Effects of baking on cyanidin-3-glucoside content and antioxidant properties of black and yellow soybean crackers

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1. Introduction

Food sources of anthocyanins are of continued interest due to growing evidence of their health benefits (Tsuda, 2012; Wallace, 2011), most notably their recent association with decreased risk of myocardial infarction in women (Cassidy et al., 2013). Berries and red cabbage are among the most widely recognised sources of anthocyanins, but anthocyanins are also present in deeply pigmented varieties of soybeans, corn and rice (Li, Walker, & Faubion, 2011; Slavin, Kenworthy, & Yu, 2009; Zhang, Zhang, Zhang, & Liu, 2010). Though levels of anthocyanins vary greatly between black soybean varieties, the average levels in seed coats may be comparable to or greater than berries on a dry weight basis.

Reported values of total anthocyanin content in black soybeans ranged from 158 to 2018 mg/100 g black soy seed coat in 10 varieties (Choung et al., 2001) and a range of 98.8–2132.5 mg/100 g seed coat across 60 varieties, with an average of 770 mg/100 g seed coat (Zhang et al., 2011). Cyanidin-3-glucoside (C3G) is the primary anthocyanin detected in black soybean seed coats, though other anthocyanins are also present, including peonidin-3-glucoside, peonidin-3-glucoside and delphinidin-3-glucoside (Lee et al., 2009; Zhang et al., 2011). We have previously reported the amount of the individual anthocyanin C3G on a whole bean basis, ranging from 3 to 60 mg/100 g whole black soybean (Slavin et al., 2009). Levels of anthocyanins in the whole bean are necessarily lower than the seed coat alone, but values of total anthocyanins are not commonly available on a whole bean basis. Nonetheless, the levels of anthocyanin in black soybean seed coats are considerable when compared to that for berries: 73–430 mg total anthocyanins/100 g blueberries, 464–627 mg/100 g black raspberries, 80–230 mg/100 g blackberries, and 52 and 230 mg/100 g raspberries, all on a fresh-frozen weight basis (Moyer, Hummer, Finn, Frei, & Wrolstad, 2002). The presence of anthocyanin in a stable, staple food form represents an interesting opportunity for inclusion in the diet when consumption of fresh fruits and vegetables is limited and/or cost-prohibitive.

Anthocyanins are known to be particularly unstable during heat processing (Patras, Brunton, O’Donnell, & Tiwari, 2010). Presumably because of anthocyanins’ prominence in fruit and solubility in water (which allows for applications as a natural colourant in beverage products), most research regarding their stability has been performed in liquid-based foods such as juice, fruit pulp, and jam. However, black soybeans may be processed into flour and used in solid food applications, such as baked goods. A study of blue corn cookies indicated that manipulating baking conditions and the use of acidulant may retard the loss of total anthocyanins during baking (Li et al., 2011). Limited research exists regarding stability of anthocyanins in black soybeans during storage and heat treatment (Lee & Cho, 2012; Xu & Chang, 2008), but research is...
lacking as to black soybean anthocyanin stability in a dry heat processed food model.

This paper reports the effects of various baking conditions on the C3G content in black soybean-whole wheat crackers. Isoflavone, hydroxymethylfurufural (HMF), phenolic acid, and total phenolic content were measured, in addition to several indicators of antioxidant activity: oxygen radical absorbance capacity (ORAC), hydroxyl radical scavenging capacity (HOSC), and ABTS+ scavenging. To our knowledge, it is the first report of baking conditions on a black soybean food model.

2. Materials and methods

2.1. Materials

ABTS salt, gallic acid, iron (III) chloride, fluorescein (FL), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), hydroxymethylfurfural (HMF), and all isoflavone and phenolic acid standards were purchased from Sigma Aldrich (St. Louis, MO, USA). 2,2’-Azinobis (2-amidinopropane) dihydrochloride (AAPH) was obtained from Wako Chemicals USA (Richmond, VA, USA). Cyanidin-3-glucoside was purchased from Polyphenols AS (Norway). Thirty percent ACS-grade H₂O₂ and ultrapure water was obtained from Fisher Scientific (Fair Lawn, NJ, USA). All other chemicals and solvents were of the highest commercial grade and used without further purification.

Soybean samples were provided by Dr. William Kenworthy, Department of Plant Science and Landscape Architecture, University of Maryland, College Park, MD. Soybeans were grown at a single location in Maryland during the 2009 growing season. Yellow Manokin and black Peking cultivars were used. We have previously reported that Peking soybeans contain significantly higher TPC than yellow Manokin soybeans, and Peking were among those black soybeans particularly high in isoflavones (Slavin, Kenworthy, et al., 2009). In short, 50 g of defatted cracker was precisely weighed and vortexed with 10 mL 50% acetone (v/v) and sonicated for 15 min. The headspace of the tube was filled with N₂, and samples were allowed to extract for 18 h in the dark at room temperature. The extracts were then filtered through a 0.45 μm nylon membrane filter and held under refrigeration (4 °C) until testing.

