Comparison of Proximate Compositions, Resistant Starch Content, and Pasting Properties of Different Colored Cowpeas (*Vigna unguiculata*) and Red Kidney Bean (*Phaseolus vulgaris*)

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Abstract**—**Four different colors of cowpeas (*Vigna unguiculata*) (black, white, red and black/white speckled) and red kidney bean (*Phaseolus vulgaris*) were used to evaluate proximate compositions, starch content, and pasting properties. There were no significant differences of moisture, protein, ash, fat, and carbohydrate contents of all bean types. The kidney bean had significantly lower amounts of total starch and solubilized starch compared to those of other cowpeas ($p \leq 0.05$), whereas the red cowpea and red kidney bean had highest content of resistant starch (9-10%). Decortication indicated no significant effect on the proximate compositions of all samples, but it significantly decreased the resistant starch content in cowpeas and increased the solubilized starch and total starch content in all types of cowpeas. The highest values of pasting properties, generally observed in flours obtained from black and black/white speckled cowpea.

Keywords**—**Cowpea, Decortication, Red kidney bean, Resistant starch

I. INTRODUCTION

COWPEA (*Vigna unguiculata*) is a legume originated in Africa. It is recognized as an inexpensive source of calories and proteins, particularly for developing countries since their seeds contain complex carbohydrate (50-67%) and protein (23-25%) [1].This plant is widely grown in the humid tropical and subtropical regions and consumed in various regions of the world. Main production areas include west and central Africa, southern Africa, central and South America and southern Asia. The total world production of dry cowpea seeds was nearly 5 million tonnes in 2002 [2].Cowpeas can be used at all growth stages. Their young leaves, immature pods, and seeds are utilized in fresh form as vegetables. Seeds are often boiled and roasted for human consumption or processed into flour for snack chips, cereal-based, and bakery products [3]. Seeds of cowpea vary in shapes, from kidney-shaped to round shape, depending on the specific cultivars, which may be in solid colors (red, black, brown, tan or white), speckled, spotted, or marbled.

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These seeds are commonly utilized either as whole seed or as decorticated grains. The simple method for decortication is to soak the seeds for a short time in water and the seed coat was removed by squeezing the soaked seeds between the palms or by gentle abrasion using grinding stone.

Numerous scientific data indicate that the consumption of grains is associated with a lower risk of several chronic diseases, such as cancers and cardiovascular diseases. The preventive effect is often associated to naturally occurring antioxidant components, such as anthocyanins, flavonoids, and other phytochemicals that are predominantly present in the seed coat [4] ,[5]. Reference [6] studied on antioxidant activity and phenolic content of two varieties of cowpeas. The results showed high percentage of radical scavenging (74.3–84.6%) in all bean extracts.

A number of legumes contain a large amount of resistant starch, which is defined as starch or starch products that are resistant to digestion by enzymes in the small intestine, and pass into the large intestine where they become the substrate for bacterial fermentation producing short chain fatty acids [7]. Starch and non-starch are the major constituents in cowpeas, with smaller, but significant amounts of oligosaccharides [9]. However, the resistant starch content of cowpea seeds is not yet gained much attention. Therefore, the objective of the present study was to determine chemical compositions and starch contents of different colors of cowpeas. The study was conducted in both in bean meats, where carbohydrates are concentrated, and whole dry seeds. The basic knowledge gained from the present study would be useful information to design and to develop new food based products.

II. MATERIALS AND METHODS

A. Sample Collection

Four local cowpea seeds with various colors were used in this study. Cowpea seeds with black, white, black/white speckled seed colors (*Vigna unguiculata*) were purchased from the local market in Mahasarakham province, Thailand whereas red bean seeds (*Vigna angularis*) and red kidney beans (*Phaseolus vulgaris*) were purchased from Big C supermarket, Mahasarakham, Thailand. All seeds were packed in plastic bags and stored at 5°C until further use.

B. Preparation of Decorticated Samples

Process for decorticating cowpea seeds and red kidney beans was adopted a modified method of [10]. Briefly, the seed coats covering any of seed meats were removed manually after soaking 250 g legume grains in 750 ml. tap water for 5 min. The soaked seeds were gently squeezed between the two palms before the seed coats were spitted off the bean meats by knife. The process of decortication of 250 g seed had to finish in 15 min. The whole seed and decorticated seeds of all bean samples were tray-dried at 40°C for 48 h, ground into powder using a stainless hammer mill and passed through a sieve (100 mm mesh size). The powdered samples were packed in glass bottles and stored at room temperature until use for chemical analyses.

