TITLE:

High antioxidant activity of coffee silverskin extracts obtained by the treatment of coffee silverskin with subcritical water.

AUTHOR(S):

Narita, Yusaku; Inouye, Kuniyo

CITATION:

Narita, Yusaku ...[et al]. High antioxidant activity of coffee silverskin extracts obtained by the treatment of coffee silverskin with subcritical water.. Food Chemistry 2012, 135(3): 943-949

ISSUE DATE:

2012-12

URL:

<http://hdl.handle.net/2433/160217>

RIGHT:

© 2012 Elsevier Ltd.; この論文は出版社版ではありません。引用の際には出版社版をご確認ご利用ください。; This is not the published version. Please cite only the published version.

High Antioxidant Activity of Coffee Silverskin Extracts Obtained by the Treatment of Coffee Silverskin with Subcritical Water

Yusaku Narita^{a,b} and Kuniyo Inouye^{a,}*

^a Division of Food Science and Biotechnology, Graduate School of Agriculture, Kyoto University,
Sakyo-ku, Kyoto 606-8502, Japan

^b R&D Center, UCC Ueshima Coffee Co., Ltd., 3-1-4 Zushi, Takatsuki-shi, Osaka 569-0036,
Japan

AUTHOR EMAIL ADDRESS: inouye@kais.kyoto-u.ac.jp

TITLE RUNNING HEAD: Antioxidant capacities of coffee silverskin subcritical water extracts.

CORRESPONDING AUTHOR FOOTNOTE:

*corresponding author. Tel.: +81-75-753-6266. Fax: +81-75-753-6265. E-mail address:
inouye@kais.kyoto-u.ac.jp. Postal address: Division of Food Science and Biotechnology,
Graduate School of Agriculture, Kyoto University, Sakyo-ku, Kyoto 606-8502, Japan.

^aKyoto University.

^bUCC Ueshima Coffee Co., Ltd..

18 Abstract

19 Coffee silverskin (CS) is a thin tegument of the outer layer of green coffee beans and a major
20 by-product of the roasting process to produce roasted coffee beans. CS extracts obtained by the
21 treatment of CS with subcritical water at 25–270 °C were investigated on their antioxidant
22 activity using hydrophilic oxygen radical absorption capacity (H-ORAC) and DPPH radical
23 scavenging capacity assays. The antioxidant activity increased with increasing the extraction
24 temperature and the highest activity was observed with the extracts obtained at 270 °C. The H-
25 ORAC and DPPH values of the extracts were 2629 ± 193 and 379 ± 36 $\mu\text{mol TE/g}$ of CS extract,
26 respectively. High correlation ($R = 0.999$) was observed between H-ORAC and DPPH values for
27 the CS extracts. High correlation of the antioxidant activity was also observed with protein and
28 phenolic contents in the extracts. The CS extracts could be useful as a good source of
29 antioxidative materials.

30

31 Keywords: Antioxidant; Biomass; Coffee; Coffee silverskin; Subcritical water

32

33 1. Introduction

34

35 Coffee is one of the most consumed popular beverages throughout the world. Coffee silverskin
36 (CS) is a thin tegument of the outer layer of green coffee beans and a major by-product of the
37 roasting process to produce roasted coffee beans. Most CS is disposed of as industrial waste
38 because the effective utilizing of CS has not yet been developed. Therefore CS can be regarded as
39 biomass which is expected to be utilized. Many investigators have reported the physiological
40 functions of green and roasted coffee beans, such as α -amylase inhibition (Narita & Inouye, 2009;
41 Narita & Inouye, 2011), tyrosinase inhibition (Iwai, Kishimoto, Kakino, Mochida, & Fujita,
42 2004), and antioxidant activity (Richelle, Tavazzi, & Offord, 2001), etc. On the other hand, the
43 physiological functions of CS reported until now are antioxidant activity (Borrelli, Esposito,
44 Napolitano, Ritieni, & Fogliano, 2004), prebiotic property (Borrelli et al., 2004), and
45 hyaluronidase inhibition (Furusawa, Narita, Iwai, Fukunaga, & Nakagiri, 2011), but the number
46 of the reports is much less than that for coffee bean.

47 The water maintained in the liquid state by pressurizing in the temperature ranging between
48 100 and 374 °C is called subcritical water. The specific inductive capacity or dielectric constant
49 of water remarkably lowered with increasing temperature (Miller & Hawthorne, 1998). The
50 specific inductive capacity of subcritical water in the temperature range of between 200 °C and
51 300 °C is comparable as polar organic solvents such as methanol and acetone. Moreover,
52 subcritical water has the characteristic which functions as acid or alkali catalyst, because the ionic
53 product of the subcritical water is higher than water under normal temperature and pressure. From
54 these features, research using subcritical water is advanced especially for treatment of food waste,
55 such as grape seeds, okara (Wakita et al., 2004), wheat bran (Kataoka, Wiboonsirikul, Kimura, &
56 Adachi, 2008), defatted rice bran (Wiboonsirikul, Kimura, Kadota, Morita, Tsuno, & Adachi,
57 2007a). It is expected to be environment-friendly to extract active ingredients, such as proteins
58 and carbohydrates, from food waste using subcritical water without using organic solvents and

59 other catalysts. Furthermore, it is excellent in safety not to use the substances harmful to human at
60 the extraction process.

61 It is well known that the oxidative stress must be a factor to cause various diseases, such as
62 cancer (Lambert & Yang, 2003), cardiovascular disease (Diaz, Frei, Vita, & Keaney, 1997), type
63 2 diabetes (Takayanagi, Inoguchi, & Ohnaka, 2011), Alzheimer's disease (Christen, 2000), and
64 Parkinson's disease (Lang & Lozano, 1998). Antioxidants exhibit important effects for human
65 health by reducing oxidative stress, and also are used to prevent food from discoloring and
66 changing flavor. Therefore, antioxidants have recently attracted attention against oxidative stress.
67 Antioxidants are divided roughly into natural and synthesized products. Consumers generally
68 prefer natural antioxidants to synthetic ones because of higher safety of the former than the latter.
69 It is well known that polyphenols such as chlorogenic acids richly contained in coffee (Iwai et al.,
70 2004), and catechins contained in tea (Gardner, McPhail, & Duthie, 1998), and ascorbic acid (Gil,
71 Tomas-Barberan, Hess-Pierce, & Kader, 2000) have strong antioxidant activity.