2.2. Cracker preparation

Cracker samples were prepared according to a recipe of fresh ground, whole soybean (24%), commercial whole wheat flour (31%), commercial corn meal (12%), sugar (1%), salt (2%), and water (30%). Ingredients were mixed into a cohesive dough, rolled into a consistent, thin sheet using a pasta roller, cut into pieces, and baked according to the following time/temperature schedule. Thermal treatments were selected based on their ability to produce an acceptable product, while also varying the thermal treatments as much as possible to increase the potential for eliciting a difference among treatments. Five combinations of time and temperature were used: a low temperature/long time (121 °C × 60 min), a moderate temperature at three different times (149 °C × 10, 13 or 16 min), and a high temperature/short time (177 °C × 10 min) combination were used. In addition, an unbaked control (UC) consisting of mixed dough that was rolled and freeze-dried was also tested. Each time/temperature combination was baked in triplicate. Baked samples were then cooled at ambient temperature, ground in a standard coffee grinder to pass through a 40-mesh sieve, and stored in sealed bags in desiccators at room temperature.

2.3. Water content

Ground cracker samples were precisely weighed into aluminum weigh boats and heated in an 80 °C oven, removed and immediately transferred to a desiccator, then weighed after cooling to room temperature. The process was repeated until a constant weight was achieved in three consecutive readings. Water content was calculated as the percent difference in weight between the original sample and the dried cracker sample. Results of the following assays were calculated on a dry-weight basis.

2.4. Oil extraction and antioxidant extraction

Oil and lipids were removed from samples via room temperature extraction with petroleum ether. Room temperature extractions were used to prevent thermal degradation of phenolics in soybeans. A 5 g sample of ground crackers was precisely weighed into a glass test tube, and 20 mL petroleum ether (PE; 30–60 °C boiling point) was added. Tubes were vortexed and allowed to sit overnight and centrifuged. The supernatant was removed to a clean tube. The PE extraction was repeated twice, and the solid cracker residual was kept in a chemical fume hood to allow solvent to evaporate.

The defatted cracker residue was extracted with 50% acetone (v/v) for antioxidant assays (Moore et al., 2005; Slavin, Cheng, Luther, Kenworthy, & Yu, 2009). One g of defatted cracker was precisely weighed and vortexed with 10 mL 50% acetone (v/v) and sonicated for 15 min. The headspace of the tube was filled with N₂, and samples were allowed to extract for 18 h in the dark at room temperature. The extracts were then filtered through a 0.45 μm nylon membrane filter and held under refrigeration (4 °C) until testing.

2.5. Total phenolic contents (TPC)

The TPC of each 50% acetone extract was measured according to a previously reported laboratory procedure with minor modifications (Slavin, Kenworthy, et al., 2009). In short, 50 μL of soybean cracker extract, 250 μL of the Folin–Ciocalteu reagent, 750 μL of 20% sodium carbonate, and 3.0 mL ultrapure water comprised the reaction mixture. After 2 h reaction at ambient temperature, absorbance was read at 765 nm on a Genesys 20 spectrophotometer (Thermo Scientific, Waltham, MA, USA). Reactions were conducted in triplicate and compared against the results of a standard, gallic acid. Results were reported as gallic acid equivalents (GAE) per g of dry cracker.

2.6. Antioxidant activity

2.6.1. Oxygen radical absorbance capacity (ORAC)

The ORAC assay was conducted to assess peroxyl radical scavenging ability according to a previously reported laboratory protocol (Slavin, Kenworthy, et al., 2009). All reagents were prepared in 75 mM sodium phosphate buffer (pH 7.4), except the Trolox standard was prepared in the same solvent as sample extracts (50% acetone). The initial reaction mixture contained 225 μL of freshly made 81.6 nM fluorescein and 30 μL of sample, standard, or blank solution. Initial reaction mixtures were preheated in a 96-well plate at 37 °C for 20 min. Then, 25 μL of freshly made 0.36 M AAPH was added to each well. The fluorescence of the assay mixture was recorded on a Victor® multilabel plate reader (Perkin–Elmer, Turku, Finland) once every 2 min over 3 h at 37 °C (λex = 485 nm and λem = 535 nm). Trolox equivalents (TE) were calculated for samples on the basis of area under the curve. Results are expressed as micromoles of TE per g of dry cracker.