C. Proximate Analysis

All samples were analyzed for contents of ash (Direct – drying method), fat (Solvent extraction method), crude protein (Kjeldahl method; Nx6.25) and moisture (Oven-drying method). All determinations were carried out in triplicates, following the official methods [11] and average values were reported. Carbohydrate content was calculated from 100 – (% protein+% fat + %moisture +% protein).

D. Determination of Solubilized Starch, Resistant Starch and Total starch

The enzymatic, spectrophotmetric method described by [12] was used of quantitative determination of solubilized starch, resistant starch and total starch in samples. All samples were analyzed using the Megazyme resistant starch assay kit (Megazyme International Ireland Ltd, Bray, Ireland). In summary, 100 ± 5 mg of the sample was weighed individually into screw cap tube, added with 4.0 ml of pancreatic α-amylase solution (10 mg/ml) containing amyloglucosidase (AMG) (3 U/ml) and subsequently incubated at 37°C with continuous shaking (200 strokes/min) for exactly 16 hr. To aid dispersion, 4.0 ml of 50% industrial methylated spirits (IMS) was added to the tube with vigorous stirring. The tube was centrifuged at 1,500 *g* for 10 min. The supernatants were drawn and kept. The pellets were re-suspended in 2 ml of 50% IMS, washed with 6 ml of 50 % IMS and then centrifuged at 1,500 *g* for 10 min. The process was repeated twice. The pellet was kept for resistant starch determination whereas the centrifuged washings were combined with the original decanted supernatant and used for solubilized starch determination.

1. Determination of Solubilized Starch (Non-Resistant Starch)

The supernatants from centrifugation were adjusted the final volume to 100 ml with 100 mM sodium acetate buffer (pH 4.5). The resulting aliquots (0.1 ml), in duplicate, were incubated with 10 µl of dilute AMG solution (300 U/ml) in 100 mM sodium maleate buffer (pH 6.0) for 20 min at 50^{o}C and followed by second incubation with 3.0 ml of glucose oxidase plus peroxidas (GOPOD) reagent for a further 20 min at 50°C. The absorbance of all solutions was measured at 510 nm against a reagent blank. The average absorbance was used in the calculation of solubilized starch content.

2. Determination of Resistant Starch

The pellets obtained from the subsequent two 50% ethanol washings and centrifugation were resuspended with 2 ml of 2 M KOH. After continuously stirring for 20 min in an ice bath, 8 ml of 1.2 M sodium acetate buffer (pH 3.8) and 0.1 ml of AMG (3300 U/ml) were added respectively and the tubes were placed in a water bath at 50°C for 30 min. At the end of the treatment, the solution was brought to a total volume of 100 ml with distilled water, mixed and centrifuged at 1,500 *g* for 10 min. A 0.1 ml aliquot (in duplicate) of supernatants was treated with 3.0 ml GOPOD reagent, vortex-mixed and kept at 50°C for 20 min. Blank (0.1 ml of 100 mM sodium acetate buffer pH 4.5) and 3.0 ml of GOPOD and glucose standards 0.1 ml of D-glucose (1 mg/ml) and 3.0 ml of GOPOD, in quadruplicate, were incubated concurrently. The absorbance of all solutions was read with a spectrophotometer at 510 nm.

3. Determination of Total Starch

The total starch content of the tested sample was calculated as sum of resistant starch and solubilized starch fractions.

E. Pasting properties

Pasting properties of cowpea flour were determined using the Rapid Visco Analyzer (RVA 4, Newport Scientific,Australia). Each sample (approximately 3g, 14% moisture basis) was dispersed in 25 mL of distilled water before measurement.

F. Statistical Analysis

All results of triplicate samples were statistically analyzed using SPSS for windows. In reporting data, the results of individual samples are reported as the mean ± standard deviation. The Duncan's multiple range test (DMRT) was applied for mean comparison when analysis of variance showed significant differences at 95% confidence level.

III. Results And Discussion

Proximate compositions

The results of the proximate analysis of whole seeds and decorticated seeds of cowpeas and red kidney bean are presented in Table 1. Overall, the compositions of cowpea seeds were found close to red kidney bean. The results indicate that the decortication did not adversely affect the chemical compositions of cowpea as decorticated cowpea beans contained similar level of fat, crude protein and ash contents to the corresponding whole seeds. The moisture contents of all cowpea seeds varied from 7.80 to 9.94%. Only seeds of black cowpea and black/white speckled cowpea showed a significant difference when compared to red kidney bean (7.8 compared to 12.4%). For decorticated beans, their moisture contents were found similar to the corresponding whole seeds, both in cowpeas and red kidney bean.