72 Recently, it has been reported that antioxidants are contained in the by-product of some food
73 such as defatted rice bran (Wiboonsirikul et al., 2007a) and black rice bran (Wiboonsirikul, Hata,
74 Tsuno, Kimura, & Adachi, 2007b). The higher the radical scavenging activity of defatted rice
75 bran extracts, the higher the temperature for extraction ranging from 50 to 250 °C (Wiboonsirikul
76 et al., 2007a). CS extracts that is obtained by the treatments with methanol and water have
77 antioxidant activity (Borrelli et al., 2004). However, the research on the effect of the extraction
78 temperature on the antioxidant activity of CS extracts has not yet been reported. It is expected
79 that the antioxidant activity of CS extracts would be improved by subcritical water treatments.

80 The purpose of the present study is to evaluate the antioxidant activity of CS extracts obtained
81 by the treatment with water at various temperatures and to investigate the correlation between the
82 antioxidant capacities and the amount of antioxidant components such as protein and total
83 phenolic compounds in the extracts.

84

2. Materials and methods

2.1. Materials and reagents

Coffee silverskin (CS) produced by roasting coffee beans (*Coffea arabica* cv. Brazil, *C. arabica* cv. Colombia, *C. canephora* var. *robusta* cv. Vietnam, and *C. canephora* var. *robusta* cv. Indonesia) was obtained from UCC Ueshima Coffee Co., Kobe, Japan. CS easily peels off roasted coffee beans in the roasting process of green coffee beans. The function to separate CS and roasted coffee beans is attached to most industrial coffee roasting machines. CS separated from roasted coffee beans in the iron pot of roasting machine is collected by aspiration of air to another container. Trolox (lot 648471) was purchased from Calbiochem (San Diego, CA, USA). Fluorescein sodium salt (lot 079K0141V) was from Sigma (St. Louis, MO, USA). 2,2'-Azobis(2-amidinopropane) dihydrochloride (AAPH, lot STN0525) was from Wako Pure Chemical (Osaka, Japan). 1,1-Diphenyl-2-picrylhydrazyl (DPPH, lot M9R6579), gallic acid (lot M9R9781), phenol reagent solution (Folin-Ciocalteu's reagent solution, lot L2286), chlorogenic acid hemihydrate (5-CQA, lot M8N8455), caffeine, anhydrous (lot M7T9075), 5-(hydroxymethyl)-2-furfural (5-HMF, lot M9M3597). Bovine serum albumin (BSA, lot M6P3104) and all other chemicals were of reagent grade and were from Nacalai Tesque (Kyoto, Japan).

2.2. Preparation of CS extracts by the treatments with water, 0.1 M HCl, and 0.1 M NaCl at 25 °C and 80 °C.

One gram of CS was added to 50 ml of distilled water, 0.1 M HCl or 0.1 M NaCl, and was constantly stirred with a magnetic stirrer at 25 °C or 80 °C for 1 h. The mixture was filtered firstly through Kiriyama No. 3 filter paper (Tokyo, Japan), and the filtrate was filtered secondly through

110 Kiriya No. 5C filter paper. The filtrate was concentrated with a rotary evaporator. The
111 concentrated sample was freeze-dried and stored at -20°C .

113 2.3. Subcritical water treatment of CS.

115 One gram of CS and 50 ml of distilled water were put in a reaction vessel of SUS-316 stainless
116 steel (Taiatsu Techno Co., Osaka, Japan). The vessel was set in a portable reactor model TPR-1
117 (TVS-N2 specification) (Taiatsu Techno Co.) and heated to a prescribed temperature (180, 210,
118 240, and 270°C) and held for 10 min at each temperature. The inner pressures of the vessel at 180,
119 210, 240, and 270°C were 1.0, 1.9, 3.2, and 5.3 MPa, respectively. It takes 17, 22, 31, and 42 min
120 to reach the temperatures of 180, 210, 240, and 270°C , respectively, from 25°C . Then the
121 mixture in the vessel was filtered firstly through Kiriya No. 3 filter paper, and the filtrate was
122 filtered secondly through Kiriya No. 5C filter paper. The filtrate was concentrated with a
123 rotary evaporator. The concentrated sample was freeze-dried and stored at -20°C .

125 2.4. HPLC analysis of caffeine, 5-CQA, and 5-HMF.

127 HPLC analysis of caffeine, 5-CQA, and 5-HMF in CS extracts was performed according to the
128 procedures previously reported (Narita & Inouye, 2011) with some modifications. The CS extract
129 solution (10 mg/ml) was applied to reversed-phase column chromatography in a preparative
130 HPLC 7400 system (GL Science, Tokyo, Japan) on an Inertsil ODS-3 [4.6 mm (inner diameter or
131 ID) $\times$ 15.0 cm] column (GL Science) at the column temperature of 35°C . The mobile phase was
132 composed of solvents A (50 mM acetic acid in H_2O) and B (50 mM acetic acid in acetonitrile),
133 and the gradient program was as follows: 0–30.0 min, 0–20% (v/v) of B; 30.0–45.0 min, 20–35%
134 (v/v) of B; 45.0–50.0 min, 35–80% (v/v) of B; 50.0–50.1 min, 80–5% (v/v) of B; and 50.1–60
135 min, 0% (v/v) of B. The injection volume of the CS extract solution was 10 μl and a flow-rate

136 was 1.0 ml/min. Caffeine, 5-CQA, and 5-HMF were detected by respective absorption at 270, 325,
137 and 284 nm with a photodiode array. Caffeine, 5-CQA, and 5-HMF in the CS extract were
138 identified by comparing the retention times and the UV spectra of the standard materials. The
139 detection limits of all quantitative analyses were 10 µg/ml.

140

141 2.5. *Determination of total sugar contents.*

142

143 Total sugar contents of CS extracts were determined by the phenol-sulfuric method (Dubois,
144 Gilles, Hamilton, Rebers, & Smith, 1956). The CS extract solution was prepared to the
145 concentration of 1.0 mg/ml using distilled water. Zero-point-five ml of 5% (w/w) phenol was
146 added to 0.5 ml of the CS extract solution of 1.0 mg/ml, followed by adding quickly 2.0 ml of
147 H₂SO₄, and the mixture was mixed well. The mixture was left for 40 min at 25 °C in a water bath.
148 The absorbance at 490 nm was measured using a spectrophotometer. The total sugar contents of
149 the CS extracts were determined using standard curves obtained with D-glucose.

