CHARACTERIZATION OF FLOUR, STARCH AND PROTEIN FROM WHITE AND BLACK ADLAY CULTIVARS

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Abstract

Adlay or Job's tears (*Coix lachryma-jobi* L.) from white (WA) and black (BA) adlay cultivars were used to study the physical, chemical and physicochemical properties of adlay flour (F), starch (S) and protein (P). Whiteness indexes of adlay flour (WAF, BAF) were lower than adlay starch (WAS, BAS) and protein (WAP, BAP). In addition, the whiteness index of flour and protein from BA was lower than that of WA. While morphology of adlay starch, flour and protein from both cultivars was similar; however, the size of protein bodies was slightly different. Additionally, the molecular weight of protein in WAP and BAP has similar pattern which consisted of the α-, β- and γ-coixin. In addition, adlay flours (WAF, BAF) composed of carbohydrate (79.74-81.71%), protein (13.28-14.88%) and lipid (3.21-3.53%). Moreover, the protein content of flour, protein and starch from black adlay cultivar seemed to be higher than that of white adlay cultivar. Furthermore, the amylose content of BAS (29.22%) was significantly higher than that of WAS (11.55%). Additionally, the pasting viscosity of adlay starches (WAS, BAS) was higher than adlay flours (WAF, BAF). In addition, the WAS and WAF had peak viscosity and breakdown value higher than BAS and BAF. However, the BAS had final viscosity and setback value higher than WAS.

Keywords : adlay, flour, properties, protein, starch

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Introduction

Adlay or Job's tears (Coix *lachrymal-jobi* L.) is widely cultivated in Asia as a grain crop. In Thailand, adlay is mainly cultivated in the northern and northeastern regions for export. Adlay is normally used as a human food and animal feed since adlay has the nutritional and medicinal properties such as prevent and inhibit the growth of lung and liver cancer cell (Yao et al., 2011) and prevent coronary heart disease (Yu, Gao, Zeng and Liu, 2011). In adlay flour, it consisted of carbohydrate (77.10-77.52%), protein (14.18-15.18%) and lipid (5.51-6.18%), respectively (Pronkitprasan, 1987). Furthermore, the major adlay protein is alcohol soluble protein (coixin) and its molecular weights are similar to protein from corn and sorghum (Tatham et al., 1996).

Adlay flour has similar chemical composition as corn and sorghum flour; therefore, it also has potential to use as a gluten-free alternative flour. However, there is only few research investigating the adlay flour properties, causing the limit utilization in the present. Therefore, the objective of this study was to investigate the physical, chemical and physicochemical properties of flour, starch and protein from two adlay cultivars for better understanding. In addition, the knowledge from this study can be applied for industrial utilization of adlay flour in many products.

Objective

To investigate the physical, chemical and physicochemical properties of flour, starch and protein from white and black adlay cultivars

Materials and methods

Materials

Black adlay (BA) and white adlay (WA) grains were cultivated and obtained from Loie province in Thailand. The term of BA and WA was defined by their shell color.

Adlay flour and starch preparation

Adlay flour was prepared from decorticated adlay grains by using dry milling method (Hammer mill, SK1, Retsch, Germany) and passing through a 100 mesh sieve. For starch preparation, the decorticated adlay grains were soaked in 0.25% (w/v) NaOH solution at 25 ^{o}C for 18 hrs prior to wet milled process (Sira and Amaiz, 2004). Adlay grains were ground by blender and then the adlay slurry was passed through 80, 200 and 270 mesh sieves, respectively prior to centrifuging at 460 xg for 20 minutes. After centrifuged, the brown layer (or protein layer) at the top part were removed and this step was repeated several times until there was no protein layer presence. The remaining starch (white layer) was then dried by hot air oven at 45 ^{o}C for 18 hrs. before grinding and passing through a 100 mesh sieve.