Whole seeds of cowpea and red kidney bean had higher ash content than those of the corresponding decorticated bean, except for red cowpea. As can be seen from Table 1, the ash contents of cowpea varied from 3.67-4.25% for whole seed to 3.32-4.22% for decorticated bean. In a similar study, [13] found 3.1% ash in cowpea seed.

TABLE I
PROXIMATE COMPOSITION AND ANALYSIS OF COWPEA AND RED KIDNEY BEAN

Samples	Percentage of dry mater				
	Moisture	Ash	Protein	Crude fat	Carbohydrate
Decotricated bean					
Black cowpea	$8.80 \pm 0.05^{a,b}$	3.32 ± 0.35^{b}	17.53 ± 2.36^{c}	1.40 ± 0.44^{c}	$66.41 \pm 3.18^{a,b}$
White cowpea	$8.58 \pm 0.08^{a,b}$	$3.71 \pm 0.25^{a,b}$	$21.10 \pm 3.51^{b,c}$	1.84 ± 0.08^{a}	$64.70 \pm 3.66^{a,b}$
Black / white speckled cowpea	$10.58 \pm 0.07^{a,b}$	4.22 ± 0.16^{a}	$25.95 \pm 1.69^{a,b}$	1.30 ± 0.02^{b}	55.60 ± 1.10^{c}
Red cowpea	$9.60 \pm 0.04^{a,b}$	4.22 ± 0.95^{a}	$20.54 \pm 4.96^{b,c}$	0.37 ± 0.04^{c}	$65.27 \pm 5.92^{a,b}$
Red kidney bean	$11.68 \pm 4.35^{a,b}$	$3.64 \pm 0.19^{a,b}$	$20.08 \pm 0.33^{b,c}$	1.22 ± 0.44^{b}	$63.30 \pm 4.17^{a,b}$
Whole seed					
Black cowpea	7.80 ± 0.17^{b}	4.25 ± 0.13^{a}	19.98 ± 3.28^{c}	1.48 ± 0.33^{b}	69.01 ± 1.90^{a}
White cowpea	$8.90 \pm 0.38^{a,b}$	4.03 ± 0.11^{a}	$22.34 \pm 4.50^{b,c}$	1.88 ± 0.08^{a}	$62.87 \pm 4.95^{a,b}$
Black /white speckled cowpea	7.81 ± 0.01^{b}	4.23 ± 0.05^{a}	28.25 ± 1.23^{a}	1.34 ± 0.17^{b}	$60.70 \pm 1.73^{a,b}$
Red cowpea	$9.94 \pm 0.06^{a,b}$	$3.67 \pm 0.06^{a,b}$	$20.61 \pm 2.62^{b,c}$	0.39 ± 0.02^{c}	$65.37 \pm 2.64^{a,b}$
Red kidney bean	12.39 ± 4.60^{a}	$3.90 \pm 0.12^{a,b}$	$21.83 \pm 2.80^{b,c}$	1.30 ± 0.33^{b}	$60.65 \pm 2.21^{b,c}$

Values are means ± SD of three different experiments.
Values in the same column with different superscripts are significantly different ($p \leq 0.05$) by DMRT.
Carbohydrate content was calculated from 100 – (%ash+%fat + %moisture +% protein).

Among four types of cowpeas, black/white speckled seeds had the highest protein content ($p \leq 0.05$), showing 28.25% for whole seed and 25.95% for decorticated bean. The lowest protein concentration was found in black cowpeas (17.53% for decorticated bean and 19.98% for whole seed). Only the protein of speckled seeds was statistically significant higher than that of red kidney bean. Similar results were reported by [14] who found the protein content of 24.8% in cowpea seeds.

The decorticated cowpea had similar fat contents to the corresponding whole seeds, ranging from 0.39 to 1.88%. This finding was in agreement with [15] who reported the fat content of 1.9% .Of four types of cowpea studied, seeds with red color had the lowest ash content whereas seeds with white color contained the largest amount.

