# STRUCTURE AND CHEMICAL AND PHYSICOCHEMICAL PROPERTIES OF JOB'S TEAR (*COIX LACRYMA-JOBI* L.) KERNELS AND FLOURS

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## Abstract

**In Job's tear, the germ is enfolded inside the kernel and comprises *ca.* one third of the whole grain. To assess the effect of germ on flour properties, degermed flour was manually prepared from grains of white and black husk cultivars. The protein of whole grain and degermed flours was not significantly different ($p > 0.05$). The antioxidant properties, which were total phenolic content, DPPH radical scavenging and reducing power, and coixenolide of whole grain and degermed flours of both cultivars were in the ranges of 7.33-8.18 mgGAE/g, 5.40-7.53%, 2.56-2.88 and 0.02-0.53 $\mu$g/g, respectively. The Job's tear starch granules showed a round and polyglonal shape and their average size was 11.68-12.29 $\mu$m. The gelatinization temperature range of white and black Job's tear starch was 67-81°C and the retrogradation behavior of Job's tear starch occurred after storage at 4°C for 39 days as monitored by differential scanning calorimetry. The peak viscosity of the white Job's tear flours was greater than those of the black cultivar flours but the setback and pasting temperature was lower. The swelling power of the white Job's tear flours was higher than that of the black cultivar flours, but their solubility was lower and the opposite result was found in the starch.**

**Keywords: Job's tear, structure, antioxidant properties, physicochemical properties**

## Introduction

*Coix lacryma-jobi L.* is a distant relative of maize in the Maydae tribe of the grass family, Poaceae or Graminaeae. It is commonly named Job's tear, adlay, mayuen, Chinese pearl barley and hatomugi. Job's tear seeds are mainly produced in East and South-East Asia, including China, Japan, the Philippines, Burma, and Thailand. The seeds of Job's tear are oval or egg shaped with 5 mm diameter and have a milky white to black outer surface after the dehulling process. Job's tear has long been used in traditional Chinese medicine and as a nourishing cereal. It is added in soups and broths in the form of flour or whole grain. In Japan and Thailand, a non-dairy drink from Job's tears is available in the market as an alternative health food. Animal and human clinical trials demonstrated that the consumption

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of dehulled flour and seed of Job's tear can improve lipid metabolism, thereby decreasing the risk of heart diseases. In addition, it could reduce liver fat accumulation and protect from tumor stimulating compounds (Chang *et al*., 2003; Yu *et al*., 2005). Some bioactive compounds in Job's tear, especially coixenolide, inhibited tumors, prevented cancer and protected against viral infection (Hung and Chang, 2003; Chun *et al*., 2004).

At present, Job's tear is used as polished grains for food applications. An increase in Job's tear utilization can take place if milling is employed to transform it into Job's tear flour, which could be incorporated into several food products as a major food ingredient. Recently, the consumption of whole grain has become popular due to its retention of functional components. However, this full-fat flour has limitations in making food products. The use of defatted Job's tear flour is necessary to make Job's tear more amenable to several food applications. Therefore, the investigation of the functional properties of this flour is essential. In addition, a structural study of Job's tear kernel, which is important for milling process, has not yet been published. There are 2 important cultivars of Job's tear that are commercially available in Thailand, one with white husk and one with black husk. Therefore, the objective of this study was to investigate the structure of the grain, chemical compositions, and chemical and physicochemical properties of whole grain flour, degermed flour, and starch of white and black husk Job's tear.

## Materials and Methods

### Materials

The black husk Job's tear was a gift from CCP Northern Co., Ltd, Phayao, Thailand. The white husk Job's tear was purchased from Yongsawadpudpol Wang Saphung, Co., Ltd, Loei, Thailand. All chemical reagents were of analytical reagent grade. The enzymes, porcine pancreatin (P-1750), amyloglucosidase (A-7095), cellulase (C-1184), alkaline protease (P-486), and PGO enzyme assay kit (P-7119) were purchased from Sigma-Aldrich Chemicals, Inc. (St. Louis, MO, U.S.A.) and isoamylase was purchased from Megazyme International Ireland Ltd. (Wicklow, Ireland)

### Structure of Grains

The Job's tear grains were steeped in distilled water for 4 hr. The grain was sliced in longitudinal and cross-sectional planes, placed on a slide, and then dyed with Congo red. The slide was placed on a stereo microscope (Nikon SMZ-2T, Nikon Corp., Japan). The images were captured with a color CCD camera (model MTV-62V1P, MEIJI, Japan).