Adlay protein (coixin) extraction

Adlay flour was extracted with 70% (w/w) aqueous ethanol (consisting of 0.5% (w/w) sodium metabisulfite and 0.35% (w/w) sodium hydroxide) at 70 °C with constant stirring for an hour by following Taylor, Taylor, Dutton and Kock (2005) method. Then the suspension was centrifuged at 3000 rpm for 5 mins. The supernatant (containing coixin protein) was separated and then placed in a fume cupboard at ambient temperature to evaporate solvent overnight. Then the protein was washed with minimal amount of cold distilled water (<10 °C), and sequentially the solution was adjusted to pH 5. Then the protein was recovered by filtrating and freeze drying, respectively. Consequently, the protein was defatted with hexane at ambient temperature with a protein to hexane ratio of 1:10 (w/w) (Taylor et al., 2005). Additionally, the percent yield of adlay starch and protein was calculated.

Physical properties

Color of flour, starch and protein: The sample color was measured by a spectrophotometer (model UltraScan XE, Hunter Lab Reston, USA). The data were recorded as L*, a*, b* values; L* values range from 0 (black) and 100 (white); a* values range from +a* (reddish) and –a* (greenish) and b* values range from +b*(yellowish) and –b* (bluish). The values were used to calculate whiteness index by following equation (Li and Lee, 1996).

$$\text{Whiteness Index} = 100 - \sqrt{((100 - L^*)^2 + a^{*2} + b^{*2})} \quad (1)$$

Morphology of starch, flour and protein: The morphology of samples was observed by scanning electron microscope (SEM) (model JSM-5600LV, JEOL, Japan). The samples were fixed onto an aluminum stub by using a double-adhesive sided carbon tape and then coated with gold prior to observing by SEM.

Chemical properties

Chemical compositions and amylose content: The chemical compositions of adlay flour, starch and protein (coixin) were determined by using AOAC method (2000). In addition, the amylose content in adlay starch was determined by colorimetric amylose-iodine complex (Juliano, 1971).

SDS-PAGE analysis of adlay protein: Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) was used to analyze the characteristic protein in coixin and starch by using Mini-Protein II Electrophoresis cell (Bio-Rad Laboratories, Inc., USA) by Laemmli (1970) and Ottoboni, Leite, Targon, Crozier and Arruda (1990) methods. The separating and stacking gel were 15% and 4%, respectively. The standard protein marker with low molecular weight range (6.5-66 kDa) (Sigma-Aldrich, Chemie Gmbh, Belgium) was used. Electrophoresis was conducted at constant voltage (100 V) for 1 hr and 45 min.

Physicochemical properties

Pasting properties: The pasting profiles of adlay flour and starch were measured by Rapid Visco Analyzer (RVA) (model RVA3D, Newport Scientific Instrument and Engineering, Australia). The suspension (8.9% solid content) was performed with the set-up temperature profile as followed (Li and Corke, 1999).

Statistical Analysis

All measurements were analyzed by using the SPSS software version 12. Duncan's new multiple range test was used to compare mean difference ($p \leq 0.05$).

Results and Discussion

Physical properties of flour, starch and protein from black and white adlay cultivars

The starch yields of white and black adlay were 23.38 and 30.38%, respectively. While the protein yields of white adlay and black adlay were 9.30 and 9.27%, respectively. In Table 1, the results show that WAF and BAF had lower L* and whiteness index but higher a* and b* values than adlay proteins and starches ($p \leq 0.05$). These indicated that the adlay flour were more brownish than protein and starch. When comparing between cultivars, the results (Table 1) show that BAF and BAP had lower L* but higher b* values than WAF and WAP significantly. These results indicated that the adlay flour and protein prepared from black adlay cultivar were more brownish than white adlay cultivar. However, the whiteness index of WAS and BAS was not significantly different ($p > 0.05$). From these results, they show that the extraction process of starch and protein could remove the other components from raw materials since the whiteness index was increased.

Table 1 Color values (L*, a*, b*) and whiteness index of flour, starch and protein from white and black adlay cultivars

Sample	Color value			Whiteness index
	L*	a*	b*	
WAF	$82.52^{d} \pm 0.73$	$0.35^{b} \pm 0.02$	$7.63^{b} \pm 0.42$	$80.92^{d} \pm 0.84$
BAF	$77.93^{e} \pm 0.39$	$0.72^{a} \pm 0.05$	$8.98^{a} \pm 0.18$	$76.16^{e} \pm 0.43$
WAS	$91.60^{a} \pm 0.14$	$-0.20^{c} \pm 0.04$	$2.58^{e} \pm 0.16$	$91.21^{a} \pm 0.18$
BAS	$91.36^{a} \pm 0.13$	$-0.24^{cd} \pm 0.06$	$2.48^{e} \pm 0.06$	$91.00^{a} \pm 0.14$
WAP	$88.72^{b} \pm 0.37$	$-0.28^{d} \pm 0.03$	$5.02^{d} \pm 0.56$	$87.65^{b} \pm 0.56$
BAP	$87.48^{c} \pm 1.06$	$-0.17^{c} \pm 0.10$	$5.57^{c} \pm 0.63$	$86.29^{c} \pm 1.22$