Resistant starch, solubilized starch and total starch contents

Figs. 1(a) and 1(b) show the resistant starch, solubilized starch and total starch contents of whole seeds and decorticated seeds of cowpea and red kidney bean. Decortication had no effect on the starch contents of red kidney bean. The contents of resistant starch, solubilized starch and total starch of undecorticated red kidney bean were 9.54, 24.54 and 34.10%, respectively and remained constant at 8.97, 24.13 and 33.10% in decorticated bean. However, decortication significantly decreased the resistant starch and total starch contents and increased the solubilized starch content of selected types of cowpeas ($p \leq 0.05$).The red cowpea seeds had the highest level of resistant starch content (10.63±0.11%) whereas the lowest amount was found in black cowpea (4.59±0.24%). In the studies of [15], similar content of resistant starch to our study was found, ranging from 2.78 to 6.44%. The effect of decortications on starch contents of cowpea was shown in Fig. 1(b). After decortications, there was a significant decrease ($p \leq 0.05$) in resistant starch content of white cowpea from 7.84 to 4.63% and of black/white speckled cowpea from 18 to 4.11%. On the other hand, insignificant changes in resistant starch content as a consequence of decortications were observed in black cowpea and red cowpea.When compared among groups of cowpeas, it was found that red cowpea had the smallest amount of solubilized starch content (*ca*. 30%). The other three types of colored cowpea had a substantial amount of solubilized starch, ranged between 41.73% and 48.19%. Obviously, the decortication has led to an increase in solubilized starch content of cowpea. Particularly, the contents of solubilized starch increased nearly 1.5-2 times from 29.52 to 54.04% for red cowpea and from 48.19 to 62.91% for black/white speckled cowpea after decortication.The total starch content was estimated from the sum of resistant starch and solubilized starch. In comparison to corresponding whole seeds, the total starch contents of all decorticated cowpea bean, except white cowpea increased significantly ($p \leq 0.05$). All decorticated cowpeas contained higher amount of total starch than red kidney bean (53.32-66.02 cf. 33.10%) as shown in Fig. 2 In previous study by [14] the total starch content of cowpea was 63.6%.

Pasting properties

The pasting properties of cowpea flour samples namely peak viscosity, trough, breakdown, final viscosities, setback time, and pasting temperature are showed in Table II. The highest values of peak viscosity and trough were found in decorticated flour of black and black/white specked cowpea, whereas the lowest values were in whole seed and decorticated flour of red kidney bean. The breakdown values were highest in whole seed flour of black and black/white specked cowpea whilst the highest final viscosity and pasting temperature was observed in the decorticated black cowpea and the whole seed flour of black/white specked cowpea, respectively.

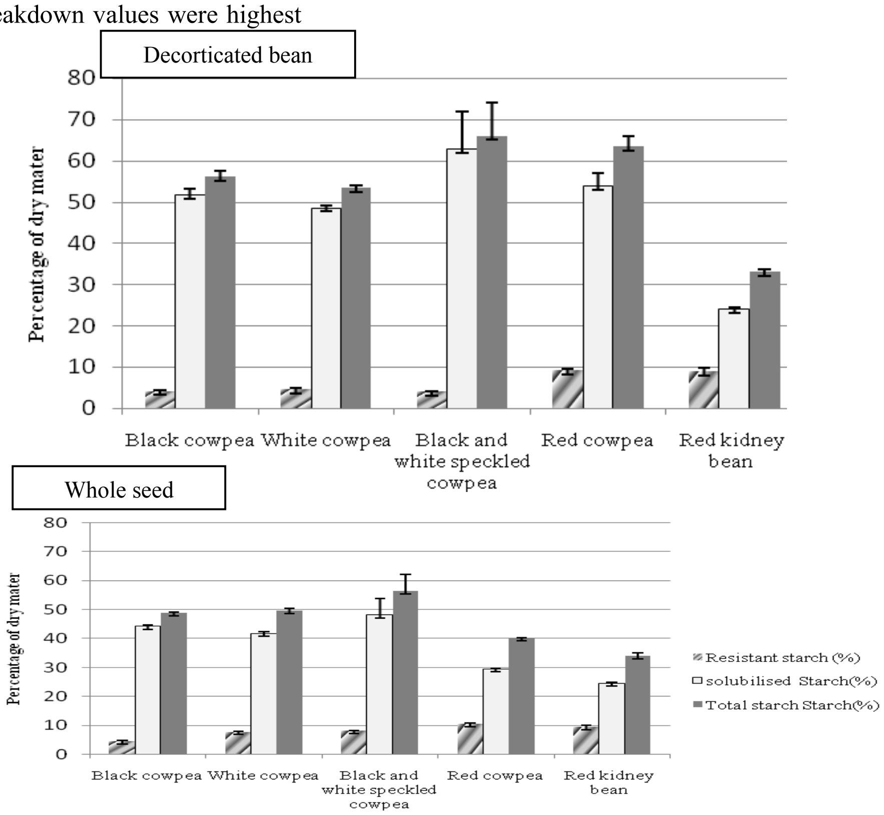

Fig. 1 Resistant Starch, Solubilized Starch and Total Starch Contents of and Whole Seeds of Different Colored Cowpeas and Red Kidney Bean

