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Physicochemical Characteristics, Antioxidant and Antibacterial Activities of Selected Raw and Roasted Legumes

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Abstract

The effects of roasting on the color, major and minor compositions of mung bean (*Vigna radiata*), adzuki bean (*Vigna angularis*), chickpea (*Cicer arietinum L.*), and yellow split pea (*Pisum sativum L.*) were studied. The legume seeds were roasted at 200°C for up to 20 min. The corresponding water extracts of both raw and roasted seeds were analyzed for their total phenolic and flavonoid contents, antioxidant and antibacterial activities. Results showed that roasting caused significant changes in color difference index (ΔE^*_{ab}) of seeds compared to the corresponding flours. Chemical analysis showed that the crude protein, fat, carbohydrate and ash contents increased with roasting time; whereas the moisture and crude fiber contents decreased due to roasting. Results suggested that roasting promoted the structural changes of phenolic compounds and formation of insoluble browning complexes and conjugated compounds, with resultant to the overall decrease in yield percentage and the amount of soluble phenolic contents. Although the formation of Maillard-type reaction products contributed the increase of flavonoid contents and antioxidant activities in chickpea and yellow split pea extracts, they did not exhibit antibacterial activity. Roasting generally increased the availability of nutrients in addition to the enhancement of appearance, but it affected their antioxidant and antibacterial activities.

Keywords

Legume seeds; Roasting; Color measurement; Chemical compositions; Antioxidant activity; Antibacterial activity

Introduction

Legumes have played a major role in traditional diets for centuries due to high amount of nutrients, such as carbohydrates, proteins, polyunsaturated fatty acids, dietary fibers, minerals and vitamins, as well as their beneficial effects in health, medicine and others [1]. Many studies focus on the beneficial properties of legume seeds as functional food ingredients, in an attempt to increase the commercial values of legume seed products. In Malaysia, the most common legumes in the daily diets include but not limited to mung bean (*Vigna radiata*), adzuki bean (*Vigna angularis*), chickpea (*Cicer arietinum L.*) and yellow split pea (*Pisum sativum L.*). The legumes are commonly processed by moist or dry heating for cooking purposes and also to remove the antinutritional factors that present in the legume seeds [2]. The processed legume seeds, after roasting, would be ground into flour. The heating process is assumed to be adequate for improving palatability before being incorporated into foods, but experimental evidence justifying this assumption is currently lacking.

Apart from the major nutritional components, phenolic compounds are naturally found in legume seeds. These compounds are categorized as secondary metabolites of plants and can be considered as anti-nutrients, simultaneously conferring health benefits [3]. They have been found to chelate metal catalysts, neutralize free radicals, activate antioxidative enzymes, reduce alpha tocopherol radicals and restrain oxidases thus effectively prevent oxidation in food products, and also act as a protective factor in the human body against oxidative diseases, such as atherosclerosis, cancer, and diabetes [4,5]. Other than antioxidant properties, phenolic compounds are also one of the biggest groups of plant secondary metabolites that possess antimicrobial activity. They disrupt the membrane proton motive force and result in cell lysis, oxidative phosphorylation, electron transport inhibition, perturbation of cell homeostasis, coagulation of cytoplasmic constituents, and affect the biosynthetic processes of macromolecules [6]. Hence, the potential health benefits of phenolic compounds in legume seeds have attracted increasing attention.

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Roasting is one of the common thermal processing methods which involves high temperature in order to reduce the moisture content, inhibit the growth of microorganisms and inactivate the enzymes present in legume seeds and thus enhance the shelf life of the products [7]. Roasting can affect the nutrients present in legume seeds where the complex protein, carbohydrate and fat could be broken down into peptides or amino acids, simple sugars and fatty acids, respectively, increase the availability of nutrients [8,9]. Besides, roasting may also promote Maillard reactions, which will lead to the formation of antioxidants [10]. Nevertheless, some studies demonstrated that prolonged roasting would lead to the inactivation of bioactive compounds and reduction in the total phenolic content [11,12]. In addition to different nature of legume seeds, such a processing step could have great impact on their nutritional values and potential health benefits. Therefore, this study was conducted to analyze the changes in characteristics of roasted legume seeds and their corresponding flours, including color, proximate composition and soluble phenolics, as well as to evaluate the antioxidant and antibacterial activities of their crude water extracts.

Materials and Methods

Materials

Mung beans (*Vigna radiata*), adzuki beans (*Vigna angularis*), chickpeas (*Cicer arietinum* L.), and yellow split peas (*Pisum sativum* L.) were obtained from a local supermarket in Kampar, Perak, Malaysia. All reagents and chemicals were of analytical grade and purchased from Sigma Aldrich, USA, Merck, Germany, and QRE©, Singapore, unless stated otherwise.

Sample preparation

Whole legume seeds (500 g) were spread out in an aluminum tray and roasted using a fan-force oven (Roller Grill, UK). The seeds were roasted at 200°C for 10 and 20 min, cooled immediately at room temperature and ground into flour using an A11 basic analytical mill (IKA, Netherlands).