The sliced grain samples were placed on a stub and coated with gold using an ion sputtering device (JFC -110E, JEOL Ltd., Japan). Then, they were examined with a scanning electron microscope (SEM) (JSM-6400, JEOL Ltd., Japan) operated at 10 kV.

### Sample Preparation

Job's tear whole grain flour was prepared by dry milling. The degermed Job's tear flour was obtained from manually degermed grains and then dry milled. The Job's tear starch was isolated by the method of Puchongkavarin *et al*. (2005). The cellulase was added into a slurry of degermed flour at pH 5 and 50°C. After it was centrifuged for 5 min, the pellet was resuspended in distilled water. Then, the alkaline protease was added. After centrifugation, the supernatant and dark tailing layer were discarded and the residual pellet was dried and milled to yield the starch. All samples were passed through a 60 mesh sieve.

### Chemical Composition

The moisture, protein and ash contents were determined according to AOAC (2000). The conversion factor of protein was 5.7. Lipid content was analyzed with an auto fat extraction system (2050 Soxtec, Foss Tecator, Skåne län, Sweden). The amylose content was determined by Juliano's amylose-iodine complex method (1979). All determinations were conducted in triplicate.

The starch fractions, which are rapidly

digested starch (RDS), slowly digested starch (SDS), and resistant starch (RS), of the samples were measured by the methods of Englyst *et al*. (1992) with slight modification. The sample (400 mg) and guar gum (50 mg) were suspended in 20 mL of 0.1 *M* acetate buffer (pH 5.2), mixed by vortexing, and incubated at 37°C for 45 min in a shaking water bath (SW22, Julabo Labortechnik GMBH, Seelbach, Germany). Then, 1.6 mL of a mixture of enzymes (porcine pancreatin and amyloglucosidase, 1.5:1) was added. After 20 and 120 min of incubation, a 0.4 mL aliquot was removed into 8 mL of absolute ethanol, mixed well and centrifuged at 3,000 xg for 5 min. The glucose content in the supernatant was determined with the PGO enzyme kit. The glucose contents at 20 and 120 min were designated G20 and G120 respectively. The RDS is defined as the glucose released after 20 min. The glucose released in the second period (after a further 100 min incubation) is defined as SDS. The RS was measured as the starch that remained unhydrolysed after 120 min of incubation.

**Chain-Length Distribution of Amylopectin**

Starch (6-7 mg) was dissolved in 10 mL DI water and debranched with isoamylase (700 units) in 0.01 *M* sodium acetate buffer (pH 3.5). The starch solution was incubated at 27°C overnight and the enzyme was inactivated by boiling for 5 min. The chain-length distribution of amylopectin was analyzed by a high-performance anion exchange chromatography on a Dionex ICS-3000 equipped with a pulsed amperometric detector (HPAEC-PAD) (Dionex, Sunnyvale, CA, USA). The sample was filtered and injected onto a 250 × 3 mm Dionex CarboPac PA-200 column with a 50 × 3 mm guard column (50 × 3 mm). The sample was eluted with a gradient between eluent A (150 m*M* sodium hydroxide) and eluent B (150 m*M* sodium hydroxide containing 500 m*M* sodium acetate) at a flow rate of 0.5 mL/min. The proportion of eluent B was changed as follows: 20% at 0 min, 40% at 10 min, 50% at 20 min and 70% at 50 min.

**Morphology and Particle Size Distribution of Starch Granules**

The starch was coated with gold as described for the grains. The microstructure of the starch granules was examined with a JSM-6400 SEM (JEOL Ltd., Japan) operated at 20 kV. The particle size distribution was determined with a Mastersizer S diffraction particle size analyzer (Malvern Instruments Ltd., Malvern, UK) in a wet-cell mode using water.

**X-ray Diffraction (XRD)**

The XRD analysis was performed on a Bruker D5005X-ray diffractometer (Bruker GmbH, Germany) with 1.54 Å Cu $K_\alpha$ radiation. The sample was exposed to the X-ray beam with the X-ray generator running at 40 kV and 40 mA. The Bragg's angle ($2\theta$) was scanned from 4 – 30°. The relative degree of crystallinity of the sample was quantitatively estimated according to Hermans and Weidinger (1961).