2.6.2. Hydroxyl radical scavenging capacity (HOSC)

The HOSC assay was conducted also using fluorescein (FL) as the fluorescent probe and a Victor® multilabel plate reader according to a previously reported laboratory protocol (Moore, Yin, & Yu, 2006). Trolox standards were prepared in the same solvent as sample extracts (50% acetone). The reaction mixture contained 170 μL
of 92.8 nM FL; 30 μL of sample, standard or blank; 40 μL of 0.1990 M H₂O₂; and 60 μL of 3.43 M FeCl₃. The FL working solution was prepared fresh for each assay from stock solution and 75 mM sodium phosphate buffer (pH 7.4). The fluorescence of the reaction mixture was recorded approximately once every 4 min over 7 h at ambient temperature (λₑₓ = 485 nm and λₑₛ = 535 nm). Trolox equivalents were calculated for samples using the same AUC calculations as in ORAC (Moore et al., 2006). Results are expressed as micromoles of TE per g of dry cracker.

2.6.3. ABTS radical cation scavenging capacity

The scavenging capacity against ABTS⁺ was measured according to a previously reported method (Slavin, Kenworthy, et al., 2009). Briefly, ABTS⁺ working solution was prepared by reacting 2,2’-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diazoni-um salt with manganese oxide (MnO₂) in solution, which was filtered and diluted to an absorbance of 0.700 at 734 nm on a Genesys 20 spectrophotometer (Thermo Scientific, Waltham, MA, USA). The reaction mixture contained 80 μL of sample or standard plus 1 mL of the working ABTS⁺ solution. Mixtures were vortexed for 30 s and absorbance was read at 734 nm at 90 s reaction time. Results were compared to a standard curve constructed with trolox in 50% acetone.

2.7. Cyanidin-3-glucoside content

Cyanidin-3-glucoside (C3G) is the primary anthocyanin present in black soybeans, and was therefore chosen as the one anthocy-a-nin to analyse in this study (Lee et al., 2009; Zhang et al., 2011). To assess C3G content, the 50% acetone extracts were injected directly onto the HPLC for analysis according to the method employed in our previous work (Slavin, Kenworthy, et al., 2009). The Shimadzu Promence HPLC system consisted of an autosampler, a Phenom-ex Gemini C18 column (150 mm × 4.6 mm × 5 μm particle size) at 25 °C and UV/Vis detector at 530 nm. Separation was achieved via a gradient elution at 1 mL/min, using 0.1% acetic acid in aceto-nitrile (A) and 0.1% acetic acid in water (B). The initial condition was 100% B, which then followed a linear gradient over 10 min to 85% B, then another linear gradient to 75% D at 20 min, held for 5 min at 10% B, and finally re-equilibrated at initial conditions for 5 min. Total run time was 30 min, and C3G elution was identified and quantified as compared to the elution time and area of an external standard. Results were expressed as µg/g of soybean cracker on a dry weight basis.

2.8. Isoflavone content

Prior to HPLC analysis of isoflavones, samples were hydrolyzed with base (via the method of Klump, Allred, MacDonald, & Ballam, 2001) to leave only the β-glycosides and aglycones. In short, defatted cracker was extracted with 80% methanol at 65 °C in a shaking water bath for 2 h, then hydrolyzed with 2 M KOH, neutralised with acetic acid, centrifuged and filtered through a 0.45 μm poly-tetrafluoroethylene (PTFE) membrane syringe filter prior to injection.

Analytical conditions were determined by the method of Collison (2008), as follows. A Phenomenex C18(2) Luna column (250 mm × 4.6 mm × 5 μm) was held at 40 °C. Solvents were 0.05% phosphoric acid in water (A), and acetonitrile (B). A linear gradient started at 10% B and reached 30% B in 60 min, followed by a 3 min wash at 90% B and 10 min re-equilibration at 10% B. The flow rate was 1.5 mL/min. Isoflavone elution was monitored at 260 nm, and peaks were identified and quantified as compared to the elution time and areas of external standards. Results were expressed as µg/g of soybean cracker on a dry weight basis.

2.9. Hydroxymethylfurfural (HMF) content

HMF in samples was determined by a previously reported method, with modifications (Garcia-Villanova, Guerra-Hernandez, Martinez-Gomez, & Montilla, 1993). In short, 0.3 g of sample was vortexed with 3.5 mL ultrapure water, centrifuged, and the supernatant removed. The extraction was repeated twice, followed by clarification with 0.5 mL each 15% potassium ferrocyanide (w/v) and 30% zinc acetate (w/v). The supernatant after centrifugation was removed and diluted to exactly 10 mL filtered through a 0.45 μm syringe filter, and 20 μL was injected for HPLC analysis. Separation was achieved on a C18(2) Phenomenex Luna column (250 mm × 4.6 mm × 5 μm) held in a 25 °C oven with a 1 mL/min isocratic elution of acetonitrile: water (95:5). HMF elution was monitored via UV absorption at 285 nm. HMF was identified and quantified as compared to the elution time and area of an external standard. Results were expressed as µg/g of soybean cracker on a dry weight basis.