Means ± standard deviation (n = 3) with different letters within a column are significantly different ($p \leq 0.05$).
L* as 0 and 100 corresponded to black and white, a* values- +a*(reddish) and –a* (greenish) and b* values - +b*(yellowish) and–b* (bluish)

Morphology of flour, starch and protein from white and black adlay cultivars

The morphology of adlay flours, starches and proteins was observed by SEM as shown in Figure 1. In Figure 1a and 1d, they showed that flour particles had different sizes and were aggregated together. In addition, the isolated starch granules were observed in Figure 1b and 1e and they appeared in spherical and polygonal shape with some dents, especially BAS (Figure 1e). Adlay starch morphology looks similar to corn and sorghum starch granules (Mishra and Rai, 2006; Olayinka, Adebowale and Olu-Owolabi, 2013). In addition, the morphology of adlay flours and starches from two cultivars had similar characters.

The extracted adlay protein, mainly coixin protein, (Figure 1c and 1f) had spherical shape in a form of protein body similarly to protein from corn and sorghum. In addition, the protein bodies of adlay protein aggregated together. Coixin from black adlay (Figure 1f) seemed to be bigger than that of white adlay (Figure 1c).

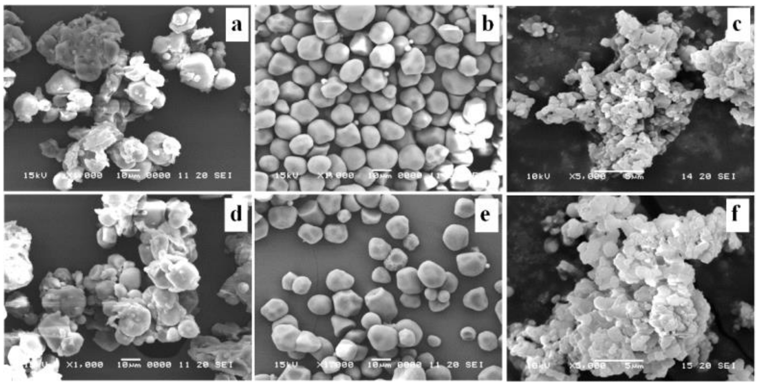

Figure 1 Scanning electron micrographs of flour (a, d), starch (b, d) and protein (c, f) from white adlay (a, b, c) and black adlay (d, e, f) cultivars with different magnifications. Scale bar is 10 μm for flour and starch and 5 μm for protein.

Chemical properties of starch, flour and protein from white and black adlay cultivars

Chemical compositions of adlay flours, starches and proteins were presented in Table 2. The result revealed that the main chemical component of both adlay flours was carbohydrate (79.74-81.71%), protein (13.28-14.88%) and lipid (3.21-3.53%), respectively. When comparing the chemical compositions among flour, starch and protein, the results showed that

WAF and BAF had higher protein, lipid and ash content than WAS and BAS. In addition, the major component of WAP and BAP is protein (81.80 and 85.84 % respectively), indicated that the extracted protein (majorly coixin) had high purity. Additionally, the results show that BAF and BAP had higher protein content than WAF and WAP, significantly. When comparing the lipid content, the result showed that the lipid content of BAF and BAS was slightly higher than that of WAF and WAS. From these results, they were indicated that BA has higher protein and lipid content than WA cultivar, possibly resulted in the different physicochemical properties of adlay flour and starch since the protein and lipid could restrict the swelling of starch granules (Li, Dhital and Hasjim, 2014).