150

151 2.6. *Determination of reducing sugar contents.*

152

153 Reducing sugar contents of CS extracts were determined by the 3,5-dinitrosalicylic acid (DNS)
154 method (Borel, Hostettler, & Deuel, 1952). The DNS reagent solution consisting of 10 g DNS,
155 0.5 g sodium sulfate, 2 g phenol, and 10 g sodium hydroxide in 1,000 ml distilled water was
156 prepared. The CS extract solutions of 1.0 mg/ml were prepared using distilled water. One ml of
157 the extract solution and 3 ml of the DNS reagent solution were mixed and heated in boiling water
158 for 5 min. The mixture was cooled to room temperature, followed by the addition of 21 ml of
159 distilled water. The absorbance at 550 nm was measured with a spectrophotometer. The reducing
160 sugar contents of the CS extracts were calculated using standard curves obtained with D-glucose.

161

162 2.7. *Determination of protein.*

163

164 Protein content of CS extracts was determined by the Lowry-Folin method (Lowly,
165 Rosebrough, Farr, & Randall, 1951). The Lowry's solution was prepared by mixing 50 ml of 5%
166 (w/v) Na_2CO_3 aqueous solution, 0.5 ml of 2% (w/v) sodium potassium tartrate aqueous solution,
167 and 0.5 ml of 1% (w/v) CuSO_4 aqueous solution. Zero-point-two ml of the CS extract solution
168 and 0.2 ml of 2 M NaOH were mixed and stood for 20 min at 25 °C. Then the mixture was added
169 to 2.0 ml of the Lowry's solution and stood for 20 min at 25 °C. Next, the Folin-reagent was
170 added to the mixture and left for 20 min at 25 °C. The absorbance at 750 nm was measured using
171 a spectrophotometer. The protein contents of the CS extracts were determined using standard
172 curves obtained with BSA.

173

174 2.8. *Determination of total phenolic contents.*

175

176 Total phenolic contents of CS extracts were determined using a previously reported procedure
177 with the Folin-Ciocalteu's reagent (Singleton & Rossi, 1965). Zero-point-two ml of 1.0 mg/ml CS
178 extract was added to 1.8 ml of distilled water followed by 1.0 ml of the Folin-Ciocalteu's reagent.
179 The mixture was left for 3 min at 25 °C. Then 5.0 ml of 0.4 M sodium carbonate was added to the
180 mixture. The mixture was incubated at 25 °C for 1 h. The absorbance at 765 nm (A_{765}) was
181 measured using a spectrophotometer and the phenolic contents in CS extracts were evaluated by a
182 standard curve obtained with gallic acid.

183

184 2.9. *Measurement of DPPH radical scavenging activity*

185

186 The DPPH radical scavenging activity of CS extracts was assayed by the previously reported
187 method (Aoshima & Ayabe, 2007) with some modifications. Zero-point-two ml of various

188 concentrations of CS extract solution prepared using 50% (v/v) ethanol aqueous solution were
189 mixed with 0.3 ml of 0.2 mM DPPH in ethanol and 0.5 ml of 0.5 M acetic acid buffer at pH 5.5.
190 The mixture was shaken vigorously and placed in the dark for 30 min at 25 °C. The decrease in
191 the absorbance at 517 nm (A_{517}) of DPPH was measured using a spectrophotometer. The DPPH
192 radical scavenging activity was calculated by equation 1.

193

$$194 \quad \text{DPPH radical scavenging activity (\%)} = [1 - (A_s - A_b) / (A_0 - A_b)] \times 100 \quad (1)$$

195

196 where A_s is A_{517} of the mixture in the presence of the sample and DPPH, A_b is A_{517} of the mixture
197 in the absence of the sample and DPPH (blank), and A_0 is A_{517} of the mixture in the absence of the
198 sample and in the presence of DPPH. The DPPH values of the each sample are expressed as the
199 amount (μmol) of trolox equivalents (TE) per gram of the CS extract.

200

201 *2.10. Measurement of hydrophilic oxygen radical absorbance capacity (H-ORAC)*

202

203 The H-ORAC assay was carried out according to the previously reported method (Prior et al.,
204 2003) with some modifications. Fluorescein and AAPH solutions were prepared to the
205 concentrations of 94.4 nM and 31.7 mM, respectively, using 75 mM potassium phosphate buffer
206 (pH 7.4, buffer A). Twenty μl of various concentrations of the CS extract solutions and 200 μl of
207 94.4 nM fluorescein solution were placed in a well of a 96-well microplate and mixed well. The
208 initial fluorescence ($f_{0 \text{ min}}$) of each well was measured at the excitation and emission wavelengths
209 of 492 and 530 nm, respectively. An MTP-800Lab micro-titer-plate reader (Corona Electric Co.,
210 Ibaraki, Japan) with 492 excitation and 530 emission cut-off filters was used for fluorescence
211 measuring. The mixture was pre-incubated at 37 °C for 10 min, and the reaction was initiated by
212 adding 75 μl of 31.7 mM AAPH solution. The fluorescence depletion was monitored every 2 min
213 for 90 min (from $f_{2 \text{ min}}$ to $f_{90 \text{ min}}$) at the excitation and emission wavelengths of 492 and 530 nm,

214 respectively. The area under the fluorescence decay curve (AUC) was calculated according to the
215 following equation 2.

216

$$217 \quad \text{AUC} = (0.5 \times f_{8 \text{ min}} + f_{10 \text{ min}} + f_{14 \text{ min}} + \dots + f_{88 \text{ min}} + 0.5 \times f_{90 \text{ min}}) / f_{0 \text{ min}} \times 2 \quad (2)$$

218

219 The net AUC was calculated as follows:

220

$$221 \quad \text{net AUC} = \text{AUC} - \text{AUC}_{\text{blank}} \quad (3)$$

222

223 where $\text{AUC}_{\text{blank}}$ is the AUC value obtained with buffer A instead of the CS extract solution. The
224 secondary regression equation between the concentration of trolox standard solutions and the
225 AUC was calculated. The H-ORAC values of the CS extract solutions were calculated according
226 to equation 4, and were expressed as μmol of TE per gram of the CS extract.

227

$$228 \quad \text{H-ORAC} (\mu\text{mol TE/g of CS extract}) = a \times (\text{net AUC})^2 + b \times \text{net AUC} + c \quad (4)$$

229

230 where a, b, and c were constants of secondary regression of equation 4.