2.10. Phenolic acids

Each soybean cracker sample was analysed for their soluble free, soluble conjugated, and insoluble bound phenolic acid compositions, according to a previously reported method (Moore et al., 2005). The soluble free, soluble conjugated, and insoluble bound phenolic acids were extracted following a combined solvent and pH extraction and fractionation, and alkaline-catalysed release of bound phenolic acids from the solid grain matrix. After evaporation of solvents, each phenolic acid extract was redissolved in MeOH. Phenolic acid composition in the MeOH solution was analysed by HPLC using a Phenomenex C18(2) Luna column (250 mm × 4.6 mm × 5 μm). Phenolic acids were separated using a linear gradient elution program with a mobile phase containing solvent A (water/acetonic, 98:2, v/v) and solvent B (water/acetoni-trile/aceticoic, 68:30:2, v/v/v). The solvent gradient was increased from 10% to 100% B over 42 min with a constant flow rate of 1.0 mL/min and absorbance was monitored at 290 nm. Identification and quantification of individual phenolic acids including ferulic acid (FA), p-coumaric acid (p-CA), syringic acid (SA) and vanillic acid (VA) was accomplished by comparing the retention time and area under the curve of sample peaks to those of the external standards. Results were expressed as µg/g of soybean cracker on a dry weight basis.

2.11. Statistical analysis

Data were reported as means ± standard deviations (SD) for triplicate treatments. One-way analysis of variation (ANOVA) and Tukey’s tests were performed using SPSS (SPSS for Windows, version 10.0.5, SPSS Inc., Chicago, IL, USA). Correlation analyses were performed using a two-tailed Pearson’s correlation test. Statistical significance was declared at P < 0.05.

3. Results and discussion

3.1. Water content

The water content of soybean cracker samples is shown in Table 1. Similar trends were seen in both yellow and black soybean crackers. Across temperature treatments, the low (121C60m) and moderate (149C13m) temperature treatments retained similar amounts of water content. The high temperature treatment (177C10m) lost the most water during baking in yellow soybeans, whereas the moderate temperature/long time combination (149C16m) held this distinction in black soybean crackers due to
high variation in the high temperature treatment. Across time treatments at a constant temperature, the short time (149°C10m) predictably contained the highest water content and the long time contained the lowest water. (Unbaked control is not considered in comparison of water content because it was freeze-dried after dough mixing to ensure adequate solvent extraction in later steps.) In general, it is not surprising to see that higher temperatures and longer times produced crackers containing lower water content.

Table 1

<table>
<thead>
<tr>
<th>Sample</th>
<th>Water content (%)</th>
<th>HMF (μg/g)</th>
<th>C3G (μg/g)</th>
<th>C3G Loss (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yellow</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>121°C60m</td>
<td>3.4b±0.1</td>
<td>ND</td>
<td>ND</td>
<td>–</td>
</tr>
<tr>
<td>149°C13m</td>
<td>3.5bcd±0.1</td>
<td>ND</td>
<td>ND</td>
<td>–</td>
</tr>
<tr>
<td>177°C10m</td>
<td>0.4a±0.2</td>
<td>39a±28</td>
<td>ND</td>
<td>–</td>
</tr>
<tr>
<td>149°C10m</td>
<td>4.6d±0.7</td>
<td>ND</td>
<td>ND</td>
<td>–</td>
</tr>
<tr>
<td>149°C16m</td>
<td>1.4abc±0.2</td>
<td>3a±0.5</td>
<td>ND</td>
<td>–</td>
</tr>
<tr>
<td>UC</td>
<td>4.0cd±0.04</td>
<td>ND</td>
<td>ND</td>
<td>–</td>
</tr>
<tr>
<td>Black</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>121°C60m</td>
<td>3.5bcd±0.3</td>
<td>ND</td>
<td>1000±0.5</td>
<td>–69%</td>
</tr>
<tr>
<td>149°C13m</td>
<td>3.5bcd±0.5</td>
<td>ND</td>
<td>192±14</td>
<td>–40%</td>
</tr>
<tr>
<td>177°C10m</td>
<td>2.2abc±0.8</td>
<td>32a±21</td>
<td>83±29</td>
<td>–74%</td>
</tr>
<tr>
<td>149°C10m</td>
<td>4.8d±0.2</td>
<td>ND</td>
<td>251±10</td>
<td>–21%</td>
</tr>
<tr>
<td>149°C16m</td>
<td>1.2ab±0.4</td>
<td>3a±1</td>
<td>102±2</td>
<td>–68%</td>
</tr>
<tr>
<td>UC</td>
<td>3.5bcd±0.2</td>
<td>ND</td>
<td>319±8</td>
<td>–</td>
</tr>
</tbody>
</table>

All values are expressed as amounts per grams of dry cracker. ND = not detected; Values marked by the same letter within a column are not statistically different. UC represents an unbaked control, consisting of mixed dough that was freeze-dried.