**Antioxidant Properties**

*Sample extraction*. The sample extracts for the determination of antioxidant activities were prepared by the modified method of Tseng *et al*. (2006). Approximately 10 g of finely ground sample was extracted in 100 mL of methanol on a shaker at 25°C for 24 h. The extract was filtered through Whatman no.4 filter paper. The residue was then re-extracted with two additional 100 mL portions of methanol by the same procedure. The combined filtrate was rotary evaporated to dryness at 35°C. The dried extract was re-dissolved in methanol to a concentration of 20 mg/mL and stored at 4°C for further uses.

*Determination of total phenolic content*. The total phenolic content (TPC) of sample was determined using the Folin-Ciocalteu assay (Marinova *et al*., 2005). A 0.2 mL of the extract or standard solution of gallic acid (20-200 mg/l) was added to test tubes containing 1.8 mL of distilled deionized (DI) water. A reagent blank of DI water was prepared. Folin- Ciocalteu's phenol reagent (0.2 mL) was added to the mixture and shaken. After 5

min, 2 mL of 7% $Na_2CO_3$ solution was added to the mixture. The solution was diluted to 5mL with DI water and mixed. After incubation for 90 min at 25°C, the absorbance at 750 nm was determined. The TPC of sample was expressed as mg gallic acid equivalents (GAE)/g extracted sample.

*DPPH radical scavenging ability.* The 1,1'-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging ability of sample was estimated following the method of Choi *et al.* (2007). Aliquots of 0.2 m*M* DPPH in methanol (0.8 mL) were mixed with 0.2 mL of the extracts. The mixtures were vigorously shaken and left to stand for 10 min in the dark. The absorbance at 517 nm was measured against water and methanol as the blank and control respectively. The scavenging ability was calculated as follows: scavenging ability (%) = [(△of control-△ of sample)/△ of control] × 100; where △ = absorbance of sample or control – absorbance of blank.

*Reducing power.* The reducing power of sample extracts was determined according to Oyaizu (1986). The extract in methanol (2.5 mL) was mixed with 2.5 mL of 200 mM phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide. The mixture was incubated at 50°C for 20 min. Then, the 2.5 mL of 10% trichloroacetic acid (w/v) was added to the mixture and it was centrifuged at 200xg for 10 min. The supernatant (5 mL) was then mixed with an equal volume of DI water and ferric chloride solution (0.1% w/v). The absorbance at 700 nm was measured against water and methanol as the blank and control respectively.

**Coixenolide Assay**

The coixenolide content was determined by measuring 2,3-butanediol liberated from coixenolide by acid-catalyzed transesterification according to the method of Yang *et al.* (2004) with slight modification. The crude oil of sample was extracted in a Soxhlet apparatus using diethyl ether according to the AOAC (2000) method. The crude oil of sample (1 g) was dissolved in 20 mL of 7% (w/w) methanolic HCl solution, and refluxed in a water bath at 100°C for 4 h. The mixture was cooled and neutralized with 30% methanolic sodium methoxide. The salt was then precipitated and filtrated and the filtrate was rotary evaporated to a final volume of 2 mL, and it was mixed with 1,5 pentanediol (Sigma Chemical Co, St. Louis, MO, USA) as an internal standard. The concentrated methanolic solution was analyzed using a gas chromatograph equipped with flame ionization detector (Varian CP3800, Varian, Inc., Middelburg, Netherland). A CP7420, WCOT fused silica, CP-select CB for FAME 100 m × 0.25 mm, 0.25 $\mu$m film thickness column was used. The column temperature was programmed to increase from 100 to 250°C at 5°C/min. The injector and detector temperatures were set at 250°C. The coixenolide content was calculated from the 2,3-butanediol content by comparing the area under the peak with that of the internal standard.

