

Effects of Roasting Temperature on Anti-Nutritional Factors and Antioxidant Property of Adzuki Bean (*Vigna angularis*) Flour

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ABSTRACT

Roasting process could be used to reduce the anti-nutritional factors (ANFs) of adzuki bean (*Vigna angularis*). In this research, adzuki beans were roasted for 20 min at different temperatures of 150 °C (R150), 165 °C (R165) and 180 °C (R180). The effects of roasting temperature on ANFs and antioxidant activity of resultant adzuki bean flour were investigated. Results indicate that roasting resulted in significant decreases in antinutrients including tannins (30 – 37%), and antitrypsin inhibitors (63 – 70%). Additionally, the reduction in trypsin inhibitors was proportional to the increase in roasting temperature. However, this trend was not observed in the reduction of tannins. Roasting also caused the loss of about 10 – 20% anthocyanin content, and hence the resultant antioxidant capacity of roasted samples also decreased as compared to that of raw bean flour. The results also imply that with low levels of ANFs and relatively high anthocyanin content, roasted adzuki bean flour could be potentially used in combination with or as substitutes for cereal flour in the preparation of bakery products.

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1. Introduction

Pulses are important staple food around the world due to their nutritional values and excellent source of natural bioactive compounds [1]. Among them, adzuki bean or red bean (*Vigna angularis*) is a kind of traditional legume food that is widely cultivated and used throughout many countries of Asia [2-4]. Its high receptivity is not only attributed to the maroon seed colour, enjoyable taste, but also the richness in carbohydrate (62.9%) and dietary fiber (12.7%), protein (19.8%), minerals, vitamins, and polyphenols [4, 5]. Hence, adzuki beans have been widely used in preparation of traditional bean pastes in confectionery products, and a variety of foods such as adzuki rice, porridge, adzuki milk, desserts, cake, jelly, and ice cream [4]. Due to the distinct nutritional values, adzuki bean has also become a research interest in food product development, such as adzuki bean flour was used in sourdough fermentation to improve bread-making quality [6], or as meat extender and fat replacer in beef meatballs [7], or extruded adzuki bean flour was used in substitution for wheat flour in Chinese steamed bread [8].

In spite of the nutritional values, adzuki bean, like many other raw pulses, contains ANFs such as tannins, saponins, phytates, and protease inhibitors which could hinder the absorption and digestibility of dietary carbohydrates, proteins and minerals [9, 10]. The presence of ANFs hence can limit the applicability of adzuki bean in food products [6]. Few studies reported that the ANFs in pulses can be removed or reduced by a number of treatments including soaking, blanching, cooking, roasting, autoclaving or germination [11, 12]. Our previous study revealed that cooking and autoclaving completely removed the trypsin inhibitors and efficiently decreased tannin and saponin contents of green-kernel black gram (*Vigna cylindrica* (L.) Skeels) flour [13]. However, treatments in combination with water (i.e. soaking, germination, cooking, autoclaving) could cause the loss of anthocyanins, a group of antioxidants, due to their high water-solubility. On the other hand, roasting seems to be a more acceptable technique in consideration of preserving the anthocyanin content of adzuki bean, and thereby its antioxidant capacity. In addition, the formation of a great number of volatile compounds during roasting could change the beany off-flavor of raw bean to more pleasant odorants in roasted bean [14].

Although some studies successfully applied roasting to reduce the trypsin inhibitor activity of soybean (*Glycine max*) [15], and cowpea (*Vigna unguiculata* L. Walp) [16], or reduce the saponins and trypsin inhibitors in mung bean (*Vigna radiata*) [17], and sufficiently reduce saponins, tannins and trypsin inhibitors in green-kernel black bean [13], research on using roasting to reduce anti-nutrients in red bean is still limited. This study hence aimed to investigate the ANFs and antioxidant property of adzuki bean flour (ABF) roasted at different temperatures. Comparison among samples were also conducted to evaluate the applicability of roasted ABF in food products.

2. Materials and Methods

2.1. Materials and chemicals

Adzuki bean (*Vigna angularis*) was purchased from local market in Dong Nai province. Chemicals and standards of analytical grade such as trypsin, N_{α} -Benzoyl-L-arginine 4-nitroanilide hydrochloride (L-BAPA), Tris(hydroxymethyl)aminomethane, 2,2-Diphenyl-1-picrylhydrazyl (DPPH), (\pm)-6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (trolox) were purchased from Sigma-Aldrich or Merck. Other chemicals were purchased from Xilong Scientific Co., Ltd.