3.2. Total phenolic content

Black soybean crackers contained significantly higher TPC than yellow crackers at all time and temperature treatments. We have previously reported that Peking soybeans contain significantly higher TPC than yellow Manokin soybeans (Slavin, Kenworthy, et al., 2009), and this trait has carried over to the baked crackers. The range of TPC in baked soybean crackers matches expectations, based on the range detected previously in yellow soybeans at 1–2 mg GAE/g soybean flour, black soybeans ranging from 3 to 12 mg GAE/g flour, with Peking black soybeans at the top of the range (Slavin, Kenworthy, et al., 2009) and that detected in baked whole wheat pizza crusts (approximately 1 mg GAE/g, Moore, Luther, Cheng, & Yu, 2009). Given the ratios of whole wheat and soy ingredients used in the cracker recipe in this study, values of approximately 2.5 and 5 mg GAE/g dry cracker are in agreement with these previous values.

Both colours of soybean showed some indication of loss of extractable TPC during baking. TPC decreased significantly under all time/temperature combinations for black soybean crackers (Fig. 1). A trend was noted within the length of time of moderate heat treatment of black soybean crackers: longer treatment resulted in lower TPC, such that UC > 149°C10m > 149°C13m > 149°C16m. The 149°C16m treatment resulted in the greatest loss of TPC in black crackers, a decrease of 12% from control. In addition, low temperature with longer time (121°C60m) and high temperature with shorter time (177°C10m) have less losses of TPC as compared to the 149°C16m treatment, but show more losses of TPC than moderate temperature with short time (149°C10m), suggesting that shorter thermal treatment with moderate temperature is best able to preserve TPC in black crackers.

Differences were less apparent in yellow soybean crackers, and significant losses of TPC compared to UC were only seen for 149°C10m and 149°C13m treatments (Fig. 1). Interestingly, Moore et al. (2009) also noted a decrease in TPC values with milder heat treatments in baked whole wheat pizza crust, whereas the high temperature and long baking time treatments were equivalent to unbaked control. These results suggest that baking may initially destroy available phenolics, but additional chemical shifts that release phenolics may occur during extended or higher temperature baking to make up for this initial loss.

Given that only black soybean crackers contain anthocyanin, and they are apparently subject to greater degradation during thermal processing, the more consistent loss of TPC in black soybean crackers may be due in large part to loss of anthocyanins. Though results of C3G do not follow exactly the same trend, black soybeans contain several other anthocyanins which are also heat labile and were not monitored in the current work (Lee et al., 2009; Xu & Chang, 2008; Zhang et al., 2011), whose loss may also contribute to the overall loss of phenolics.

3.3. Antioxidant activity

The soybean crackers of the present study contain multiple sources of antioxidants, including anthocyanins and isoflavones in soybeans, as well as phenolic acids in both soybean and whole wheat. On a weight basis of the whole grain, yellow soy and wheat have remarkably similar antioxidant properties in terms of ABTS radical cation and peroxyl radical (ORAC) scavenging, as well as total phenolic contents (Moore et al., 2005; Slavin, Kenworthy, et al., 2009). Black soybeans, however, exhibited significantly greater antioxidant activity in these assays, exhibiting a ten-fold difference in TPC and ORAC values. Though it is increasingly apparent that health effects of the antioxidant components are mitigated by biological responses beyond direct radical scavenging, assessment of antioxidants in foods remains of interest for several reasons. First, testing provides a quick assessment of the presence of these compounds, and in this study, whether they degraded. Second, particularly with anthocyanins, the presence of other pigments/antioxidants in a food has been associated with a greater stability of anthocyanins overall (Patras et al., 2010). Thus, monitoring of antioxidant abilities of a food may provide insight into stability and preservation of these components.

Given that the mechanisms of anthocyanin breakdown are incompletely understood, it has been suggested that the breakdown products of anthocyanins maintain antioxidant properties (Patras et al., 2010). Therefore, changes in antioxidant activity may not be evident upon their breakdown. Furthermore, anthocyanins make a small fraction of the total phenolics in the black soybean crackers (319 μg C3G/g compared to approximately 5000 μg GAE/g of total phenolics) in the samples. Small losses of antioxidant ability due to anthocyanin degradation may only represent a small fraction of the total chemical antioxidant ability.

In all antioxidant activity assays, black soybean crackers exhibited significantly higher radical scavenging than yellow soybean crackers at all time and temperature combinations (Fig. 2). This could be explained by their higher level of total isoflavones and the presence of anthocyanins in black soybeans but not yellow, though the yellow soybean crackers had comparable levels of phenolic acids (Table 3).
3.3.1. ABTS⁺ scavenging

Scavenging of ABTS radical cations is used among the battery of antioxidant assays as an indicator of reducing ability of stable, nitrogen-centered radical cations (Magalhaes, Segundo, Reis, & Lima, 2008). The assay was previously called the Trolox equivalent antioxidant capacity (TEAC) prior to the use of trolox in other antioxidant assays.