Color measurements

The color of raw and roasted whole legume seeds and their corresponding flours were analyzed using a Konica Minolta spectrophotometer CM-600d (Konica Minolta, Japan). The instrument was calibrated using CM-A177 White Calibration Cap before each series of measurement. Color is defined by the L^* (0 = black, 100 = white), a^* (+value = redness, -value = greenness), and b^* (+value = yellowness, -value = blueness) tristimulus system (CEILAB). In order to ascertain the practical significance of changes in legume seed and flour color before and after roasting at various periods of time, a color difference index (ΔE^*_{ab}) was calculated from L^* , a^* , and b^* color coordinates using the following equation:

$$\Delta E^*_{ab} = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$$

Where, $\Delta L^* = L^*_0 - L^*_1$, $\Delta a^* = a^*_0 - a^*_1$, $\Delta b^* = b^*_0 - b^*_1$. Raw seed L^* , a^* and b^* values (subscript 0) and values at each roasting interval (subscript 1) were used to derive ΔE^*_{ab} values.

Proximate analysis

Moisture content of raw and roasted legume flour was determined using a moisture analyzer MX-50 (A&D Company Limited, Japan) by following the manufacturer's instruction. Total protein content was determined using Kjeldahl method with a nitrogen determination system consisting of K-436 Speed Digester, K-355 Distillation Unit and K-415 Scrubber (BÜCHI, Switzerland). Crude fiber content was analyzed using Fiber Bag-System, FBS6, crude fiber analyzer (C. Gerhardt GmbH & Co. KG, Germany). Total fat content was determined using SOX THERM® rapid extraction system (C. Gerhardt GmbH & Co. KG, Germany). Ash content was evaluated gravimetrically based on the weight of the sample after burning at 600°C for 24 h in a high temperature chamber furnace RHF 16/35 (Carbolite Gero, UK). All aforementioned procedures were carried out according to Association of Official Analytical Chemists (AOAC, 2006) methods,

while total carbohydrates were estimated by the difference of 100%.

Extraction of water soluble matter

Water soluble matter was extracted from raw and roasted legume flour in 10 volumes of distilled water with constant stirring at room temperature for 2 h. After centrifugation using a Refrigerated Tabletop Centrifuge (Sartorius, Germany) at 4000 rpm for 10 min, the supernatant (crude extract) was collected and filtered through Whatman No. 1 filter paper prior to lyophilization using a ScanVac CoolSafe freeze dryer (Labogene™, Denmark). The total yield was obtained by weighing the freeze-dried extracts.

Determination of total soluble phenolics

Total soluble phenolic content in the crude extract of legume flour was determined using the Folin-Ciocalteu procedure as previously described with modification [13]. Briefly, 200 μ L of Folin-Ciocalteu phenol-reagent was mixed with 100 μ L of extract (5 – 10 mg/mL of dry matter in distilled water) and left for 3 min at room temperature. After that, 800 μ L of 7% (w/v) of Na_2CO_3 solution was pipetted to the mixture and it was kept in the dark for 2 h. The absorbance of the mixture was read at 750 nm using a GENESYS™ 10S UV-Vis spectrophotometer (Thermo Fisher Scientific, USA). This assay was standardized against a calibration curve of gallic acid (20 – 100 μ g/mL) in 80% (v/v) aqueous ethanol. The results were expressed as milligram of gallic acid equivalents (GAE) per gram of dry matter. The linearity of the standard curve was determined to be $R^2 = 0.9961$, giving an absorbance range of 0.033 – 0.212 AU.

Determination of total soluble flavonoids

Total soluble flavonoid content in the crude extract of legume flour was determined according to the procedure as previously described with modification [13]. Briefly, 250 μ L of extract (5 – 10 mg/mL of dry matter in distilled water) was mixed with 1.25 mL distilled water and 75 μ L of 5% (w/v) of NaNO_2 solution in a test tube. After standing at room temperature for 6 min, 150 μ L of 10% (w/v) $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$ was added, and the mixture was left for further 5 min at room temperature, followed by the addition of 0.5 mL of 1 M NaOH solution. The absorbance of the mixture was read at 510 nm using a GENESYS™ 10S UV-Vis spectrophotometer (Thermo Fisher Scientific, USA). The concentration of flavonoids was obtained by referring to a standard curve of quercetin (20 – 100 μ g/mL) in 80% (v/v) aqueous methanol, with a linearity of 0.9917 within absorbance range of 0.101 – 0.331 AU. Total flavonoids were expressed as milligrams of quercetin equivalents (QE) per gram of dry matter.

Determination of antioxidant activity

ABTS free radical scavenging assay

The antioxidant activity of legume seed extract was determined by measuring the percentage of inhibition of peroxidation of ABTS radical cation using the procedure as previously described with minor modification [14]. A solution of 7 mM ABTS diammonium salt was mixed with 2.45 mM potassium persulfate ($\text{K}_2\text{S}_2\text{O}_8$) solution. The mixture was kept in the dark for 12 – 16 h at room temperature to produce a dark blue solution with stable absorbance. This stock solution of ABTS was then diluted with distilled water until an absorbance of 0.7 ± 0.02 at 734 nm. The resulting ABTS solution (1 mL) was then mixed with 100 μ L of crude extract (10 – 100 mg/mL of dry matter in distilled water). The mixtures were incubated for 5 min before the absorbance was read at 734 nm. The diluted ABTS solution without adding sample was served as control in this assay. Trolox standard solutions (0 – 75 μ g/mL) in 80% (v/v) ethanol were prepared and assayed under the same conditions as mentioned above. Results were expressed as milligram of Trolox equivalent (TE) per gram of dry matter. The 50% of inhibitory concentrations (IC_{50}) of the crude extracts were also reported.