231

232 3. Results and discussion

233

234 3.1. Yields of CS extracts obtained by the treatments of CS with water, 0.1 M HCl, and 0.1 M 235 NaOH at various temperatures

236

237 CS extracts were obtained by the treatments with water, 0.1 M HCl, and 0.1 M NaOH at
238 various temperatures, and the yields of the CS extracts from 1 g CS were shown Table 1. The
239 yield of the CS extract obtained by the treatment with water increased with increasing the

240 extraction temperatures from 25 to 210 °C, although it decreased with increasing the temperature
241 exceeding 210 °C. The highest yield of the CS extracts by the treatment with water was given at
242 the extraction temperature of 210 °C, and the yield was 29% (w/w), being one point eight times as
243 high as that (16%, w/w) obtained at 25 °C (Table 1). It was reported previously that the yields of
244 the CS extracts by the treatment with water at 25 °C and 121 °C were 14% and 20%, respectively
245 (Furusawa et al., 2011). The yield at 121 °C was estimated to be 21% by extrapolating the data in
246 Table 1, and this value is in reasonable agreement with that of Furusawa et al. The yield was
247 considerably different by changing the extracting solvent from water to 0.1 M HCl and 0.1 M
248 NaOH, although the extraction temperatures with those solvents were allowed only at 25 °C and
249 80 °C (Table 1). The apparent highest yield (44%) was given by the treatment with 0.1 M NaOH
250 at 80 °C among the conditions examined. This apparent highest yield shows the dry weight of CS
251 extracts obtained by freeze-drying filtration of mixture incubated 1g CS and 50 ml of 0.1 M
252 NaOH at 80 °C for 1 h. Therefore, it is thought that about 20% of the apparent yield was re-
253 solidified NaOH or sodium salt given by the treatment of 1g CS with 50 ml of 0.1 M NaOH at 80
254 °C for 1 h.

255

256 (Table 1)

257

258 3.2. HPLC of caffeine, 5-CQA, and 5-HMF of CS extracts.

259

260 It is reported that hexose and pentose are decomposed to mainly 5-HMF and furfural in the
261 process by subcritical water treatment (Khajavi, Kimura, Oomori, Matsuno, & Adachi, 2005;
262 Usuki, Kimura, & Adachi, 2008). We analyzed caffeine and 5-CQA which are the main
263 polyphenols in coffee beans and 5-HMF in the CS extracts by reversed-phase HPLC (Fig. 1).

264 The peak of caffeine was observed at the elution time of 13.8 min. The amounts of caffeine
265 extracted from 1 g CS by the treatment with water were in the range of 4.1–4.4 mg, being

substantially the same without depending on the extraction temperature from 180 to 270 °C (Table 2). It was reported that the percentage yield of caffeine extracted by subcritical water extraction from tea waste increased depending on the increase in the extraction temperature from 100 °C to 175 °C, and the highest value obtained at 175 °C was 0.77% (w/w) namely 7.7 mg/g (Shalmashi, Abedi, Golmohammad, & Eikani, 2010). This difference might be due to that most of all caffeine is contained in CS could be extracted even at 25 °C, although caffeine in tea waste is located in the state so as to be extracted by the degradation of cell walls and other cell components (Table 2). Another point is the extraction time with subcritical water. In the present study, we applied 10 min for extraction to CS, whereas 120 min was applied to tea waste. At least, it was suggested that caffeine in CS extracts is not decomposed by subcritical water at 180–270 °C from this experimental result. The amounts (4.1–4.2 mg) of caffeine extracted from 1 g CS by the treatment with 0.1 M HCl at 25 and 80 °C were the same. The amounts (1.7–1.8 mg) of caffeine extracted from 1 g CS by the treatment with 0.1 M NaOH at 25 and 80 °C were almost the same, and were lower than those of the CS extracts obtained by treatment with water and 0.1M HCl. This cause seems that the solubility of caffeine is lowered in 0.1 M NaOH at high pH (pH is around 13) because it is a basic material (Table 2).

5-CQA was detected in the CS extracts obtained by the treatments with water at 25, 80, and 180 °C (Table 2), although it was not detected in the CS extracts obtained by the treatments with water at 210, 240, and 270 °C (Table 2). These results suggest that 5-CQA in CS treated by subcritical water above 210 °C was decomposed. It is known that a progressive destruction and transformation of chlorogenic acid with 8–10% being lost for every 1% loss of dry matter during roasting of coffee beans (Clifford, 1999).

No difference was observed in the amounts (1.1 mg) of 5-CQA extracted from 1 g CS with 0.1 M HCl at 25 °C and 80 °C were the same. However, 5-CQA was not detected in the CS extracts obtained with 0.1 M NaOH at 25 °C and 80 °C. It was reported that caffeic acid and chlorogenic acid are stable in phosphate or acetate buffer in acid pH (3–6) (Friedman & Jurgens, 2000).

292 However, they are unstable in borate buffer (pH 7–11) with an increase in pH, and their structural
293 changes are time-dependent and nonreversible (Friedman & Jurgens, 2000). The cause that 5-
294 CQA was not detected in CS extracts obtained with 0.1 M NaOH (pH is around 13) at 25 °C and
295 80 °C might be based on the stability of 5-CQA at alkaline pH.

296 The amount of 5-HMF extracted from 1 g CS increased with increasing the extraction
297 temperature from 25 to 210 °C and reached the maximum (2.0 mg), while steeply decreased at the
298 temperature over 210 °C (Table 2). 5-HMF is considered to be a main degradation product formed
299 by dehydration of hexoses through hydrothermolysis (Khajavi et al., 2005; Usuki et al., 2008) and
300 its content in general is almost none. In the present study, CS produced as a by-product of the
301 roasting process of coffee beans over 200 °C. 5-HMF detected in the CS extract obtained by the
302 treatment of CS with water at 25 °C could be derived from roasting of coffee beans. The amounts
303 of 5-HMF extracted from 1 g CS obtained with 0.1 M HCl at 25 and 80 °C were almost the same
304 (0.4–0.5 mg), but 5-HMF was not detected in the extract obtained by the treatment with 0.1 M
305 NaOH at 25 and 80 °C (Table 2).

306

307 (Fig. 1)

308 (Table 2)

309

310 3.3. Total sugar and reducing sugar contents of CS extracts.