Yellow soybean cracker samples in the current study followed a consistent trend of increasing ABTS⁺ scavenging with higher temperature and longer treatments (Fig. 2A). The trend was particularly evident across time of treatment in samples baked at 149 °C. Average values increased from 5.2 μmol TE/g for the unbaked yellow control to 5.6, 5.9 and 7.5 μmol TE/g for baking times of 10, 13 and 16 min, respectively. Also, the yellow crackers baked at the low temperature for an extended time (121 °C × 60 min) showed a significant 35% increase in ABTS⁺ scavenging, as did the high temperature–low time combination (177 °C × 10 min) with an even greater 77% increase.

Interestingly, black soybean crackers did not exhibit a similar trend. The low temperature–long time combination (121 °C × 60 min) and medium temperature–high time combination (149 °C × 16 min) both lowered ABTS⁺ scavenging by 12%, whereas these treatments had increased scavenging in their yellow counterparts. The increases of scavenging seen in yellow samples may be due to liberation of phenolics from esters and other side chains, as is suspected to be the case with isoflavones, which would increase their direct scavenging ability. It is suspected that the instability of anthocyanins during the more extreme heat treatments (Table 1) may be contributing to the loss of scavenging ability and masking potential patterns of antioxidant activity occurring due to alteration of other chemical species.

3.4. Cyanidin-3-glucoside

C3G is the predominant anthocyanin in black soybeans (Lee et al., 2009; Zhang et al., 2011), and is not detected in other colours of soybean. Since the majority of anthocyanin stability data pertain to fruit juices and fruit purees, dry heat processing of this unique anthocyanin food source was studied in a food model appropriate for soybeans—baked crackers. The unbaked control (UC) contained the highest amount of C3G, at 319 μg/g dry cracker (Table 1). C3G degradation was noted under all baking conditions, but was most severe at extreme temperature (177°C10m) and time (121C60m, 149C16m) conditions, where losses of 74%, 69%, and 68% were detected, showing that the higher baking temperature even with limited time exposure resulted in the greatest loss of C3G and longer baking time also degraded the C3G in a high amount.
Loss of C3G expectedly increased with increasing time, however, the magnitude of loss was striking over short time intervals. Interestingly, 3 min intervals between treatments at the moderate temperature of 149 °C showed that a 21% loss of C3G at 10 min jumped to a 40% loss at 13 min and 68% loss at 16 min. Any benefits which would expected from a lower baking temperature appear to be negated by the extended baking time necessary to achieve a desirable product, as in treatment 121°C60m.

Results indicate that the best baking conditions for preserving C3G would be minimal baking time at a moderate temperature. Still, baking conditions must be selected carefully due to the significant drop in C3G from just 10–13 min.

The effects of thermal processing on food anthocyanin stability have been reviewed (Patras et al., 2010), however, the authors note the limited available data on the topic. Additionally, methods for which there is data include almost exclusively moist heat processing: boiling, steaming, blanching, pasteurisation, and canning. Nonetheless, this previous research presents strong evidence for degradation of anthocyanins with increasing magnitude and duration of heating. The current research agrees with these previous trends, though a more direct comparison would be necessary to determine if baking is more or less detrimental than the moist heat methods. Still, the current results suggest that a baked soybean product has potential to retain significant amounts of C3G if exposure to heat is controlled, providing a potentially significant alternate source of anthocyanins in the diet. In summary, minimal baking time at moderate temperature produced the smallest loss in C3G.