DPPH free radical scavenging assay

Free radical scavenging activities of legume seed extract was measured using the procedure as previously described with minor modification [14]. Ten milligram of DPPH was dissolved in 250 mL of 80%

(v/v) aqueous methanol. For assay, 500 μ L of crude extract (10 – 100 mg/mL of dry matter in distilled water) was added to 700 μ L DPPH radical solution. The mixture was shaken vigorously and left in the dark for 30 min at room temperature prior to measurement of absorbance at 517 nm. The mixture of 500 μ L distilled water and 700 μ L DPPH radical solution was served as control in this assay. Trolox standard solutions (0 – 15 μ g/mL) in 80% (v/v) ethanol solution were prepared and assayed under the same conditions as mentioned above. Results were reported as milligram of Trolox equivalents (TE) per gram of dry matter. The 50% of inhibitory concentrations (IC_{50}) of the crude extracts were also reported.

Ferric reducing antioxidant power (FRAP) assay

FRAP assay was performed according to the procedure of Nithiyanantham et al. with modification [15]. Briefly, sodium acetate trihydrate (0.155 g) was added into distilled water (50 mL) and glacial acetic acid (0.8 mL) to prepare acetate buffer (300 mM). The acetate buffer was kept at pH 3.6. Then, FRAP reagent was prepared by mixing 50 mL of acetate buffer with 5 mL of 2,4,6-tri[2-pyridyl]-s-triazine (TPTZ) solution (10 mM in 40 mM HCl solution) and 5 mL of 20 mM $FeCl_3 \cdot 6H_2O$ solution. Next, the FRAP reagent (1.5 mL) was warmed at 37°C for 30 min before mixing with 30 μ L of crude extract containing 10 – 100 mg/mL. The mixtures were then incubated at 37°C for 30 min prior to the measurement of absorbance at 593 nm. Ferrous sulfate standard solution (200 μ L, 100 – 1000 μ M) were prepared and assayed under the same conditions as mentioned above. The assay was triplicated and the average FRAP value was reported as milligram Fe (II) equivalent per gram of dry matter accordance to the following equation:

$$FRAP \text{ value} = \frac{\text{ferrous sulfate} \left(\frac{\mu M}{mL} \right) \times \text{crude extract (mL)}}{\text{dry matter (g)}}$$

Well-diffusion antibacterial assay

Antibacterial activity of crude extract was evaluated using Muller-Hinton agar well-diffusion method as described by Mohapatra et al, with minor modification [16]. Briefly, bacteria culture (*Escherichia coli* ATCC 35218 and *Staphylococcus aureus* ATCC 33591) was inoculated in 2 mL of normal saline to achieve turbidity of 0.5 McFarland with optical density (OD) reading within 0.08 to 0.1 at 625 nm. The culture suspension was then streaked evenly on the surface of Muller Hinton agar using a sterile cotton swab. After that, wells with 6-mm diameter were prepared on the agar with a sterile cork borer. Each well was then filled with 60 μ L of crude extracts with concentrations corresponding to the IC_{50} values obtained from antioxidant assays. After incubation at 37°C for 24 h, the diameter of inhibition zone was measured in millimeters. The assay was carried out in triplicate and the results were recorded as mean $\pm$ standard deviation. Ciprofloxacin and gentamicin were used as positive controls, while distilled water was used as negative control.

Statistical analysis

All extractions and analyses were carried out in triplicate and results were reported in means and standard deviations. Means of collected data were subjected to analysis of variance (ANOVA) and t-test and the significant differences were reported at $p < 0.05$.

Results and Discussion

Effect of roasting on the color of legume seeds and flours

Figure-1 shows the changes of color in raw and roasted mung beans, adzuki beans, chickpeas, yellow split peas and their corresponding flours. Results showed that roasting at 200°C for up to 20 min had significant impact on the L^* , a^* , b^* and ΔE^*_{ab} values of all the tested legume seeds. When examined visually, all legume seeds was observed to change gradually from initial brighter to darker color, as shown in L^* values, indicating the loss of surface moisture that decreased the sample luminosity [17]. Similarly, the L^* values of their corresponding flours showed steadily decline after