**Swelling Power and Solubility**

Swelling power and solubility were determined according to Li and Corke (1999) with modification. The sample (0.3 g dry basis) and 15 mL of distilled water were added into a centrifuge tube with screw cap. The centrifuge tube was heated at 65, 75, 85, and 95°C for 30 min in a shaking water bath (SWB20, Ratek Instruments Pty. Ltd., Australia) and then centrifuged at 2000 × g for 15 min. The clear supernatant was removed into a preweighed dish and dried at 105°C until a constant weight was obtained. The swollen sediment and dried supernatant were weighed to determine the swelling power and solubility as follows: Swelling power (g/g) = [sediment weight / (initial sample weight x (100% - %solubility))] × 100; Solubility (%) = [dry supernatant weight / initial sample weight] × 100

**Pasting Properties**

The pasting properties were measured with a Rapid Visco Analyzer (RVA, Newport Scientific, Warriewood, Australia), according to Li and Corke (1999) with slight modification. A 3 g of sample (db) was weighed into a RVA

canister and DI water was added to obtain the total sample weight of 28 g. The time-temperature profile was: holding for 1 min at 50°C, heating to 95°C in 8.3 min, holding for 5 min 95°C, cooling to 50°C in 7.7 min, and holding for 2 min at 50°C, which was a standard profile 2.

### Differential Scanning Calorimetry (DSC)

The gelatinization and retrogradation properties of the samples were investigated by DSC (DSC 7, PerkinElmer Inc., Shelton, CT, USA). The sample (10 mg) was weighed into a 60 $\mu$l stainless steel pan and distilled water was added to obtain starch-water suspension containing 75% water. Indium and an empty stainless steel pan were used for the standard and reference respectively. The pans were heated from 25 to 125°C at a heating rate of 10°C / min. After heating, the sample was aged at 4°C for 7, 14, 21, 28, and 39 days to monitor retrogradation. The retrograded samples were reheated again with the same procedure. The onset temperature (To), peak temperature ($T_p$), completion temperature ($T_c$), gelatinization temperature range ($T_c$- $T_o$) and enthalpy ($\Delta H$) were determined with Pyris software.

### Statistical Analysis

A completely randomized design (CRD) was performed. Analysis of variance (ANOVA) was performed with SPSS version 13 (SPSS Inc., IL, USA). Each experiment was conducted in triplicate. The differences between mean values were established by Duncan's multiple-range test at the 95% significance level.

## Results and Discussion

### Structural Morphology of Job's Tear Grain

The size of white husk Job's tear is slightly bigger than that of the black husk variety but their shapes are similar (data not shown). The cross and longitudinal sections of white and black husk Job's tear grains are shown in Figure 1. The kernel structural morphology is similar to that of barley and wheat in that a crease exists in the middle of the kernel. The germ is mostly entrapped in the endosperm and makes up ca. one third of the whole grain, which is quite large, compared with other cereals. Figure 1a and b illustrates the acrospire and root location in the germ part. The SEM image showed that the endosperm and germ could clearly be distinguished by their different structural morphologies (Figure 2(a), (b)). A network appeared in the germ part that was likely to be protein matrix (Figure 2(c), (d)), while the endosperm exhibited starch granules and protein bodies (Figure 2(e), (f)). It revealed that Job's tear kernels contain a large portion of germ embedded beneath the crease of the seed where it is difficult to separate.

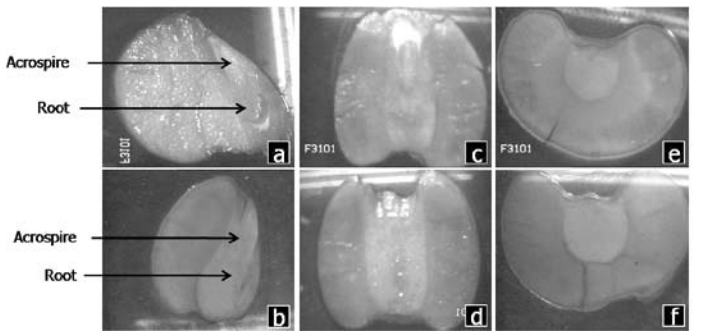


**Figure 1. Cross (a, b) and longitudinal (c, d, e, f) sections of white (a, c, e) and black (b, d, f) husk Job's tear kernels, as viewed under a stereo microscope**

**Chemical Composition of Job's Tear**

The chemical composition of whole grain flour from white and black husk of Job's tear presented in Table 1 was similar to that of Job's tear from Laos, Vietnam, and Taiwan (Wu *et al.*, 2007). The protein and lipid contents of black husk Job's tear were higher than those of the white husk variety but the ash content was lower ($p < 0.05$). After removing the germ, the lipid and ash contents of the degermed Job's tear were lower than those of the whole grain, demonstrating that the degerming process was conducted properly. However, the protein content of the whole grain was similar to that of the degermed flour. This indicated that the protein of Job's tear endosperm has a relatively high protein content, which is equally distributed along the whole kernel.