Fig. 2. Antioxidant activity of soybean cracker samples. Results of soybean cracker extracts scavenging against several different radical systems are presented: (A) ABTS•−, (B) hydroxyl radicals (HOSC), and (C) peroxyl radicals (ORAC). Results are expressed as μmol trolox equivalents (TE) per gram of dry cracker. Mean values of samples baked in triplicate are reported with standard deviation indicated with error bars. Bars marked by the same letter are not statistically different (P < 0.05).
Development of HMF was measured as an indication of the extent of Maillard, nonenzymatic browning during cracker baking. HMF has also been used as an indicator of the heat effects of thermal processing during manufacture of certain grain foods (Ramírez-Jimenez, Guerra-Hernandez, & Garcia-Villanova, 2006). HMF was detectable only in high temperature (177°C10m) and high time (177°C10m) treatments do not achieve the same result. Nonetheless, the distinction between aglycone (suffix -ein) and the aglycone and glucoside forms allows for HPLC analysis of the 6 glucoside forms (Wilson, 2004). However, a simplified analysis therefore were of interest to study in the current samples. Phenolic acids are present in both soybeans and whole wheat, having antioxidant activity, and have been noted to vary under different baking conditions in pizza crust (Moore et al., 2009), and therefore were of interest to study in the current samples. Ferulic acid is the primary phenolic acid in whole wheat (Cheng et al., 2006), and baking of whole wheat pizza crust was shown to increase amounts of soluble free ferulic acid, while decreasing soluble conjugated ferulic acid (Moore et al., 2009). To make an acceptable product, the crackers in the current study contain more wheat than soy (44% of dry ingredients by weight wheat vs. 34% soy). Combined with baking temperatures of crackers in this study (121–177 °C) were less extreme than the pizza crust study (up to 288 °C), which may partially explain why trends were less evident than in previous work. Nonetheless, a trend was noted in baked soybean cracker phenolic acid content. The highest temperature treatment (177C10m) consistently produced the highest value of all 4 soluble free phenolic acids in both yellow and black soybean (Table 3). This indicates that a threshold temperature may be necessary to encourage cleavage to the free phenolic form, whereas the free phenolic acid amounts in lower temperature/longer time treatments do not achieve the same result. Soluble free, conjugated and insoluble bound phenolic acids were detected in all samples (Table 3). Ferulic acid was the predominant phenolic acid both in soluble free and conjugated acids. The concentration of ferulic acid ranged from 0.13 to 1.75 µg/g soybean cracker in soluble free and conjugated, respectively. Our result was similar to that reported by Mattila, Pihlava, and Hellström (2005) in wheat samples in that ferulic acid was the primary phenolic acid both in insoluble bound and conjugated acids, followed by p-coumaric acid and vanillic acid. The concentrations in the current samples appeared to be more consistent with that of levels previously detected in soybeans. Insoluble bound ferulic acid ranged from 4.10 to 20.79 µg/g soybean cracker. This was consistent to the results of Taie, El-Mergawi, and Radwan (2008) that the ferulic acid content of soybean seeds after alkaline hydrolysis was between 20 and 30 µg/g. The highest insoluble bound ferulic acid was observed in the high (149C16m) samples. Furthermore, variability in these samples was extreme. HMF was therefore not a useful indicator of heat treatments for the majority of samples in this study. However, those samples that developed HMF were also those which experienced the greatest losses of C3G, suggesting that conditions suitable for developing HMF in a baked black soy product may also elicit significant loss of anthocyanin.

3.7. Phenolic acids

Table 2

<table>
<thead>
<tr>
<th>Sample</th>
<th>Total isoflavones (µmol/g)</th>
<th>Daidzin (µg/g)</th>
<th>Daidzein (µg/g)</th>
<th>Genistin (µg/g)</th>
<th>Genistein (µg/g)</th>
<th>Glycitin (µg/g)</th>
<th>Glycitein (µg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Yellow</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>121C60m</td>
<td>2.13b ± 0.04</td>
<td>400b ± 3</td>
<td>22b ± 3</td>
<td>382b ± 10</td>
<td>19ab ± 0.5</td>
<td>49c ± 1</td>
<td>6.2b ± 0.3</td>
</tr>
<tr>
<td>149C13m</td>
<td>2.11b ± 0.04</td>
<td>403b ± 9</td>
<td>16b ± 1</td>
<td>384b ± 5</td>
<td>16ab ± 0.9</td>
<td>49c ± 1</td>
<td>5.4b ± 0.4</td>
</tr>
<tr>
<td>177C10m</td>
<td>2.08b ± 0.03</td>
<td>392b ± 5</td>
<td>21b ± 4</td>
<td>368b ± 10</td>
<td>22a ± 5</td>
<td>43c ± 2</td>
<td>8.5b ± 2.3</td>
</tr>
<tr>
<td>149C16m</td>
<td>2.05b ± 0.03</td>
<td>399b ± 5</td>
<td>15b ± 3</td>
<td>371b ± 8</td>
<td>15ab ± 2</td>
<td>49c ± 0.9</td>
<td>5.3b ± 0.6</td>
</tr>
<tr>
<td>UC</td>
<td>2.06b ± 0.12</td>
<td>382b ± 25</td>
<td>27b ± 8</td>
<td>361b ± 26</td>
<td>18ab ± 5</td>
<td>50c ± 3</td>
<td>6.5b ± 0.4</td>
</tr>
<tr>
<td><strong>Black</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>121C60m</td>
<td>3.80a ± 0.04</td>
<td>817a ± 5</td>
<td>28b ± 0.4</td>
<td>572a ± 12</td>
<td>11ab ± 0.6</td>
<td>149a ± 3</td>
<td>8.7b ± 0.2</td>
</tr>
<tr>
<td>149C13m</td>
<td>3.61a ± 0.04</td>
<td>784a ± 7</td>
<td>17b ± 1</td>
<td>553a ± 13</td>
<td>6b ± 0.7</td>
<td>149a ± 5</td>
<td>7.4b ± 0.1</td>
</tr>
<tr>
<td>177C10m</td>
<td>3.75a ± 0.10</td>
<td>802a ± 27</td>
<td>30b ± 11</td>
<td>555a ± 18</td>
<td>20a ± 10</td>
<td>128b ± 14</td>
<td>18.2± 6.6</td>
</tr>
<tr>
<td>149C16m</td>
<td>3.63a ± 0.07</td>
<td>787a ± 6</td>
<td>16b ± 1</td>
<td>554a ± 15</td>
<td>5b ± 2</td>
<td>151a ± 6</td>
<td>7.4b ± 0.4</td>
</tr>
<tr>
<td>UC</td>
<td>3.77a ± 0.13</td>
<td>822a ± 20</td>
<td>21b ± 0.7</td>
<td>574a ± 17</td>
<td>9b ± 0.4</td>
<td>147a ± 11</td>
<td>9.7b ± 0.6</td>
</tr>
</tbody>
</table>