In addition, the amylose content of BAS (29.22% dry basis) was significantly higher than that of WAS (11.55%). The amylose content of BAS had slightly higher that of a normal adlay (coix) starch ranged from 15.9-25.8% (Li and Corke, 1999); while the amylose content of WAS (11.55%) was slightly below the range (Li and Corke, 1999). According to Copeland, Blazek, Salman and Tang (2009), the high amylose content and the lipid content of BAS could affect the pasting behavior of starch since these components could form amylose-lipid complex, inhibiting swelling of starch granules (Copeland et al., 2009; Li et al., 2014).

Table 2 Chemical compositions of flour, starch and protein from white and black adlay cultivars

Sample	Chemical composition (% dry basis)				
	Protein	Lipid	Ash	Fiber	Carbohydrate
WAF	$13.28^{d}\pm0.29$	$3.21^{a}\pm0.11$	$1.12^{b}\pm0.02$	$0.67^{a}\pm0.04$	$81.71^{b}\pm0.18$
BAF	$14.88^{c}\pm0.03$	$3.53^{a}\pm0.08$	$1.17^{a}\pm0.02$	$0.68^{a}\pm0.04$	$79.74^{c}\pm0.13$
WAS	$0.25^{e}\pm0.03$	$0.05^{c}\pm0.02$	$1.00^{c}\pm0.00$	$0.16^{c}\pm0.12$	$98.55^{a}\pm0.11$
BAS	$0.52^{e}\pm0.14$	$0.18^{c}\pm0.03$	$1.00^{c}\pm0.00$	$0.09^{c}\pm0.06$	$98.23^{a}\pm0.18$
WAP	$81.80^{b}\pm0.62$	$3.09^{a}\pm0.56$	$0.99^{c}\pm0.00$	$0.18^{c}\pm0.14$	$13.94^{d}\pm0.67$
BAP	$85.84^{a}\pm0.97$	$1.93^{b}\pm0.82$	$0.99^{c}\pm0.00$	$0.38^{b}\pm0.15$	$10.86^{e}\pm1.92$

Means ± standard deviation (n = 3) with different letters within a column are significantly different ($p \leq 0.05$).

Molecular weight of protein in protein and starch from white and black adlay cultivars

The characterization of protein in the extracted protein and starch of the black and white adlay cultivars was determined by SDS-PAGE. In addition, the molecular weight of protein in BAP, WAP, BAS and WAS was compared to standard protein as shown in Figure 2. The molecular weight of BAP (track 1) and WAP (track 2) consisted of γ–coixin (27-28 kDa), α–coixin (22-23 kDa) and β–coixin (16–19 kDa). The molecular weight and the amount of coixin proteins were similar to zein and kafirin protein (Ottoboni et al., 1990; Tatham et al., 1996). In addition, the molecular weight of the coixin under the reducing condition of BAP (track 3) and WAP (track 4) was similar to the non-reducing condition (track 1, 2); nonetheless, the intensity of coixin bands (α-, γ- and β-coixin) was stronger under the reducing condition by adding 2-mercaptoethanol (presented as 2-ME). It could be indicated that the coixin proteins were stabilized by disulfide cross-link.

Additionally, the protein pattern of BAS and WAS was investigated as shown in Figure 2. Under the reducing condition, the α-prolamin dimer (45 kDa) (Parris and Dickey, 2001) disappeared; while the α-, γ- and β-coixin bands were clearly observed. Furthermore, under the reducing condition, the 60 kDa band, corresponded to granule bound starch synthase (GBSS) protein, was observed in BAS but not present in WAS. From previous research, GBSS is an enzyme, synthesizing the amylose molecules and usually present in high amylose starch (Han & Hamaker, 2002). In addition, most of the protein bands had higher intensity under the reducing condition; therefore, it could imply that the disulfide bonds might be responsible for stabilizing coixin protein and GBSS protein (Han and Hamaker, 2002).

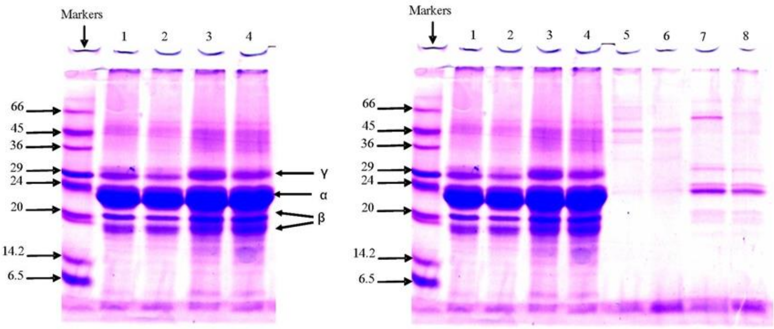

Figure 2 SDS-PAGE of protein pattern in black adlay protein (1, 3), white adlay protein (2, 4), black adlay starch (5, 7) and white adlay starch (6, 8) without the addition of 2-ME (1, 2, 5, 6) and with the addition of 2-ME (3, 4, 7, 8). Marker is standard protein.