311

312 Table 3 shows the total sugar and reducing sugar contents in 1 g CS extracts obtained by the
313 treatments under various conditions. The values of the total sugar and reducing sugar contents
314 (mg/g CS extract) were converted to their amounts (mg/g CS) contained in 1 g CS (Table 3).
315 When 1 g CS is treated by water at 25–270 °C, the amounts of total sugar and reducing sugar in 1
316 g CS increased with increasing the extraction temperature from 25 °C to reach the maximum for
317 both at 180 °C to the amounts of 121 ± 9 mg and 52 ± 2 mg, respectively (Table 3). However, the

amounts for both decreased at the temperature over 180 °C and turned to around 15 mg at 270 °C. The total sugar contents of the CS extracts obtained by the treatment with water increased with increasing temperature up to 180 °C and drastically decreased at the extraction temperature over 180 °C. It was reported that the carbohydrate contents from the defatted rice bran extract obtained by the treatment with water increased with increasing the extraction temperature up to 200 °C, and decreased at the temperature over 200 °C (Wiboonsirikul et al., 2007a). This profile of the dependence of the carbohydrate contents on the extraction temperature is similar to that shown in Table 3. The decrease in the carbohydrate contents at the temperatures over 200 °C was considered due to the hydrolysis of poly- or oligosaccharides and the degradation of monosaccharides generated by the high ionic product of water at high temperature under subcritical conditions (Wiboonsirikul et al., 2007a). The decrease in the total sugar contents of the CS extracts at the temperature over 180 °C would also be ascribed to the same cause. The total sugar and reducing sugar contents of the CS extracts obtained at 240 °C and 270 °C were almost the same, suggesting that most of the saccharides produced from the CS extracts obtained at 240 °C and 270 °C could be the mixture of monosaccharides. At the extraction temperature of 25 °C and 80 °C, the efficiency of the extraction solvent for the amounts of the total sugar extracted was in the order of 0.1 M HCl > 0.1 M NaOH > water, while that of the reducing sugar was in the order of 0.1 M HCl > water > 0.1 M NaOH.

336

337 (Table 3)

338

339 *3.4. Protein and total phenolic contents of CS extracts.*

340

341 Table 3 shows the protein and total phenolic contents of 1 g CS extracts obtained by the
342 treatments under various conditions. The values of the protein and total phenolic contents were
343 converted to their amounts (mg/g CS) contained in 1 g CS (Table 3). The protein content in the

CS extract obtained by the treatment with water increased with increasing the extraction temperature from 25 °C to 240 °C and the maximum values were observed at 240 °C being 582 ± 10 mg per g of CS extract (Table 3) and their contents extracted from 1 g CS increased with increasing the temperature, although the maximum was observed at 210 °C being 157 ± 4 mg per g CS (Table 3). The largest amount of protein was extracted from 1 g CS at 210 °C was about five times as high as that extracted with water at 25 °C (33 ± 2 mg). This high extraction degree obtained under the subcritical water condition might due to the enhanced hydrolysis of proteins, solubilization of insoluble proteins, and degradation of cell walls. It is reported that the solubility of the rice bran protein increased by the hydrolysis of proteins and cell wall by subcritical water treatment (Wiboonsirikul et al., 2007a). At the extraction temperature of 25 °C and 80 °C, the efficacy of the solvent for the protein extraction from 1 g CS was in the order of 0.1 M NaOH > 0.1 M HCl > water (Table 3). The largest amount of protein was extracted from 1 g CS in these conditions was 97 ± 8 mg, and was obtained by extraction with 0.1 M NaOH at 80 °C. However, the amount of protein was extracted from 1 g CS with subcritical water at 210 °C was about one point five times as high as that extracted with NaOH at 80 °C (Table 3).

The total phenolic content in the CS extract obtained by the treatment with water increased with increasing the extraction temperature from 25 °C to 240 °C and the maximum values were observed at 240 °C being 130 ± 6 mg per g of the CS extract (Table 3). The largest amount of total phenolic components (36 ± 3 mg) extracted from 1 g CS was also observed with water at 210 °C (Table 3). It was six times as high as it extracted from 1 g CS with water at 25 °C (6 ± 0 mg). This might also due to the hydrolysis or degradation of polyphenolic compounds such as lignin and lignan into smaller and soluble compounds. At the extraction temperature of 25 °C and 80 °C, the efficacy of the solvent in the extraction of total phenolic compounds was not much different in these treatments (Table 3). The amount of total phenolic components was 5–8 mg/ per g of CS (Table 3). The amount of total phenolic components was extracted from 1 g CS with subcritical

water at 210 °C was about five times as high as that extracted with these solvents at 25 and 80 °C (Table 3).

3.5. DPPH radical scavenging activity and H-ORAC of CS extracts.

Figure 2A and Table 4 show the results of DPPH radical scavenging activity assay on the CS extracts obtained by the treatments with water, 0.1 M HCl, and 0.1 M NaOH at various temperatures. The values of the CS extracts obtained with water increased remarkably in a sigmoid fashion with increasing the extraction temperature from 25 to 270 °C (Fig. 2A). The maximum value was given with the CS extract obtained by the treatment with water at 270 °C was 379 ± 36 μmol TE per g of CS extract (Table 4). The DPPH values of the extracts obtained with water, 0.1 M HCl or 0.1 M NaOH at 25 and 80 °C were substantially the same, and were range of 61–75 μmol TE per g of CS extract (Table 4). The DPPH values of CS extraction by treatment with subcritical water at 270 °C were about five times as high as the values of the extraction by treatment with water, 0.1 M HCl or 0.1 M NaOH at 25 and 80 °C (Table 4).

The H-ORAC values of the CS extracts obtained by the treatments by with water, 0.1 M HCl, and 0.1 M NaOH at the various temperatures were also examined (Fig. 2A & Table 4). The values of the CS extracts obtained with water increased with increasing the temperature also in a sigmoid fashion with increasing the extraction temperature from 25 °C to 270 °C (Fig. 2A). The maximum value was given at 270 °C was 2629 ± 193 μmol TE per g of CS extract (Table 4). The values of the extracts obtained with water, 0.1 M HCl, and 0.1 M NaOH at 25 °C and 80 °C were almost the same, and were range of 273–384 μmol TE per g of CS extract (Table 4). The H-ORAC values of CS extraction by treatment with subcritical water at 270 °C were about seven times as high as the values of the extraction by treatment with water, 0.1 M HCl or 0.1 M NaOH at 25 and 80 °C (Table 4).