After Job's tear starch was isolated, the protein, lipid, and ash of the starch from both cultivars were less than 1%. The amylose contents of starch from white and black husk Job's tear were 10.34 and 17.01% respectively. Regarding starch digested fractions, the slowly digested starch (SDS) content of the

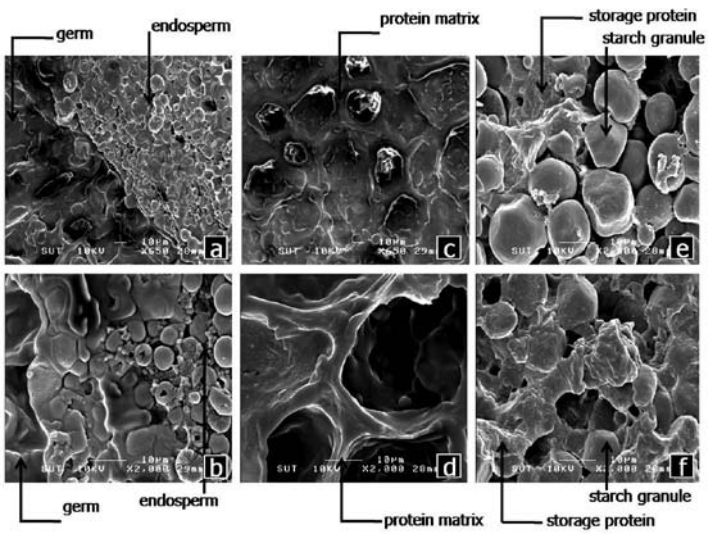


**Figure 2. Scanning electron micrograph of longitudinal sections of white (a, c, e) and black (b, d, f) husk Job's tear kernels. (a, b: endosperm vs. germ, c, d: germ, e, f: endosperm)**

**Table 1. Chemical composition of whole grain and degermed Job's tear flours**

| Composition | Whole grain flour | | Degermed flour | |
|---|---|---|---|---|
| | White | Black | White | Black |
| Protein (%,db) | $13.54 \pm 0.12^{b}$ | $16.85 \pm 0.28^{a}$ | $13.18 \pm 0.20^{b}$ | $16.46 \pm 0.33^{a}$ |
| Lipid (%,db) | $4.86 \pm 0.14^{b}$ | $5.35 \pm 0.16^{a}$ | $0.91 \pm 0.02^{c}$ | $1.09 \pm 0.01^{c}$ |
| Ash(%,db) | $1.74 \pm 0.02^{a}$ | $1.17 \pm 0.01^{b}$ | $0.59 \pm 0.02^{c}$ | $0.23 \pm 0.01^{d}$ |

$^{a, b, c, d}$ *Different letters within the same row indicate a significant difference ($p < 0.05$).*

white starch (WS) (39.93%) was higher than that of the black starch (BS) (36.20%), whereas the resistant starch (RS) content of the WS (49.29%) was lower that of the BS (52.00%) ($p < 0.05$). The rapidly digested starch (RDS) content of both Job's tear cultivars was not significantly different (10.78-11.80%) ($p > 0.05$). As compared with potato starch, the RDS and SDS content of Job's tear starch was higher than that of potato starch whereas the RS content of Job's tear starch was lower (data not shown). This suggests the structural integrity of Job's tear starch granule was weaker than that of potato starch, a B-crystalline type. This hypothesis was supported by the result that Job's tear starch exhibited an A-crystalline pattern (data not shown) which was less packed than the B-type starch (Zhang *et al*., 2006). In addition, the granular surface of Job's tear starch contained pores as shown in Figure 3, which could enhance the enzyme accessibility.