roasting, with the greatest decline between 10 to 20 min roasting in adzuki bean flour (from 71.94 to 55.04) and chickpea flour (from 58.66 to 48.72). In general, all the corresponding flours had lighter color compared to the seeds, even after roasting up to 20 min. This might be due to the exposure of beige color inner part of the legume seeds after grinding. Throughout the 20-min period of roasting, instrumental measurement also showed continuous decreases in b^* value of all legume seeds, suggesting the fading of yellow hue. The b^* values declined gradually in the first 10-min roasting, except a sharp decline in chickpea (from 11.49 to 8.16), followed by evident declines in a further 10-min roasting, especially in mung beans (from 21.87 to 11.21) and yellow split peas (from 28.77 to 21.06). The decrease of yellowness in legume seeds is mostly due to the dissociation of some heat sensitive polyphenols and the loss of carotenoids [18]. On the other hand, the a^* values increased steadily in all legume seeds after roasting, suggesting the formation of deep red color. Between 0 to 10 min, a^* values of the corresponding flours were steady, except mung bean flour (increased from 0.10 to 1.75), but began a steady rise after that. In general, all the corresponding flours had lower a^* values compared to the seeds, even after roasting up to 20 min. Similar results have been reported for roasted wattle seeds and soybeans where a^* value increased sharply in roasting up to 20 min [19,20]. This could be due to the degradation of phospholipid and the Maillard reaction that forming brown pigments in the samples [21]. Oxidative polymerization of components in roasting process could develop distinct characteristics of legume seed products in terms of color and flavor. The color difference index (ΔE^*_{ab}) increased over the entire 20-min roasting process for both the legume seeds and flour; however, the overall changes to legume seeds were slightly higher than those to the corresponding flours at any given time (Figure 1A-1D).

Proximate composition of raw and roasted legumes

Dry heat processing may cause alteration in the quality of food materials due to evaporative loss or heat-induced chemical reactions. Table 1 shows the proximate composition of mung beans, adzuki beans, chickpeas and yellow split peas over the 20-min period of roasting. As expected, moisture has been driven off significantly from the legume seeds during the roasting process. With a gradual moisture loss (about 18 – 50%) throughout the 20-min period of roasting, the mung beans, adzuki beans, chickpeas and yellow split peas remained about 36%, 50%, 40% and 20%, respectively, of the initial moisture content. Similarly, a steady decline in crude fiber content was observed in all legume seeds throughout the roasting process. This could be due to the breaking of bonds between the polysaccharide chains and also the glycosidic linkages in dietary fiber, and thus increased the solubilization of fiber [22]. Decreased moisture and crude fiber contents as a result of roasting have also been reported in similar studies on kidney beans, African walnuts and asparagus beans [9,22,23]. On the other hand, the levels of protein increased gradually during the roasting process. Breaking down of crude protein to smaller polypeptides during processing might be the reason of the increment of total protein content of roasted legume seeds [24]. Besides, it has been reported that the increased activity of proteolytic enzymes in roasted food products will hydrolyze the inherent proteins to their constituent peptides and amino acids. These have also been reported in similar studies on mucuna seeds, mung beans and kenaf seeds [25,26]. Results strongly suggested the possibility of Maillard reaction between the available amino acids and reducing sugars, although the levels of carbohydrate showed a gradual increase with the roasting time, which could partly be due to the corresponding decrease in moisture. This is also in agreement with the results of inclining a^* values in the color measurement outlined above, indicating the development of browning pigments in roasting process (Figure 1B). Furthermore, it has been reported that thermal processing could break down starch granules and soften the cellulose that would consequently increase the total carbohydrates in legumes [27]. The increase of crude fat content was also indicated in all roasted legume seeds. The heat-induced destruction of cell structure and the efficient release of oil reserve could be the reasons