TABLE II
COMPARISON OF PASTING PROPERTIES (RVU) OF DIFFERENT COWPEAS AND RED KIDNEY BEAN

Samples	Percentage of dry mater						
	Peak viscosity	Trough	Breakdown	Final viscosity	Setback	Peak time (min)	Pasting temperature (°C)
Decotricated bean							
Black cowpea	$74.00 \pm 2.66^{a,b}$	72.75 ± 2.86^{a}	1.22 ± 0.26^{b}	129.86 ± 0.58^{a}	57.05 ± 3.01^{a}	7.00 ± 0.00^{a}	79.95 ± 1.42^{d}
White cowpea	60.78 ± 6.13^{c}	$59.25 \pm 5.37^{b,c}$	1.52 ± 0.86^{b}	$94.36 \pm 1.63^{c,d}$	35.11 ± 10.96^{b}	6.97 ± 0.04^{a}	79.40 ± 0.47^{d}
Black / white speckled cowpea	77.16 ± 6.42^{a}	74.10 ± 4.83^{a}	2.20 ± 1.56^{b}	$103.92 \pm 0.36^{b,c}$	$28.91 \pm 1.46^{b,c}$	6.42 ± 0.65^{a}	82.38 ± 0.05^{b}
Red cowpea	$65.24 \pm 1.80^{b,c}$	63.78 ± 1.63^{b}	1.47 ± 0.17^{b}	$119.22 \pm 0.91^{a,b}$	55.44 ± 7.51^{a}	6.86 ± 0.13^{a}	77.80 ± 0.47^{e}
Red kidney bean	48.56 ± 0.11^{d}	$46.06 \pm 0.13^{d,e}$	2.50 ± 0.21^{b}	69.38 ± 0.39^{e}	$23.11 \pm 0.72^{c,d}$	6.97 ± 0.03^{a}	83.30 ± 0.10^{b}
Whole seed							
Black cowpea	61.20 ± 5.43^{c}	$57.72 \pm 6.13^{b,c}$	$3.47 \pm 0.84^{a,b}$	$84.90 \pm 0.96^{d,e}$	$27.16 \pm 3.60^{b,c}$	4.77 ± 0.16^{c}	$80.76 \pm 0.02^{c,d}$
White cowpea	$69.30 \pm 3.30^{a,b,c}$	$66.90 \pm 3.50^{a,b}$	2.41 ± 0.30^{b}	$95.50 \pm 0.21^{c,d}$	$28.61 \pm 1.70^{b,c}$	6.77 ± 0.20^{a}	$81.83 \pm 0.93^{b,c}$
Black /white speckled cowpea	59.17 ± 11.0^{c}	$53.52 \pm 6.93^{c,d}$	5.63 ± 4.16^{a}	67.83 ± 0.71^{e}	14.30 ± 0.21^{d}	5.71 ± 0.77^{b}	85.61 ± 1.65^{a}
Red cowpea	$63.94 \pm 9.75^{b,c}$	$62.47 \pm 9.25^{b,c}$	1.47 ± 0.53^{b}	$97.16 \pm 1.95^{c,d}$	34.69 ± 10.33^{b}	6.82 ± 0.04^{a}	77.80 ± 1.24^{e}
Red kidney bean	42.38 ± 0.19^{d}	40.88 ± 0.07^{e}	1.33 ± 2.32^{b}	76.18 ± 0.04^{e}	35.52 ± 0.32^{b}	6.89 ± 0.04^{a}	75.40 ± 0.20^{f}

Values are means ± SD of three different experiments.
Values in the same column with different superscripts are significantly different ($p \leq 0.05$) by DMRT.

IV. CONCLUSIONS

It can be concluded that decortication significantly affected starch contents of colored cowpeas ($p \leq 0.05$), but it had little effect on other chemical compositions of the beans. Prior to decortication, cow peas had a resistant starch content of 4.59-10.63%, a solubilized starch content of 29.52-48.18 and a total starch content of 40.15-56.37%. Dependening on the types, decorticated beans of cowpea contained lower resistant starch content (4.20-9.32%) and higher solubilized starch (48.67-62.91%). The total starch content of decorticated cowpea also significant increased to 53.32-66.02%. As starch and non-starch polysaccharides are the major constituents in cowpea and also since it contained the significant amount resistant starch, the important bioactive constituent of cowpea, this study suggested that it is possible to employ cowpea in varieties of food products, food based, or to process into flour for industrial uses.

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