roasting. Roasting significantly affects the chemical composition of coffee beans (Vignoli et al., 2011; Wang and Lim, 2017). Along with excessive alteration of physical properties, the alteration of the bioactive composition of coffee beans also occurs with such roasting, which also determines the functionality (Hečimović et al., 2011; Vignoli et al., 2014).

**Bioactive compound composition of the coffee brews from the first and second crack roasted beans**

The question about the alteration of the bioactive compound composition of coffee beans roasted at different levels was further evaluated. The $^1$H NMR spectra of the coffee brews from the first and second crack roasted beans are presented in Figures 1. The samples had different $^1$H NMR spectra: the first crack samples’ spectrum had a higher $^1$H signal than that of the second crack samples. The $^1$H signals in chemical shifts at $\delta$ 6 - 7 ppm, at approximately 5 ppm and approximately 2 ppm in the first crack sample (Figure 1A), were much more intense than the signals in the second crack sample (Figure 1B). The $^1$H signals in those chemicals shifts belong to the $^1$H that are bonded to carbon atoms in chlorogenic acid structures. Chlorogenic acid isomers are frequently reported to be native phenolic acids in coffee beans (Fujioka and Shibamoto, 2008; Mills et al., 2013; Jeszka-Skowron et al., 2015; 2016; Zanin et al., 2016).

The $^1$H signals in the second crack sample with the same chemical shift positions as mentioned above ($\delta$ 6 - 7, approximately 5 ppm and approximately 2 ppm) almost disappeared. This means that the chlorogenic acid isomers in the second crack sample had been greatly degraded. The most notable $^1$H signals in the second crack sample at $\delta$ 3 - 3.5 and 7.8 ppm belonged to $^1$H bonded to carbon atoms in caffeine (Okuom et al., 2013). This result confirms the stability of caffeine during roasting, as stated by previous studies (Hečimović et al., 2011; Rodrigues and Bragagnolo, 2013).

Although many bioactive compounds in the second crack sample were degraded, it was found that the functional properties of the sample were still comparable to those of the first crack sample. Previous studies suggest that caffeine does not strongly protect against free radicals (Anesini et al., 2012; Peres et al., 2013; Xu et al., 2015), and caffeine...
may not be an important contributor to anti-glycation activity (Mesías et al., 2014). Therefore, the bioactive compounds responsible for the observed functional properties in the second crack coffee sample were likely different from the compounds observed in the first crack sample. However, these compounds in the second crack sample were not detected by NMR spectroscopy.

Further analysis of the bioactive compounds in the coffee brew samples was conducted using LC-MS and HPLC. The phenolic acid composition of the samples is presented in Table 3. There were at least 10 phenolic acids identified in the coffee brew from the first crack sample. Previous studies identified nine native phenolic acids in green coffee bean extract: 3-caffeoylquinic acid (3-CQA); 5-caffeoylquinic acid (5-CQA); 4-caffeoylquinic acid (4-CQA); 3-feruoylquinic acid (3-FQA); 5-O-feruoylquinic acid (5-FQA); 5-p-coumaroylquinic acid (5-pCoQA); 3,4-dicaffeoylquinic acid (3,4-diCQA); 3,5-dicaffeoylquinic acid (3,5-diCQA); and 4,5-dicaffeoylquinic acid (4,5-diCQA) (Fujioka and Shibamoto, 2008; Gawlik-Dziki et al., 2014). Mills et al. (2013) also found these phenolic acids in commercial coffee brews; only 3-FQA was absent in the samples.

The coffee brew from the second crack sample only contained four native phenolic acids (3 CQAs and 1 FQA) with less than 40% retention as compared to the first crack roasted beans. The excessive degradation of phenolic acid in the second crack sample was also represented by the number of small and unidentified peaks. The second crack sample had more small and unidentified peaks than the first crack sample. Phenolic acid, especially chlorogenic acid isomers, partly transform into their derivatives such as caffeoylshikimic acids and chlorogenic acid lactone (Jaiswal et al., 2014).

The bioactive compounds in the first crack sample responsible for antioxidant, anti-α-glucosidase, and anti-glycation activities are likely the phenolic acids. Previous reports stated that the coffee brew compounds responsible for AA are phenolics (Hečimović et al., 2011; Jeszka-Skowron et al., 2016; Moreira et al., 2017). The hydroxyl group from phenolic acid donates a hydrogen atom to free radicals (Brezová et al., 2009; Vignoli et al., 2011).

Previous studies have mentioned that phenolic acids in Ipomoea batatas leaves have anti-α-glucosidase activity (Zhang et al., 2016). This finding indicates that phenolic acids in coffee brew may also have an anti-α-glucosidase activity. Since phenolic acids are the major bioactive compounds in the coffee brew from the 1st crack sample, those compounds may also be responsible for the anti-glycation activity. Phenolic acids in the coffee brew, such as 3-CQA, 4-CQA, and 5-CQA, are polar compounds; therefore, they have very high accessibility (Świeca et al., 2017). In addition, phenolic acids are highly bioavailable in humans (Farah et al., 2008). Therefore, it is possible that phenolic acids in the first crack sample are effective vehicles for the prevention and/or management of T2DM through their antioxidant, anti-α-glucosidase and anti-glycation activities. However, an in vivo study should be conducted in order to have more representative data of the effectiveness of these compounds.