## Morphology, Size Distribution, and XRD of Starch Granules

The starch granules of Job's tears from both cultivars were round, polyglonal in shape with porous surfaces (Figure 3). The particle size distribution of Job's tear starch was bimodal (Figure 4). The small granules of white and black

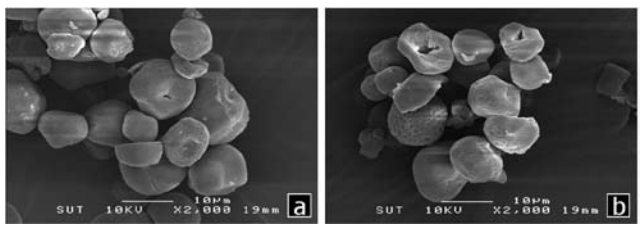


**Figure 3. Scanning electron micrograph of starch granules of white (a) and black (b) Job's tear**

**Table 2. Antioxidant properties of methanolic extracts of whole grain and degermed Job's tear flours**

| Sample | Antioxidant Properties | | |
|---|---|---|---|
| | Total Phenolic content (mgGAE/g) | DPPH radical scavenging activity (%) | Reducing power ($A_{700}$) |
| WGWF[1] | 7.33 ± 0.14[b] | 5.40 ± 1.00[b] | 2.88 ± 0.29 |
| WGBF[2] | 7.93 ± 0.18[a] | 6.49 ± 1.17[ab] | 2.56 ± 0.28 |
| DGWF[3] | 7.05 ± 0.41[b] | 6.56 ± 0.41[ab] | 2.69 ± 0.23 |
| DGBF[4] | 8.18 ± 0.39[a] | 7.53 ± 0.85[a] | 2.64 ± 0.23 |

[a, b] *Different letters within the same column indicate a significant difference ($p < 0.05$).*
[1] *whole grain white Job's tear flour*
[2] *whole grain black Job's tear flour*
[3] *degermed white Job's tear flour*
[4] *degermed black Job's tear flour*

Job's tear starch had diameters in the range of 0.25-3 and 0.2-3 $\mu$m respectively and those of the large granules were 3-25 and 3-30 $\mu$m respectively. The average sizes of granules of white and black Job's tear starch were 11.68 and 12.29 $\mu$m respectively, so they may be considered to have relatively small granules.

The XRD pattern of Job's tear is similar to that of rice starch (data not shown), which is an A-type starch, a typical pattern of cereal starch. The relative crystallinities of white and black Job's tear starch were 24.56 and 22.86% respectively.

**Chain-Length Distribution of Amylopectin**

The chain-length distribution of debranched amylopectin from both cultivars is shown in Figure 5. The maximum proportion of chain-length distribution of Job's tear was found at degree of polymerization (DP) 13-24, which was similar to wheat, barley, and other A-type starches (Jane *et al.*, 1999). The average chain-length distribution of the WS and BS was DP 21.0 and 20.8 respectively which are shorter than that reported in other A-type starches, such as wheat (23.3), barley (25.7), and triticle (23.8) (Ao and Jane, 2007).

**Antioxidant Properties**

The TPC of methanolic extracts of white and black husk Job's tear are shown in Table 2. The TPC of the white husk Job's tear was less than that of the black husk one for both whole grain and degermed flours ($p < 0.05$). As compared with other cereals, the TPC of Job's tear was lower than those of millet (13.87 mg/g),