394 It is reported that the CS extracts obtained by the treatments with distilled water and methanol
395 have antioxidant activities by two methods, *N,N*-dimethyl-*p*-phenylenediamine (DMPD) for the
396 water extracts and 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radical
397 scavenging activity for the methanol extracts (Borrelli et al., 2004). It has not been studied
398 whether the antioxidant activity of the CS extracted with water changes depending on the
399 extraction temperature (Borrelli et al., 2004). There are many methods reported for measuring
400 antioxidative activity. These methods are mainly classified into two types. One is the assay based
401 on hydrogen atom transfer (HAT) which includes the H-ORAC assay and the other is that based
402 on electron transfer (ET) which includes DPPH, ABTS, superoxide dismutase, and ferric reducing
403 antioxidant potential assays (Dudonne, Vitrac, Coutiere, Woillez, & Merillon, 2009). In the
404 present study, we evaluated the antioxidant activities of CS extracts using DPPH assay and H-
405 ORAC assay. The H-ORAC and DPPH values of CS extracts obtained by the treatment with
406 water at 25–270 °C, and both values increased remarkably with increasing the extraction
407 temperatures (Fig. 2A & Table 4) and the highest values (2629 ± 193 and 379 ± 36 $\mu\text{mol TE/g}$ of
408 CS extract) of H-ORAC and DPPH are observed at 270 °C, respectively (Fig. 2A). There is very
409 good correlation between the DPPH and H-ORAC values ($R = 0.999$) (Fig. 2B). The good
410 correlation of the antioxidant activities measured by H-ORAC and DPPH methods was reported
411 also with sorghums (Awika, Rooney, Wu, Prior, & Cisneros-Zevallos, 2003). Generally, phenolic
412 compounds contribute highly to the antioxidant activity of food, actually the total phenolic
413 contents of CS extracts have high correlation with their H-ORAC and DPPH values with the
414 correlation coefficients (R) of 0.987 and 0.982, respectively (Fig. 3). It is worthy to note that there
415 is also high correlation between protein contents and their H-ORAC and DPPH values, with the R
416 values of 0.994 and 0.990, respectively (Fig. 3). Peptides produced by the decomposition of
417 soybean protein and wheat gluten were reported to exhibit strong DPPH radical scavenging
418 activity and antioxidant activity against linoleic acid oxidation in emulsion systems (Park,
419 Morimae, Matsumura, Nakamura, & Sato, 2008). Peptides produced by hydrolyzing the protein in

CS by subcritical water treatment are considered to show such a high antioxidant capacity. The CS extracts obtained by the treatment with water at 210–270 °C did not contain 5-CQA, which is thought to be the main antioxidant component of coffee beans, although the extracts showed high antioxidant activity (Table 2 & Fig. 2A). This antioxidant activity must due to proteins and peptides. It is necessary to clarify the component in the CS extract contributed to the antioxidant capacity by further study in the next step. This study shows that CS contains fairly strong antioxidant activity and proposes that the subcritical water treatment is the effective method for extraction of antioxidant components from CS.

(Figs. 2 & 3)

(Table 4)

4. Conclusions

The antioxidant activity of CS extracts obtained by the treatment of CS with water and subcritical water increased with increasing the temperature also in a sigmoid fashion with increasing the extraction temperature from 25 °C to 270 °C. The maximum H-ORAC and DPPH values of the extracts were given at 270 °C were 2629 ± 193 and 379 ± 36 $\mu\text{mol TE per g of CS}$ extract, respectively. On the other hand, the antioxidant activity evaluated by H-ORAC and DPPH radical scavenging activity of CS extracts obtained by the treatment with water, 0.1 M HCl, and 0.1M NaOH at 25 °C and 80 °C was almost the same. The antioxidant activity of CS extracts obtained by the treatment of CS with subcritical water was stronger than that extracted by treatment with water, 0.1 M HCl, and 0.1M NaOH at 25 °C and 80 °C.

Phenolic contents of CS extracts obtained by the treatment with water and subcritical water at 25–270 °C have high correlation with their H-ORAC and DPPH values with the correlation coefficients (*R*) of 0.987 and 0.982, respectively. It is worthy to note that there is also high correlation between protein contents and their H-ORAC and DPPH values, with the *R* values of

446 0.994 and 0.990, respectively. Peptides produced by hydrolyzing the protein in CS by subcritical
447 water treatment are considered to show such a high antioxidant capacity.

448 The CS extracts could be useful as a good source of antioxidative materials. Furthermore, the
449 treatment using subcritical water was more efficient for production of the antioxidative materials
450 from CS. Besides, it was shown that the subcritical water treatment is effective on extraction of
451 total sugar, reducing sugar, protein, phenolic components, and 5-HMF from CS by adjusting
452 treatment temperature.

453

454

455

456 References

457

458 Aoshima, H. & Ayabe, S. (2007). Prevention of the deterioration of polyphenol-rich beverages.
459 *Food Chemistry*, 100, 350-355.

460 Awika, J. M., Rooney, L. W., Wu, X., Prior, R. L., & Cisneros-Zevallos, L. (2003). Screening
461 methods to measure antioxidant activity of sorghum (*sorghum bicolor*) and sorghum products.
462 *Journal of Agricultural and Food Chemistry*, 51, 6657-6662.

463 Borel, E., Hostettler, F., & Deuel, H. (1952). Quantitative Zuckerbestimmung mit 3,5-
464 dinitrosalicylsäure und phenol. *Helvetica Chimica Acta*, 35, 115-120.

465 Borrelli, R. C., Esposito, F., Napolitano, A., Ritieni, A., & Fogliano, V. (2004). Characterization
466 of a new potential functional ingredient: coffee silverskin. *Journal of Agricultural and Food*
467 *Chemistry*, 52, 1338-1343.

468 Clifford, M. N. (1999). Chlorogenic acids and other cinnamates: Nature, occurrence and dietary
469 burden. *Journal of the Science of Food and Agriculture*, 79, 362-372.

470 Christen, Y. (2000). Oxidative stress and Alzheimer disease. *The American Journal of Clinical*
471 *Nutrition*, 71, 621S-629S.

472 Diaz, M. N., Frei, B., Vita, J. A., & Keaney, J. F., Jr. (1997). Antioxidants and atherosclerotic
473 heart disease. *The New England Journal of Medicine*, 337, 408-416.

474 Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., & Smith, F. (1956). Colorimetric
475 method for determination of sugars and related substances. *Analytical Chemistry*, 28, 350-356.