### Table 3. Phenolic compounds in the coffee brews from the first and second crack roasted beans.

<table>
<thead>
<tr>
<th>[M-H]-(m/z)</th>
<th>Compounds</th>
<th>Concentration in 1&lt;sup&gt;st&lt;/sup&gt; crack sample (mg/mL)</th>
<th>Concentration in 2&lt;sup&gt;nd&lt;/sup&gt; crack sample (mg/mL)</th>
<th>Reduction (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>353</td>
<td>CQA</td>
<td>0.51 ± 0.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.12 ± 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>73</td>
</tr>
<tr>
<td>353</td>
<td>5-CQA</td>
<td>1.27 ± 0.07&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.26 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>79</td>
</tr>
<tr>
<td>353</td>
<td>CQA</td>
<td>0.72 ± 0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.17 ± 0.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>75</td>
</tr>
<tr>
<td>367</td>
<td>FQA</td>
<td>0.21 ± 0.01</td>
<td>n.d.</td>
<td>100</td>
</tr>
<tr>
<td>367</td>
<td>FQA</td>
<td>0.25 ± 0.00</td>
<td>0.09 ± 0.00</td>
<td>64</td>
</tr>
<tr>
<td>333</td>
<td>pCoQA</td>
<td>0.02 ± 0.00</td>
<td>n.d.</td>
<td>100</td>
</tr>
<tr>
<td>515</td>
<td>diCQA</td>
<td>0.09 ± 0.00</td>
<td>n.d.</td>
<td>100</td>
</tr>
<tr>
<td>515</td>
<td>diCQAs</td>
<td>0.11 ± 0.01</td>
<td>n.d.</td>
<td>100</td>
</tr>
<tr>
<td>367</td>
<td>FQAs</td>
<td>0.08 ± 0.00</td>
<td>n.d.</td>
<td>100</td>
</tr>
<tr>
<td>515</td>
<td>diCQAs</td>
<td>0.01 ± 0.00</td>
<td>n.d.</td>
<td>100</td>
</tr>
<tr>
<td>Total identified phenolic</td>
<td>3.29 ± 0.08&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.60 ± 0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of small and unidentified peaks</td>
<td>10</td>
<td>20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soluble solid (mg/mL)</td>
<td>12.30 ± 0.22&lt;sup&gt;a&lt;/sup&gt;</td>
<td>15.50 ± 0.21&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data are means of three samples (n = 3) ± standard deviations. Means with different superscripts in the same row were significantly different according to Duncan’s test (p < 0.05). n.d. is not detected.
The simultaneous separation and assay of the second crack sample were conducted in order to have more information on their bioactive compounds. The antioxidant and anti-α-glucosidase activities of the fractions separated from the coffee brew prepared from the second crack sample are presented in Figure 2. Separation of coffee brew using an ODS Sep-Pak column resulted in five different fractions: water, 20% MeOH, 40% MeOH, MeOH, and EtOH. Meanwhile, further separation of coffee fractions using ultrafiltration resulted in two different subfractions: a subfraction containing low molecular weight compounds (LMW) with MW < 10 kDa and a high molecular weight compounds (HMW) with MW > 10 kDa.

Based on antioxidant activity determined by the H-ORAC method, the most active fraction was the 20% MeOH fraction, followed by the water, 40% MeOH, MeOH and EtOH fractions. Further separation using membrane ultrafiltration showed that the active subfractions were the LMW fraction with MWs < 10 kDa. A similar result was shown by the anti-α-glucosidase activity data, where the most active fractions were the 20% MeOH fraction and its LMW subfraction.

The bioactive compounds in the LMW subfraction should be phenolic acid and the LMW compounds that resulted from the Maillard reaction. Separation of melanoidins from the coffee brew sample used a membrane with 10-kDa cut off (Bartel et al., 2015) or 12 - 14 kDa (Vignoli et al., 2011; 2014). Since the phenolics in the sample were excessively degraded as shown in Table 3, it is likely that the important active compounds in the coffee brew from the second crack sample responsible for antioxidant and anti-α-glucosidase activities were neither phenolics nor melanoidins with molecular weights > 10 kDa. However, previous studies have also shown that melanoidins have attracted interest as a result of their potential health benefits, especially as an antioxidant (Liu and Kitts, 2011; Vignoli et al., 2011; Bartel et al., 2015). On the other hand, the LMW subfraction may also contain lipid compounds such as diterpenes, which have some biological activities including antioxidant activity (Liang and Kitts, 2014). The diterpene 16-O-methylcafestol (16-OMC) is found exclusively in Robusta coffee; thus, it serves as a reliable marker for distinguishing between Robusta and Arabica coffee (Speer and Kölling-Speer, 2006; Monakhova et al., 2015). The occurrence of 16-OMC in a lipophilic fraction of ground roasted Robusta coffee is clearly detected in NMR spectra (Monakhova et al., 2015; Defernez et al., 2017). However, it is not detected in NMR spectra of the aqueous extract of Robusta coffee due to its low solubility (Monakhova et al., 2015).

The compounds in the second crack sample may also show potential for T2DM prevention and management. Further studies are needed to identify bioactive compounds in the second crack sample and to validate their effects in vivo.

Conclusion

Roasting resulted in the alteration of the bioactive compound composition of Robusta coffee brew. Interestingly, the antioxidant, anti-α-glucosidase and anti-glycation activities of the Robusta coffee brew from the second crack coffee beans were somewhat comparable with the activities of the coffee brew prepared from the first crack sample, despite the difference in their bioactive compound compositions.
Almost all native phenolic acids were fully degraded in the brew of the second crack sample, which suggests that the formation of new compounds during roasting compensated for the loss of the biological activities of the phenolics. The compounds responsible for the antioxidant and anti-α-glucosidase activities in the second crack sample was neither phenolics nor melanoidins, and further study is needed to identify the active compounds in the second crack sample and to evaluate their effectiveness as bioactive compounds for T2DM management.

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