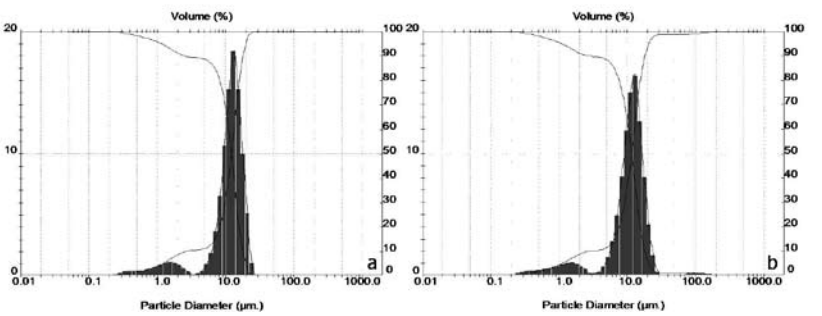


**Figure 4. Particle size distribution of white (a) and black (b) Job's tear starches**

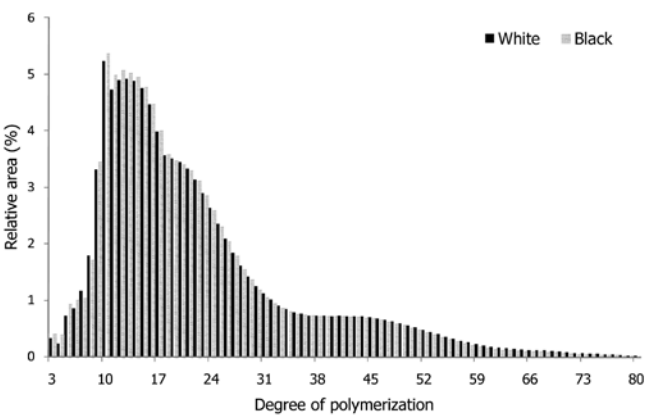


**Figure 5. Chain length distribution of white and black Job's tear amylopectin**

rye (10.26 mg/g) and sorghum (41.28 mg/g) (Ragaee *et al*., 2005) but higher than those of black rice (3.13 mg/g), brown rice (0.54 mg/g), barley (0.50 mg/g), mungbean (0.45 mg/g), foxtail millet (0.47 mg/g), and prosomillet (0.29 mg/g) (Choi *et al*., 2007). It could be possible that the TPC of each sample depended on the type of cereal, cultivar, composition and technical assay (Maisuthisakul *et al*., 2008). Moreover, comparable TPC of whole grain and degermed flour were observed for the 2 cultivars ($p > 0.05$). It could be mentioned that the phenolic compounds of Job's tear grains are evenly distributed in both the germ and endosperm portions. This could be due to the fact that the major phenolic compounds in the Job's tear grains may have both hydrophilic and hydrophobic properties in nature that are able to solubilize both in the germ and endosperm, i.e., coniferyl alcohol, syringic acid, and ferulic acid (Kuo *et al*., 2002)

The DPPH radical scavenging ability of methanolic extracts of both white and black husk Job's tear flour was not significantly different ($p > 0.05$) (Table 2). However, slightly better activity was observed for the whole grain black Job's tear flour and for the degermed flour of both cultivars. As compared with other cereals, the DPPH radical scavenging ability of Job's tear was higher than that of mungbean, foxtail millet, and prosomillet, but lower than that of brown rice, black rice, sorghum and barley (Choi *et al*., 2007). The DPPH radical scavenging ability is a measure of the conversion of the DPPH radical (DPPH•) to DPPH-H by an antioxidant giving either an electron or hydrogen atom. Stratil *et al*. (2007) reported that the total phenolic content has a positive correlation with the free radical scavenging ability in fruits and cereals. Obviously, the TPC of the black husk cultivar was slightly higher ($p < 0.05$) than that of the white one in both whole and degermed flours resulting in slightly higher DPPH activity. In addition, it could be considered that the degermed flours had a better antioxidant activity in terms of the ability to scavenge free radicals of DPPH although significant differences were not found. Antioxidant activity of carbohydrate products could be affected by other components in the sample as reported by Zhang *et al*. (2001), Zhang *et al*. (2004) and Chirinang and Intarapichet (2009) in that antioxidant activity of some mushrooms not always solely depended on the TPC content only but also depended on other components of the materials such as dietary fiber and amino acid contents.

Similar to the results of the DPPH radical scavenging ability, the reducing power of both cultivars of Job's tear was not significantly different ($p > 0.05$) (Table 2). Compared with other cereals, the reducing power of Job's tear flours of both cultivars was lower than those of sorghum and black rice, but greater than those of brown rice, white rice, mungbean, foxtail millet, and prosomillet (Choi *et al*., 2007). Therefore, with regard to reducing power, whole grain and degermed flours of white and black Job's tears were comparable.

### Coixenolide Content

Coixenolide is one of lipids in Job's tear. Job's tear oil and coixenolide were reported to provide many health benefits such as blood circulation improvement, a reduction of inflammation, purulence and pain, anti-cancer, and anti-tumor (Bao *et al*., 2005; Dharamanada, 2007). The coixenolide content of whole grain white and black Job's tear was 0.02 $\mu$g/g. After the degerming process, the coixenolide of both Job's tear flour was not detected due to a minute content of oil. Chang and But (1987) reported that the coixenolide content in Job's tear was less than 0.25%. In addition, some processing steps for breaking the structure and extraction such as microbial fermentation and supercritical fluid extraction with or without ultrasound may be needed to improve the content of coixenolide (Yang *et al*., 2004; Hu *et al*., 2007).