Pasting properties of starches and flours from white and black adlay cultivars

In Figure 3, the results showed that the pasting profiles of WAF and BAF were lower than those of WAS and BAS due to the lower starch content and the presence of other chemical compositions (Table 2). The presence of other chemical compositions such as proteins and lipids, could restrict the swelling of starch granules, responsible for the lowered viscosity (Li et al., 2014). Moreover, the pasting character of BAF showed the biphasic peaks, which could possibly due to either protein effect, like observed in rice flour, or the mixing of starch granules (Fitzgerald, Martin, Ward, Park and Shead, 2003; Sasaki, Yasui and Matsuki, 2000).

When considering the pasting profile of WAS and BAS in Figure 3, the results showed that both WAS and BAS had similar pattern. However, the breakdown viscosity of BAS was lower than that of WAS, due to the higher protein and lipid content in BAS (Table 2) led to lower swelling and less sensitive to shear force (Singh, Singh, Kaur, Sodhi and Gill, 2003; Li et al., 2014). Moreover, the GBSS protein in BAS could also restrict granule swelling and breakdown (Han, Campanella, Guan, Keeling and Hamaker, 2002). The final and setback viscosity of BAS were higher than those of WAS since there were more amylose molecules available to reassociate in BAS paste than in WAS (Whistler and BeMiller, 1999).

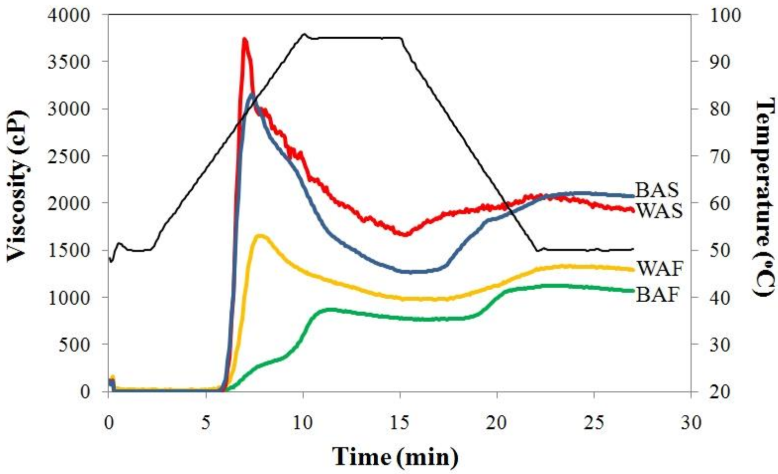

Figure 3 Pasting profiles of flour and starch from white and black adlay cultivars

In Figure 3, it showed that the pasting profile of WAF was higher than that of BAF; moreover, the WAF had a single peak viscosity (PV); while BAF had a biphasic PV. The first PV of BAF was at the same position with WAF; however, the second PV of BAF was occurred around 95 ^{o}C. The results may be indicated that the BAF consisted of starch granules, whose behavior similarly to WAF and also starch granules, which have higher heat stability.

Conclusion

Morphology of flour, starch and protein prepared from white and black adlay was similar. The protein content of black adlay cultivar was higher than white adlay cultivar. The amylose content of black adlay starch was higher than white adlay starch. Molecular weight of coixin protein consisted of α-, β- and γ-coixins. Pasting profile of adlay starches (WAS, BAS) was higher than that of flours (WAF, BAF). Moreover, peak viscosity and breakdown of WAS and WAF were higher than those of BAS and BAF; however, final and setback viscosity of BAS was higher than those of WAS. Besides, the BAF pasting profile had two peaks which were lower than WAF.

Suggestions

Decortication process should select the specific machine as suitable for adlay grains in order to prevent the damage of grain structure.

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