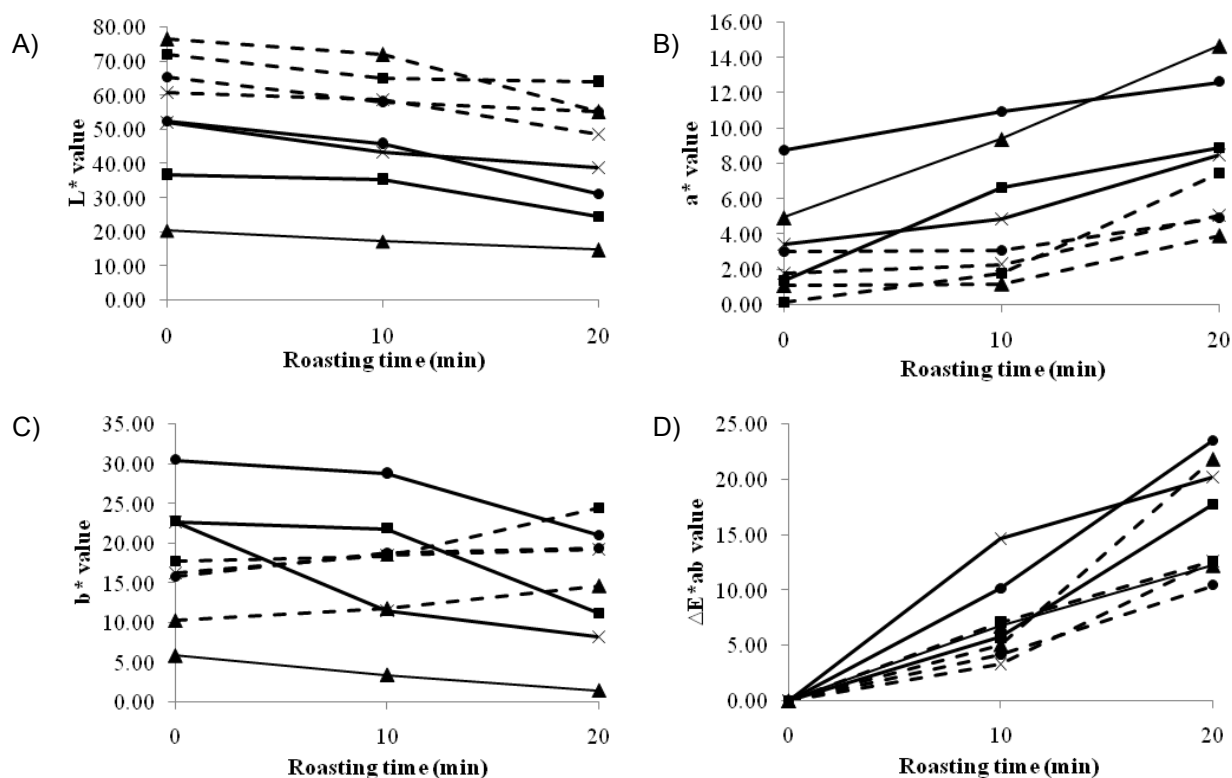


Figure 1: Effect of roasting at 200°C on (A) L*, (B) a*, (C) b* and (D) ΔE^*_{ab} values of the seeds (solid line) and flour (broken line) of mung bean (■), adzuki bean (▲), chickpea (×) and yellow split pea (●)

Legume sample	Roasting time (min)	Moisture	Protein	Carbohydrate ^B	Crude fat	Crude fiber	Ash
Mung bean	0	11.79 ± 0.19 ^a	28.94 ± 0.21 ^a	50.65 ± 0.48 ^a	0.86 ± 0.04 ^a	3.91 ± 0.08 ^a	3.85 ± 0.02 ^a
	10	8.21 ± 0.20 ^b	29.83 ± 0.05 ^{a,b}	53.01 ± 0.25 ^b	1.16 ± 0.19 ^b	3.76 ± 0.17 ^a	4.03 ± 0.01 ^b
	20	4.43 ± 0.11 ^c	30.89 ± 0.19 ^b	55.90 ± 0.20 ^c	1.24 ± 0.09 ^b	3.32 ± 0.19 ^b	4.22 ± 0.02 ^c
Adzuki bean	0	13.60 ± 0.27 ^d	26.42 ± 0.16 ^c	52.24 ± 0.35 ^b	0.73 ± 0.04 ^c	3.80 ± 0.07 ^a	3.21 ± 0.11 ^d
	10	10.50 ± 0.08 ^e	27.34 ± 0.20 ^d	54.62 ± 0.11 ^d	0.82 ± 0.04 ^d	3.37 ± 0.06 ^b	3.35 ± 0.03 ^d
	20	6.74 ± 0.06 ^f	28.52 ± 0.17 ^e	57.53 ± 0.11 ^e	0.92 ± 0.13 ^d	2.85 ± 0.10 ^c	3.44 ± 0.01 ^e
Chickpea	0	11.46 ± 0.01 ^a	20.53 ± 0.53 ^f	58.26 ± 0.91 ^e	4.72 ± 0.07 ^e	1.96 ± 0.07 ^d	3.07 ± 0.05 ^f
	10	9.67 ± 0.01 ^g	21.23 ± 0.10 ^f	59.92 ± 0.06 ^e	4.91 ± 0.11 ^f	1.27 ± 0.11 ^e	3.00 ± 0.03 ^f
	20	4.53 ± 0.01 ^h	22.17 ± 0.20 ^g	63.62 ± 0.70 ^f	5.64 ± 0.53 ^g	0.93 ± 0.06 ^f	3.11 ± 0.04 ^f
Yellow split pea	0	12.29 ± 0.01 ⁱ	22.30 ± 0.27 ^g	60.32 ± 0.15 ^g	1.33 ± 0.11 ^h	1.38 ± 0.06 ^e	2.38 ± 0.10 ^g
	10	5.94 ± 0.01 ^j	23.93 ± 0.37 ^{g,h}	65.69 ± 0.85 ^h	1.46 ± 0.12 ^h	0.89 ± 0.12 ^f	2.09 ± 0.10 ^h
	20	2.50 ± 0.01 ^k	24.45 ± 0.97 ^h	68.98 ± 0.87 ⁱ	1.53 ± 0.30 ^h	0.65 ± 0.03 ^g	1.89 ± 0.04 ⁱ

Table 1: Proximate composition of raw and roasted legume seeds^A

^A Data are means of three determinations with standard deviations and based on 100 g of seed flour

^B Values were obtained by difference

^{a-k} Data in column with the same superscripts are not significantly different while data in column with different superscripts are significantly different at $p < 0.05$

of inclined level of fat content [28]. These have also been reported in similar studies on mangrove legumes, maize and groundnuts [29,30]. Results also indicated a minor change on the ash content after roasting, but insignificant for adzuki beans and chickpeas. Overall, roasting increases the level of major nutritional components in these legume seeds.