476 Dudonne, S., Vitrac, X., Coutiere, P., Woillez, M., & Merillon, J.-M. (2009). Comparative study
477 of antioxidant properties and total phenolic content of 30 plant extracts of industrial interest using

- 478 DPPH, ABTS, FRAP, SOD, and ORAC assays. *Journal of Agricultural and Food Chemistry*, 57,
479 1768-1774.
- 480 Friedman, M., & Jurgens, H. S. (2000). Effect of pH on the stability of plant phenolic compounds.
481 *Journal of Agricultural and Food Chemistry*, 48, 2101-2110.
- 482 Furusawa, M., Narita, Y., Iwai, K., Fukunaga, T., & Nakagiri, O. (2011). Inhibitory effect of a hot
483 water extract of coffee “silverskin” on hyaluronidase. *Bioscience, Biotechnology, and*
484 *Biochemistry*, 75, 1205-1207.
- 485 Gardner, P. T., McPhail, D. B., & Duthie, G. G. (1998). Electron spin resonance spectroscopic
486 assessment of the antioxidant potential of teas in aqueous and organic media. *Journal of the*
487 *Science of Food and Agriculture*, 76, 257–262.
- 488 Gil, M. I., Tomas-Barberan, F. A., Hess-Pierce, B., & Kader, A. A. (2000). Antioxidant capacities,
489 phenolic compounds, carotenoids, and vitamin C contents of nectarine, peach, and plum cultivars
490 from California. *Journal of Agricultural and Food Chemistry*, 50, 4976-4982.
- 491 Khajavi, S. H., Kimura, Y., Oomori, T., Matsuno, R., & Adachi, S. (2005). Degradation kinetics
492 of monosaccharides in subcritical water. *Journal of Food Engineering*, 68, 309-313.
- 493 Iwai, K., Kishimoto, N., Kakino, Y., Mochida, K., & Fujita, T. (2004). In vitro antioxidative
494 effects and tyrosinase inhibitory activities of seven hydroxycinnamoyl derivatives in green coffee
495 beans. *Journal of Agricultural and Food Chemistry*, 52, 4893-4898.
- 496 Kataoka, M., Wiboonsirikul, J., Kimura, Y., & Adachi, S. (2008) Properties of extracts from
497 wheat bran by subcritical water treatment. *Food Science and Technology Research*, 14, 553-556.
- 498 Lambert, J. D., & Yang, C. S. (2003). Mechanisms of cancer prevention by tea constituents. *The*
499 *Journal of Nutrition*, 133, 3262S-3267S.

- 500 Lang, A. E., & Lozano, A. M. (1998). Parkinson's disease. First of two parts. *The New England*
501 *Journal of Medicine*, 339, 1044-1053.
- 502 Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951). Protein measurement with
503 the folin phenol reagent. *Journal of Biological Chemistry*, 193, 265-275.
- 504 Miller, D. J., & Hawthorne, S. B. (1998). Method for determining the solubilities of hydrophobic
505 organics in subcritical water. *Analytical Chemistry*, 70, 1618-1621.
- 506 Narita, Y., & Inouye, K. (2009). Kinetic analysis and mechanism on the inhibition of chlorogenic
507 acid and its components against porcine pancreas α -amylase isozymes I and II. *Journal of*
508 *Agricultural and Food Chemistry*, 57, 9218-9225.
- 509 Narita, Y., & Inouye, K. (2011). Inhibitory effects of chlorogenic acids from green coffee beans
510 and cinnamate derivatives on the activity of porcine pancreas α -amylase isozyme I. *Food*
511 *Chemistry*, 127, 1532-1539.
- 512 Park, E. Y., Morimae, M., Matsumura, Y., Nakamura, Y., & Sato, K. (2008). Antioxidant activity
513 of some protein hydrolysates and their fractions with different isoelectric points. *Journal of*
514 *Agricultural and Food Chemistry*, 56, 9246-9251.
- 515 Prior, R. L., Hoang, H., Gu, L., Wu, X., Bacchiocca, M., Howard, L., et al. (2003). Assays for
516 hydrophilic and lipophilic antioxidant capacity (oxygen radical absorbance capacity (ORAC_{FL}))
517 of plasma and other biological and food samples. *Journal of Agricultural and Food Chemistry*, 51,
518 3273-3279.
- 519 Richelle, M., Tavazzi, I., & Offord, E. (2001). Comparison of the antioxidant activity of
520 commonly consumed polyphenolic beverages (coffee, cocoa, and tea) prepared per cup serving.
521 *Journal of Agricultural and Food Chemistry*, 49, 3438-3442.

- 522 Shalmashi, A., Abedi, M., Golmohammad, F., & Eikani, M. H. (2010). Isolation of caffeine from
- 523 tea waste using subcritical water extraction. *Journal of Food Process Engineering*, 33, 701-711.
- 524 Singleton, V. L., & Rossi, J. A., Jr. (1965). Colorimetry of total phenolics with phosphomolybdic-
- 525 phosphotungstic acid reagents. *American Journal of Enology and Viticulture*, 16, 144-158.
- 526 Takayanagi, R., Inoguchi, T., & Ohnaka, K. (2011). Clinical and experimental evidence for
- 527 oxidative stress as an exacerbating factor of diabetes mellitus. *Journal of Clinical Biochemistry*
- 528 *and Nutrition*, 48, 72-77.
- 529 Usuki, C., Kimura, Y., & Adachi, S. (2008). Degradation of pentaoses and hexouronic acids in
- 530 subcritical water. *Chemical Engineering and Technology*, 31, 133-137.
- 531 Wakita, Y., Harada, O., Kuwata, M., Fujimura, T., Yamada, T., & Suzuki, M., et al. (2004).
- 532 Preparation of subcritical water-treated okara and its effect on blood pressure in spontaneously
- 533 hypertensive rats. *Food Science and Technology Research*, 10, 164-167.
- 534 Wiboonsirikul, J., Kimura, Y., Kadota, M., Morita, H., Tsuno, T., & Adachi, S. (2007a).
- 535 Properties of extracts from defatted rice bran by its subcritical water treatment. *Journal of*
- 536 *Agricultural and Food Chemistry*, 55, 8759-8765.
- 537 Wiboonsirikul, J., Hata, S., Tsuno, T., Kimura, Y., & Adachi, S. (2007b). Production of
- 538 functional substances from black rice bran by its treatment in subcritical water. *LWT – Food*
- 539 *Science and Technology*, 40, 1732-1740.
- 540

541 Figure Captions

542

543 Fig. 1. HPLC chromatograms of the CS extracts obtained by the treatments of CS with water, 0.1
544 M HCl, and 0.1 M NaOH at 25 °C and 210 °C. The extraction solvents/temperatures: 0.1 M
545 HCl/25 °C (a), 0.1 M NaOH/25 °C (b), water/25 °C (c), and water/210 °C (d). The wavelength for
546 detection: 270 nm. Peaks (retention times): 1, 5-HMF (5.4 min); 2, caffeine (13.8 min); and 3, 5-
547 CQA (16.5 min).