2.2. Sample preparation

Unqualified beans (i.e. broken or damaged beans) and foreign objects were hand-removed. After that the beans were soaked in water to remove small beans floating on water surface, and then thoroughly washed to remove dust, sand and soil. The washed beans were dried at 70 °C (Memmert UF260, Germany) until constant weight. The raw sample was then ground into flour using a grinder (800Y, China) and subsequently sieved at 0.045 mm, kept in zip bags and stored at 4 °C until further analyses.

Roasting procedure: the whole raw adzuki beans were roasted for 20 min at three different temperatures of 150 °C, 165 °C and 180 °C using a coffee bean roaster (Gene Café CBR-101, South Korea). All roasted bean samples were also ground, sieved and stored in the same conditions of raw ABF. The roasted bean flour samples were designated as R150, R165 and R180, respectively.

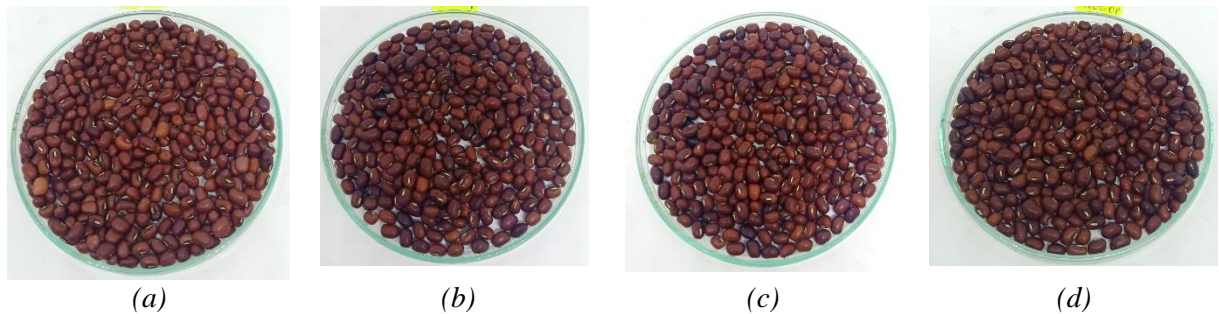


Figure 1. (a) Raw beans; and bean samples roasted at (b) 150 °C, (c) 165 °C and (d) 180 °C

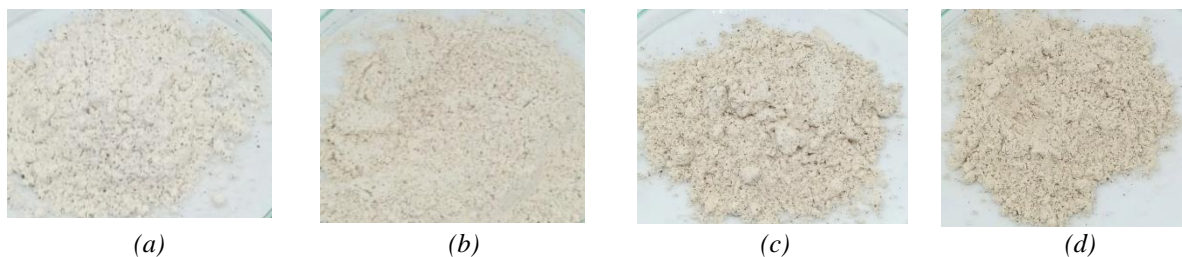


Figure 2. (a) Raw ABF, (b) R150, (c) R165, and (d) R180

2.3. Analytical methods

Moisture content of flour samples was determined by AOAC Official Method 925.10 [18]. The tannin content was analyzed by the method of Atanassova & Christova-Bagdassarian [19]. Trypsin inhibitory activity (TIA) was determined using spectrophotometric method of Nwosu (2011) with some modifications [20]. ABF of 2 g was added in 50 mL of 0.15 M NaCl solution. The mixture was shaken