Water soluble matters in raw and roasted legumes

Table 2 shows the yield of water soluble matters, total phenolic content and total flavonoid content in legume seeds before and after roasting for different durations. The yield of water soluble materials decreased significantly from the beginning of roasting for

all the legume seeds, ranging from approximately 1 to 9% of the dry matter which suggested that roasting might have caused thermal denaturation of cellular components of the seeds with subsequent formation of water insoluble materials. Intensive roasting could reduce the extraction yield due to the loss of volatile components and breakdown of products [31]. Similar to the overall decrease in water soluble materials, roasting also caused significant decrease in the total phenolic content. Results suggested that roasting might have altered the structure of some phenolic compounds, forming large non-extractable polymers, else this could be due to thermal degradation and formation of volatile acids from heat susceptible phenolic compounds, with consequent reduction in the extracts. These have been reported in similar studies on mung bean, soybean, adzuki bean[12] and kidney bean[9]. Similarly, total flavonoid content in all the legume seeds extracts decreased after roasting, except a slight increase was found in chickpeas and yellow split peas.

Solubility of flavonoid compounds might be increased after heat treatment, and some products formed by Maillard reaction could also be the possible reason of increasing flavonoid content. This has also been reported in a similar study on kidney beans and black eyed peas [32]. When taken together, these results strongly suggested that the soluble materials formed in roasting process of these legume seeds were mainly protein or peptides, simple carbohydrates and possible soluble fiber in nature. Relatively, the products of Maillard reaction and caramelization, including phenolic compounds, produced by the heat treatment, were very limited.

Antioxidant activities of raw and roasted legume seed extracts

Table 3 shows the antioxidant activities of aqueous extracts obtained from raw and roasted mung beans, adzuki beans, chickpeas and yellow split peas. A combination of three different

Legume sample	Roasting time (min)	Yield of extract ^B	Total phenolic content ^C	Total flavonoid content ^D
Mung bean	0	22.23 ± 0.45 ^a	6.66 ± 0.03 ^a	4.02 ± 0.29 ^a
	10	18.58 ± 1.39 ^b	4.02 ± 0.05 ^b	3.45 ± 0.35 ^{a,b}
	20	12.29 ± 0.14 ^c	3.24 ± 0.03 ^c	3.07 ± 0.35 ^b
Adzuki bean	0	11.60 ± 0.34 ^d	6.87 ± 0.09 ^d	6.19 ± 0.29 ^c
	10	10.22 ± 0.23 ^e	5.37 ± 0.10 ^e	4.67 ± 0.28 ^d
	20	9.77 ± 0.25 ^f	3.36 ± 0.13 ^c	3.05 ± 0.31 ^b
Chickpea	0	19.60 ± 0.62 ^b	3.91 ± 0.04 ^f	2.63 ± 0.02 ^e
	10	16.73 ± 0.49 ^g	2.97 ± 0.06 ^g	2.78 ± 0.02 ^f
	20	15.37 ± 1.08 ^g	2.20 ± 0.08 ^h	3.12 ± 0.16 ^b
Yellow split pea	0	22.80 ± 1.21 ^a	9.29 ± 0.15 ⁱ	2.24 ± 0.01 ^g
	10	21.80 ± 0.52 ^a	6.85 ± 0.14 ^{a,d}	2.49 ± 0.03 ^h
	20	14.33 ± 0.15 ^g	3.99 ± 0.03 ^j	2.77 ± 0.04 ^f

Table 2: Effect of roasting on water soluble matters of legume seed extracts^A

^AData are means of three determinations with standard deviations

^BBased on 100 g legume seed flour

^CMilligram of gallic acid equivalents per gram of dry matter (mg GAE/g)

^DMilligram of quercetin equivalents per gram of dry matter (mg QE/g)

^{a-j}Data in column with the same superscripts are not significantly different while data in column with different superscripts are significantly different at $p < 0.05$

Legume sample	Roasting time (min)	ABTS assay ^B	DPPH assay ^B	FRAP assay ^C
Mung bean	0	2.30 ± 0.01 ^a	1.07 ± 0.01 ^a	8.45 ± 0.02 ^a
	10	1.68 ± 0.01 ^b	0.57 ± 0.01 ^b	7.52 ± 0.01 ^b
	20	1.28 ± 0.01 ^c	0.41 ± 0.01 ^c	5.75 ± 0.02 ^c
Adzuki bean	0	4.94 ± 0.01 ^d	3.24 ± 0.01 ^d	20.88 ± 0.15 ^d
	10	3.82 ± 0.01 ^e	2.08 ± 0.01 ^e	14.92 ± 0.13 ^e
	20	3.46 ± 0.02 ^f	1.80 ± 0.01 ^f	12.78 ± 0.10 ^f
Chickpea	0	2.52 ± 0.01 ^g	0.22 ± 0.01 ^g	2.63 ± 0.02 ^g
	10	2.70 ± 0.02 ^h	0.23 ± 0.01 ^g	2.78 ± 0.02 ^g
	20	2.88 ± 0.05 ⁱ	0.33 ± 0.02 ^h	3.12 ± 0.16 ^h
Yellow split pea	0	3.55 ± 0.04 ^j	0.39 ± 0.01 ⁱ	2.24 ± 0.01 ⁱ
	10	3.83 ± 0.14 ^e	0.43 ± 0.02 ⁱ	2.49 ± 0.03 ^j
	20	4.29 ± 0.02 ^k	0.46 ± 0.01 ^j	2.77 ± 0.04 ^g