548

549 Fig. 2. H-ORAC and DPPH values of the CS extracts. Panel A: H-ORAC (open symbols) and
550 DPPH (solid symbols) values of the CS extracts obtained by the treatments of CS with water
551 (circles), 0.1 M HCl (triangles), and 0.1 M NaOH (squares) at various temperatures. Each point
552 represents the mean and standard deviation of triplicate experiments. Panel B: Relationship
553 between the H-ORAC and DPPH values of the CS extracts obtained by the treatments of CS with
554 water in the temperature range of 25–270 °C.

555

556 Fig. 3. Relationship between the H-ORAC or DPPH values and protein or total phenolic contents
557 of the CS extracts. The symbols, open circles, solid circles, open triangles, and solid triangles
558 represent the H-ORAC values against total phenolic contents, DPPH values against total phenolic
559 contents, H-ORAC values against total protein contents, and DPPH values against protein
560 contents of the CS extracts, respectively.

561

562

Table 1. Yield of the CS extracts^a

	Extraction		Yield
	solvent	temperature (°C)	(%, w/w)
	water	25	16 ± 1
	water	80	19 ± 1
	water	180	25 ± 1
	water	210	29 ± 1
	water	240	27 ± 1
	water	270	23 ± 1
	0.1 M HCl	25	21 ± 2
	0.1 M HCl	80	28 ± 2
	0.1 M NaOH	25	37 ± 1
	0.1 M NaOH	80	44 ± 1

^a Each value is a mean of triplicate analysis ± standard deviation.

Table 2. Caffeine, 5-CQA, and 5-HMF contents of 1 g CS or CS extracts^a

Extraction		Caffeine		5-CQA		5-HMF	
solvent	temperature (°C)	(mg/g of CS extract)	(mg/g of CS)	(mg/g of CS extract)	(mg/g of CS)	(mg/g of CS extract)	(mg/g of CS)
water	25	26.4 ± 0.1	4.1 ± 0.1	6.4 ± 0.3	1.0 ± 0.0	1.1 ± 0.1	0.2 ± 0.0
water	80	23.1 ± 0.3	4.4 ± 0.2	9.0 ± 0.4	1.7 ± 0.1	1.1 ± 0.2	0.2 ± 0.0
water	180	16.2 ± 0.2	4.1 ± 0.1	6.1 ± 0.2	1.5 ± 0.1	4.6 ± 0.3	1.2 ± 0.1
water	210	14.4 ± 0.1	4.2 ± 0.1	N. D. ^b	N. D.	6.9 ± 0.3	2.0 ± 0.1
water	240	15.8 ± 0.1	4.2 ± 0.2	N. D.	N. D.	3.8 ± 0.1	1.0 ± 0.0
water	270	17.9 ± 0.1	4.1 ± 0.2	N. D.	N. D.	1.8 ± 0.3	0.4 ± 0.1
0.1 M HCl	25	19.0 ± 0.2	4.1 ± 0.3	5.3 ± 0.2	1.1 ± 0.0	1.8 ± 0.2	0.4 ± 0.0
0.1 M HCl	80	15.3 ± 0.2	4.2 ± 0.3	3.8 ± 0.1	1.1 ± 0.1	1.7 ± 0.2	0.5 ± 0.1
0.1 M NaOH	25	5.0 ± 0.2	1.8 ± 0.1	N. D.	N. D.	N. D.	N. D.
0.1 M NaOH	80	3.9 ± 0.3	1.7 ± 0.1	N. D.	N. D.	N. D.	N. D.

^a Each value is a mean of triplicate analysis ± standard deviation.

^b Not detected.

Table 3. Total sugar, reducing sugar, protein and total phenolic contents of 1 g CS or CS extracts^a

Extraction		Total sugar		Reducing sugar		Protein		Total phenolic	
solvent	temperature (°C)	(mg/g of CS extract)	(mg/g of CS)	(mg/g of CS extract)	(mg/g of CS)	(mg/g of CS extract)	(mg/g of CS)	(mg/g of CS extract)	(mg/g of CS)
water	25	366 ± 21	57 ± 2	156 ± 4	25 ± 1	212 ± 18	33 ± 2	36 ± 3	6 ± 0
water	80	405 ± 30	77 ± 9	155 ± 9	28 ± 2	236 ± 12	45 ± 3	35 ± 1	7 ± 0
water	180	477 ± 29	121 ± 9	206 ± 6	52 ± 2	378 ± 20	95 ± 5	85 ± 5	22 ± 1
water	210	228 ± 5	67 ± 3	137 ± 3	40 ± 1	535 ± 14	157 ± 4	124 ± 9	36 ± 3
water	240	86 ± 1	23 ± 1	82 ± 10	21 ± 3	582 ± 10	155 ± 7	130 ± 6	35 ± 2
water	270	71 ± 6	16 ± 1	70 ± 4	15 ± 1	544 ± 11	125 ± 4	123 ± 9	28 ± 1
0.1 M HCl	25	304 ± 13	65 ± 7	122 ± 1	26 ± 2	189 ± 7	40 ± 4	23 ± 1	5 ± 1
0.1 M HCl	80	345 ± 10	95 ± 4	115 ± 5	32 ± 1	183 ± 13	50 ± 7	24 ± 1	7 ± 0
0.1 M NaOH	25	168 ± 14	62 ± 7	55 ± 18	20 ± 8	205 ± 15	76 ± 8	15 ± 2	5 ± 1
0.1 M NaOH	80	185 ± 28	80 ± 12	49 ± 12	21 ± 5	221 ± 17	97 ± 8	19 ± 2	8 ± 1

^a Each value is a mean of triplicate analysis ± standard deviation.

Table 4. DPPH radical scavenging activity and H-ORAC of CS extracts^a

587	Extraction		H-ORAC		DPPH	
	solvent	temperature (°C)	(μmol TE/g of CS extract)		(μmol TE/g of CS extract)	
588	water	25	354 ± 44		74 ± 13	
	water	80	384 ± 58		75 ± 18	
589	water	180	1223 ± 65		184 ± 28	
	water	210	2321 ± 169		323 ± 39	
	water	240	2611 ± 150		371 ± 33	
590	water	270	2629 ± 193		379 ± 36	
591	0.1 M HCl	25	289 ± 34		67 ± 9	
	0.1 M HCl	80	284 ± 37		71 ± 11	
	0.1 M NaOH	25	275 ± 22		61 ± 5	
	0.1 M NaOH	80	273 ± 20		63 ± 6	

^aEach value is a mean of triplicate analysis ± standard deviation.

Fig. 1

Fig. 2A

Fig. 2B

Fig. 3