at room temperature (150 rpm, 30 min) and the extract was then recovered by centrifuging at 4,500 rpm for 15 min (Hettich 1004, Germany). Standard trypsin solution was prepared and used to treat substrate (L-BAPA) solution. Standard trypsin solution of 0.4 mL was added into a test tube containing 0.4 mL of extract and 1 mL of BAPA solution. The blank sample was similarly prepared but 0.4 mL of extract was replaced by distilled water. The reaction solutions were incubated at 37 °C for 10 min, and were then stopped by adding 0.2 mL of 30% acetic acid solution. The absorbance of the solutions was measured at a wavelength of 410 nm using an ultraviolet–visible spectrophotometer (Double Beam Spectrophotometer UH5300, Hitachi, Japan). One unit of trypsin inhibitor (TIU) is defined as an increase of 0.01 absorbance unit at 410 nm [20]:

$$\text{Trypsin inhibitor} = \frac{A_u}{A_s} \times 0.01 \times \frac{V_f}{V_a \times W} \quad (1)$$

where A_u is the absorbance of test sample, A_s is the absorbance of blank sample; V_f and V_a (mL) are the total volume of extract and volume of extract analyzed, respectively; and W (g) is the weight of sample analyzed.

Anthocyanin content of ABF samples was determined by the pH-differential method [21]. ABF of 1 g was extracted in acidified ethanol aqueous solution at 60 °C for 60 min [22]. The extract was then recovered by centrifugation at 4,500 rpm for 15 mins (Hettich 1004, Germany). The ABF extract was thoroughly mixed with two buffer solutions, 0.025 M potassium chloride buffer (pH = 1.0) and 0.4 M sodium acetate buffer (pH = 4.5), at a ratio of 1 : 25 (v/v). The mixture was then spectrophotometrically measured at wavelengths of 520 nm and 700 nm, respectively. The anthocyanin content was calculated as follows [23]:

$$\text{Total anthocyanin} \left(\frac{\text{mg}}{\text{kg}} \right) = \frac{[(A_{520} - A_{700})_{\text{pH}1.0} - (A_{520} - A_{700})_{\text{pH}4.5}] \times MW \times DF \times 1000}{\epsilon \times 1 \times m} \quad (2)$$

where MW is the molecular weight (449.2 g/mol for cyanidin-3-O-glucoside), DF is the dilution factor, 1 (cm) is path length, and ϵ is the molar absorptivity ($\epsilon = 26,900$ L/mol/cm), m (g) is weight of sample analyzed.

The antioxidant capacity was evaluated through DPPH free radical scavenging ability [24]. Flour sample of 5 g was mixed with 100 mL of methanol. The content was kept at room temperature for 2 h and was subsequently centrifuged at 4,000 rpm for 15 min to remove the pellet. The 0.4 mL volume of supernatant was mixed with 4 mL of 0.1 mM of DPPH solution. The blank sample contained 0.4 mL of methanol (without any sample) and 4 mL of DPPH solution. After adding DPPH, the mixture was thoroughly mixed and kept in the dark for 30 min. Its absorbance was then measured at 517 nm (Double Beam Spectrophotometer UH5300, Hitachi, Japan). Trolox (1 mmol/L) was used as standard and the result was expressed as millimoles of Trolox equivalents per kg of sample (mmol TE/kg).

2.4. Statistical analysis

Each experiment was conducted with three replications. Statistical analyses were conducted by Minitab (version 19) using one-way ANOVA and Tukey's comparison tests with 95% confidence.

3. Results and Discussion

3.1. Moisture content

Results in Figure 3 show that moisture content significantly decreased ($p < 0.05$) from 7.5% (raw ABF) to 5.1% (R150), 4.6% (R165) and 2.7% (R180). The drop in moisture content was due to the progressive loss of water as a result of increasing roasting temperature. The low moisture content of roasted ABF samples (2.7 – 5.1%) could be beneficial in the prevention of microbial spoilage during storage. Similar trend was also observed for roasted pigeon pea (*Cajanus cajan*) flour [25]. Adeparusi (2001) also reported that the moisture content of lima bean (*Phaseolus lunatus* L.) flour significantly dropped from 7.7% (Raw) to 0.83% when lima beans were toasted at 204 °C for 20 min [26].