Table 3: Effect of roasting on antioxidant activities of legume seed extracts^A

^AData are means of three determinations with standard deviations

^BMilligram of Trolox equivalent per gram of dry matter (mg TE/g)

^CMilligram Fe (II) equivalent per gram of dry matter (mg Fe(II)/g)

^{a-k}Data in column with the same superscripts are not significantly different while data in column with different superscripts are significantly different at $p < 0.05$

well established antioxidant assays, using different free radicals, standards, and sample-to-free radical ratio, was employed for present study to provide a more reliable assessment of antioxidant activity, especially in plant-based extracts. Generally, the free radical scavenging antioxidant activities of mung beans and adzuki beans decreased with processing at 200°C for up to 20 min, but slightly increased in chickpeas and yellow split peas. Besides, FRAP assay was used to determine the reduction ability of antioxidants to decrease the oxidant, from Fe (III) to Fe (II) ions. The results obtained by the FRAP assay were highly correlated to those by both the ABTS and DPPH assays. The decrease in antioxidants, such as phenolic compounds, lead to the reducing of FRAP value. The substantial decrease in the total phenolic content (Table 2) could be the major reason for the decreasing antioxidant activities produced by roasting shown in the present study, and was similar to findings reported for several other legumes and pulses, such as faba beans, cowpea, almond skins and black soybean [33-36]. Phenolic compounds are thermal sensitive, hence applying heat treatment to legumes might cause decomposition or molecular alteration, and thus decrease chemical activity or extractability of phenolic compounds[11]. Aqueous extraction may not be efficient in extracting the less-polar phenolic compounds, such as Maillard reaction products, tannin-protein binding alternatives and other cross-linked products, which generated after the roasting process. Interestingly, the highest level of antioxidant activities in all assays was found in adzuki beans. This could be due to the high amount of water soluble anthocyanin in adzuki beans in comparison with other samples [37].

On the other hand, the dry heat treatment could have increased the antioxidant activities when the new generated antioxidant products overcome the degradation of the naturally occurring thermal labile antioxidants. This was evidenced by an increase in total flavonoid content of the roasted chickpeas and yellow split peas in present study (Table 2). Potentially, the glycoside moiety of flavonoids has been broken down in order to form aglycone, which possess higher antioxidant capacity than flavonoid glycosides [36]. In addition, the complex composition of legumes, such as active polysaccharides, amino acids, proteins, vitamins, and microelements may also affect their antioxidant capacity [38]. For instance, conformational changes caused by thermal processing increased accessibility to metal-chelating amino acids, such as lysine, arginine, aspartic acid, glutamic acid and histidine [39]. Furthermore, higher degree of roasting was indicated by color change in legumes with thin seed coat (chickpeas) or without seed coat (yellow split peas) compared to those with seed coats (Figure 1D). It promoted the formation of melanoidin intermediate compounds, such as 2, 6-dichlorophenol-indophenol, which could act as reducing agents. In addition, it has been reported that proteins of roasted chickpea provided higher antioxidant capacity, which was mainly associated with its amino acid profile [39]. These results demonstrated that the balance between the formation of new reaction antioxidant products and the thermal degradation of naturally occurring antioxidant compounds affected overall effects of roasting on capacity of antioxidant.

Antibacterial activity evaluation

IC₅₀ is the measure of the concentration of inhibitor that required to inhibit the biochemical or biological function by half. In present study, IC₅₀ values obtained from ABTS and DPPH antioxidant assays of raw and roasted legume seed extracts were employed in the evaluation of antibacterial activities (Table 4). All samples showed a general increase in IC₅₀ value after roasting, except a decrease was observed in chickpea on both ABTS and DPPH assays. *E. coli* and *S. aureus* were identified to be ampicillin resistant species, but susceptible to gentamicin and ciprofloxacin (Figure 2). In spite of the moderate levels of antioxidant activities, however, no corresponding *in vitro* antibacterial activities were observed in any of the samples (Figure 2). It has been reported that raw mung bean and pigeon pea extracts exhibited insignificant antimicrobial activity against *E. coli* and *S. aureus*, but the legume extracts had showed good inhibitory effect against *B. cereus* [40]. This could be due to the resistant