All values are expressed as amounts per g of dry cracker. Values marked by the same letter within a column are not statistically different. UC = unbaked control. Yellow soybeans are included as a reference.

3.5. Isoflavones

As with any soy food for health, isoflavone content is of interest. Black soybean crackers in this study contained significantly more isoflavones than yellow soybean crackers (Table 2), likely due to the genotype of soybean selected. As shown in our previous work (Slavin, Kenworthy, et al., 2009), black soybeans may contain higher total isoflavones on average than yellow soybeans, and Peking soybeans used here were among those black soybeans particularly high in isoflavones. Within each colour of soybean cracker, total isoflavones did not vary. Yellow crackers contained approximately 2.1 µmol isoflavones/g dry cracker while black crackers contained slightly less than twice that amount, in the range of 3.6–3.8 µmol/g dry cracker. Previous work has indicated that heat treatment of soybeans and soy food products results in significant changes in forms of isoflavones, particularly from the malonyl-glucoside form to the acetyl- or β-D-glucoside forms (Wilson, 2004). However, a simplified analysis procedure was utilised to convert glucoside ester forms of isoflavones into their glucoside forms, allowing for HPLC analysis of the 6 aglycone and β-D-glucoside forms (Klump et al., 2001), and thus preventing observation of the malonyl and acetyl-glucoside forms. Nonetheless, the distinction between aglycone (suffix -ein) and the β-D-glucoside (suffix -in) forms does allow to see minor differences during baking. Glycitin levels are lowest in the samples exposed to the extreme high temperature treatment (177C10m) and glycitein levels are correspondingly higher, indicating that the cleavage of the glucose side chain of glycinin may occur during high heat baking of the soy crackers, and leave behind glycitin. The difference is significant for glycitin/glycitein in black soybean crackers, but a similar trend is noted with yellow crackers and for genistin/genistein in both colours, though the differences do not reach statistical significance. At least two previous studies have reported a similar maintenance of total isoflavones during the baking process of a soy and wheat bread, while noting shifts in composition from malonylglucoside to acetylglucosides and β-glucosides during baking (Shao et al., 2009; Zhang, Lee, Vodovozt, & Schwartz, 2004). Shifts to increase aglycones after baking were also noted.

3.6. HMF

Development of HMF was measured as an indication of the extent of Maillard, nonenzymatic browning during cracker baking. HMF has also been used as an indicator of the heat effects of thermal processing during manufacture of certain grain foods (Ramírez-Jimenez, Guerra-Hernandez, & Garcia-Villanova, 2006). HMF was detectable only in high temperature (177C10m) and high time (149C16m) samples. Furthermore, variability in these samples was extreme. HMF was therefore not a useful indicator of heat treatments for the majority of samples in this study. However, those samples that developed HMF were also those which experienced the greatest losses of C3G, suggesting that conditions suitable for developing HMF in a baked black soy product may also elicit significant loss of anthocyanin.
temperature–short time treatment (177°C10m). Previously published data on baked whole wheat pizza crust agreed that ferulic acid was present primarily in the insoluble-bound form, though in considerably higher quantities 200–300 μg/g dry pizza crust (Moore et al., 2009). Though wheat was the predominant grain in the crackers, the lower quantity of ferulic acid noted in the current study could be due to the use of a different (commercial) source of whole wheat flour, as well as the ‘dilution effect’ of soy flour and corn meal in the recipe.

4. Conclusions

Baked crackers have been demonstrated as a potential model for studying the effects of dry-heat thermal effects on anthocyanins in black soybeans. Results showed that anthocyanin loss is dependent on time and temperature combinations of baking conditions. Isoflavones and phenolic acids are retained, but shifts in form may occur during baking. Crackers prepared with black soybeans had significantly higher TPC, total isoflavones, and per-
Acknowledgements

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References