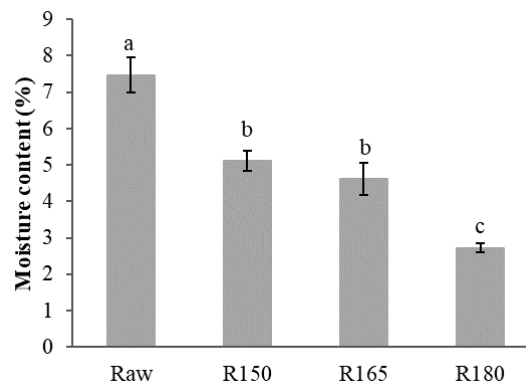


Figure 3. Moisture content of ABF samples. Different small letters indicate significant differences among samples ($p < 0.05$)

3.2. Anti-nutritional factors

3.2.1. Tannin content

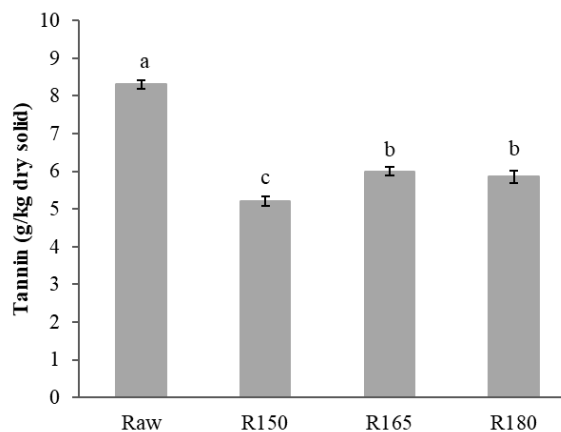


Figure 4. Tannin content of ABF after roasting at different temperatures. Different small letters indicate significant differences among samples ($p < 0.05$)

Adzuki beans have been reported to contain tannins [27]. Tannins can form insoluble complexes with proteins, and inhibit digestive enzymes such as trypsin, chymotrypsin, amylase and lipase. Therefore, consuming tannins containing foods could decrease protein digestibility or depress food intake [28, 29]. Results in Figure 4 show that tannin content of raw ABF (8.3 g/kg) was lower than that of raw green-kernel black bean flour (22.7 g/kg, [13]). Roasting pretreatment significantly decreased the tannin contents in red bean samples ($p < 0.05$). There was a slight increase in tannin content from 5.2 g/kg dry solid (R150) to about 6.0 g/kg dry solid (R165 and R180). Compared to the raw one, the tannin contents of samples R150, R165 and R180 decreased approximately 30 – 37%. The reduction in tannin content in roasted red bean flour can be explained by thermal degradation of phenolic compounds [30]. Similar reduction trend in tannins after roasting was also observed in green-kernel black gram flour (42.7%, [13]), soybean flour (approximately 83%, [31]), climbing bean (*Phaseolus vulgaris* L.) flour (45.6%, [32]).

3.2.2. Trypsin inhibitors

Trypsin inhibitors exist in a wide range of grain legumes such as soy beans, mung beans and chickpeas. Trypsin inhibitors commonly act on serine proteases, including trypsin and chymotrypsin, thereby reducing absorption and digestion of dietary proteins [33]. Thermal treatments (including roasting) are widely applied to inactivate thermolabile ANFs like trypsin inhibitors [33, 34].

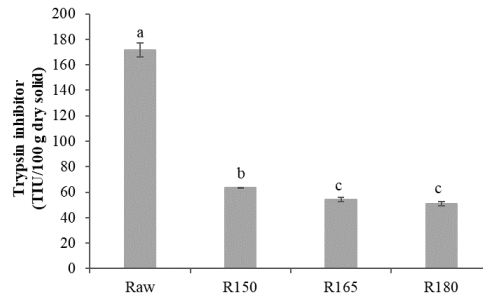


Figure 5. Trypsin inhibitors of ABF after roasting at different temperatures. Different small letters indicate significant differences among samples ($p < 0.05$)

As shown in Figure 5, roasting process can efficiently decrease trypsin inhibitor activity (TIA) of ABF. Roasting at 150 °C - 180 °C for 20 mins reduced up to 63 – 70% of total TIA. There was no significant difference in TIA among samples R165 and R180 ($p > 0.05$). Some previous studies had reported similar results on the substantial reduction of TIA using roasting technique, such as the complete removal of trypsin inhibitor in ‘oze’ (*Bosqueia angolensis*) seeds roasted at 150 °C for 45 minutes [20], or 62% decrease in total TIA of black soybean roasted at 230 °C for 25 min [34].