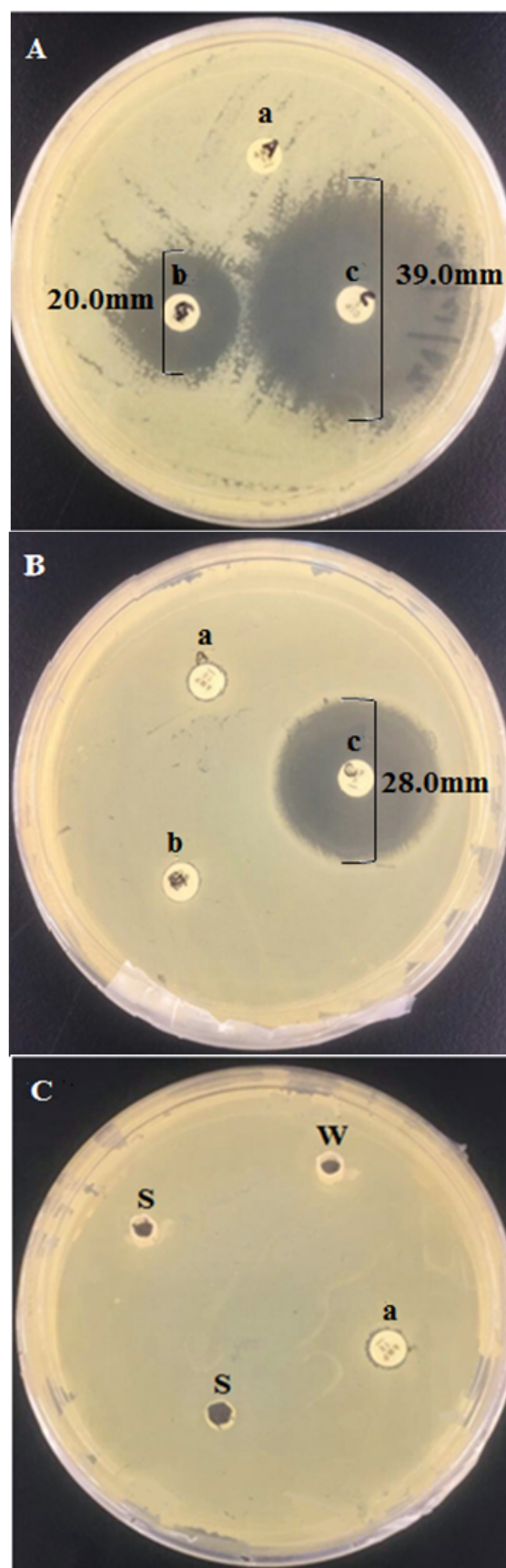


Figure 2: Well diffusion agar plates with zone of inhibition (mm): ampicillin (a), gentamicin (b) and ciprofloxacin (c), against *E. coli* ATCC 35218 (A) and *S. aureus* ATCC 33591 (B); and a typical agar plate (C) with duplicated samples (S) and distilled water (W)

Legume sample	Roasting time (min)	ABTS assay ^B	DPPH assay ^B
Mung bean	0	7.57 ± 0.07 ^a	5.25 ± 0.01 ^a
	10	10.60 ± 0.01 ^b	9.33 ± 0.01 ^b
	20	11.11 ± 0.01 ^c	10.82 ± 0.05 ^c
Adzuki bean	0	4.80 ± 0.01 ^d	2.44 ± 0.02 ^d
	10	7.71 ± 0.03 ^e	3.62 ± 0.01 ^e
	20	8.33 ± 0.02 ^f	3.97 ± 0.00 ^f
Chickpea	0	9.41 ± 0.04 ^g	30.28 ± 0.48 ^g
	10	9.39 ± 0.06 ^g	30.05 ± 0.10 ^g
	20	8.57 ± 0.14 ^h	18.33 ± 0.17 ^h
Yellow split pea	0	17.29 ± 0.12 ⁱ	5.56 ± 0.03 ⁱ
	10	10.43 ± 0.02 ^j	4.09 ± 0.06 ^j
	20	9.92 ± 0.02 ^k	4.05 ± 0.39 ^j

Table 4: IC₅₀ values of legume seed extracts on antioxidant assays^A

^AData are means of three determinations with standard deviations

^BMilligram of dry matter per milliliter distilled water (mg/mL)

^{a-k}Data in column with the same superscripts are not significantly different while data in column with different superscripts are significantly different at $p < 0.05$

characteristics of the Gram-negative bacteria. Other reasons for the low antibacterial activity in the legume seed extracts could be the types of solvent used for extraction and the concentration of the bioactive components in the extracts. It has been reported that using methanol as solvent might give high solubility and polarity effects toward active components with antibacterial properties[16]. Besides, phytic acid and flavonoids in the extracts of pulses have been reported to contribute significant activity against both Gram-positive and Gram-negative bacteria [40]. Compared with these findings, the considerable reduction of total phenolic contents in legume seeds as a result of roasting in present study could be the major reason for insignificant antibacterial activity (Table 2). The relatively low versatility of the legume seed water extracts in terms of their biological activities may also be related to the low diversity of the phytochemical constituents, such as tannins, alkaloids and saponins.

Conclusion

Processing legume seeds by roasting at a constant temperature for different time regimes affected both the chemical and physical properties of the seeds, flour, and extracts. In general, moisture and crude fiber contents decreased with relative increase in the concentration of the major components. Intensive roasting led to a substantial lowering of the water soluble matters, including total phenolic and flavonoid compounds and enhanced the color intensity in legume seeds and their corresponding flours. The decrease in its soluble phenolic content could be a result of thermally induced formation of non-polar phenolics or non-soluble conjugated compounds by chemical reactions. With the corresponding variations in the antioxidant activity of the legume seed extracts, results suggested that roasting significantly affected the nutritional value and health benefits of the legume seed products. Furthermore, with the IC₅₀ concentration obtained from ABTS and DPPH assays, raw and roasted legume seed extracts did not possess any significant *in vitro* antibacterial activities. These results should enhance the understanding of effects of thermal processing on physical and chemical properties of different legume seeds. Further research could be directed at characterizing their active phytochemical components in the extracts obtained from different solvents and exploring their health functional properties.

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