3.3. Anthocyanin content and antioxidant capacity

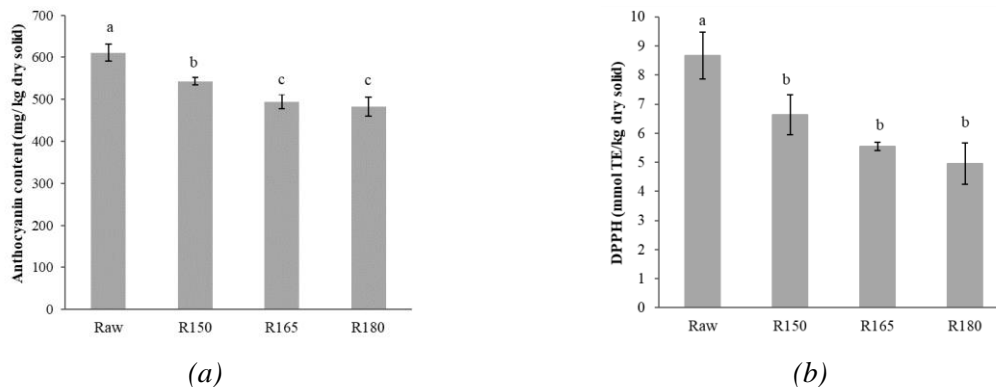


Figure 6. (a) Anthocyanin content and (b) antioxidant capacity of ABF roasted at different temperatures. Different small letters indicate significant differences among samples ($p < 0.05$)

Anthocyanins are the main pigment in the seed coat of adzuki bean [35], and they have been reported to have beneficial effects on human health due to their potent antioxidant and anti-inflammatory properties [36]. Results indicate that roasting can efficiently decrease ANFs (e.g. tannin and trypsin inhibitor) of adzuki beans, but it can also degrade their anthocyanin content. Therefore, it is necessary to determine the anthocyanin content as well as antioxidant capacity of roasted ABF samples.

As shown in Figure 6a, the anthocyanin content of raw ABF was 610.8 mg/kg dry solid, higher than those of black coat common bean (*Phaseolus vulgaris* L.) (440 mg/kg, [37]), lentil (*Lens culinaris*) (36.2 mg/kg, [38]), and lower than that of green-kernel black bean (791.6 mg/kg, [13]). Results show that all roasted ABF samples had significant reduction ($p < 0.05$) in anthocyanin content. In particular, the loss of anthocyanin content in R150 was only 10%, whereas the higher roasting temperatures of 165 °C and 180 °C resulted in higher loss of total anthocyanin content (about 20%). There was no significant difference in anthocyanin contents of R165 and R180. Previous studies also demonstrated the effectiveness of roasting in effectively reducing the ANFs of black gram flour while still preserving high levels of anthocyanin content [13, 39].

The antioxidant capacity of flour samples was measured through DPPH free radical scavenging activity. As a result of high anthocyanin content, raw ABF also possessed high value of antioxidant capacity (8.7 mmol TE/kg dry solid). Roasting at different temperatures (150 °C, 165 °C and 180 °C) significantly reduced antioxidant capacity (Figure 6b), but there was no significant difference in antioxidant activities among three roasted samples. The reduced antioxidant capacity of roasted adzuki beans were in accordance with those obtained in roasted black gram [13, 39].

4. Conclusions

Results confirm that roasting was applicable in reducing antinutrients of adzuki beans. Increased roasting temperature further decreased trypsin inhibitor activity of adzuki beans, but this trend was not observed in the reduction of tannins. Roasting also cause the reduction in anthocyanins and hence antioxidant capacity of ABF, however this reduction was still in acceptable range. In consideration of remaining antinutrient contents, anthocyanin content, and resultant pleasant flavors produced by roasting, ABF samples roasted at 150 °C and 165 °C could be potentially used as substitutes for or in combination with cereal flour in the preparation of bakery products.

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Conflict of Interest

The authors declare no conflict of interest.

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