## Physicochemical Properties

### Swelling Power and Solubility

The swelling power of Job's tear flours and starches was higher with increasing the

temperature as shown in Figure 6. Beginning from the temperature of 75°C, the swelling powers of whole grain and degermed white Job's tear flours were higher than those of the black ones due to their lower protein, lipid and amylose contents. Protein in grain could enhance the molecular interactions between protein and protein or protein and starch by heat. It may obstruct the hydration of water and could reduce or restrict the swelling volume of starch granules (Hamaker and Griffin, 1993) Amylose could interact with lipid to form an amylose-lipid complex which may affect the bonding force within starch granules, consequently decreasing the swelling volume (Adebowale *et al*., 2002). The swelling power of the degermed flour was higher than that of the whole grain flour due to the lower lipid content of the degermed flour. Lipid that surrounded the starch granules could hinder water penetration into the starch granules. In contrast, the swelling power of the WS was lower than that of the BS. It may be due to the difference in the crystallinity of the starch of both cultivars as mentioned previously. The ratio of crystalline to amorphous regions influences the interactions of water molecules with the hydroxyl groups of amylose and amylopectin in these regions; thus, a higher proportion of the amorphous region or lower relative crystallinity contributes to a higher swelling power (Tester and Morrison, 1990).

The solubility of Job's tear flour and starches is shown in Figure 7. The lower solubility of Job's tear flours may be due to the protein-amylose complex formation in Job's tear flour. According to Pomeranz (1991), the formation of protein-amylose complex in native starch and flour resulted in a decreased swelling power. The solubilities of the black Job's tear flours were higher than those of the white ones due to the higher content of amylose, lipid, and protein in the black flours. Regarding the whole grain and degermed flours, the solubility of the whole grain flour was higher, probably related to the greater lipid and ash of the whole grain flour. With increasing temperatures, the solubility of Job's tear starches was substantially increased up to 58-59%, which is relatively high. This

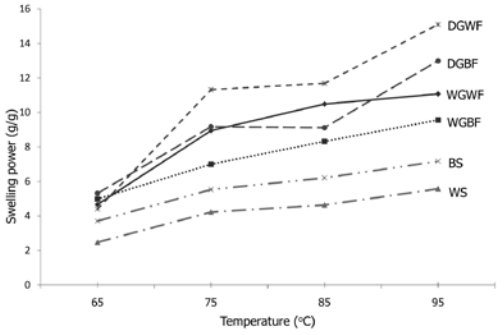


**Figure 6. Swelling power of whole grain and degermed flours and starch of Job's tear. (WGWF, whole grain white Job's tear flour; WGBF, whole grain black Job's tear flour, DGWF, degermed white Job's tear flour; DGBF, degermed black Job's tear flour; WS, white Job's tear starch, BS, black Job's tear starch)**

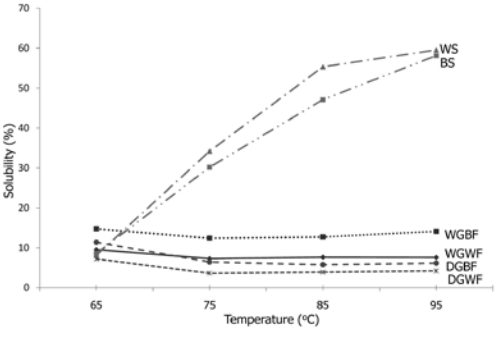


**Figure 7. Solubility of whole grain and degermed flours and starch of Job's tear. (WGWF, whole grain white Job's tear flour; WGBF, whole grain black Job's tear flour, DGWF, degermed white Job's tear flour; DGBF, degermed black Job's tear flour; WS, white Job's tear starch, BS, black Job's tear starch)**

might due to the fact that the Job's tear starch contained a high amount of small size starch molecules and some big and small holes appeared on the surface of the starch granules. Upon swelling, the surface holes may allow a number of the small starch molecules to leach out into the water.

**Pasting Properties**

Table 3 illustrates that the RVA pasting profiles of the flours from white husk Job's tear had a higher peak, trough, breakdown, and final viscosities but lower setback and pasting temperatures than those of the black flours. Since white husk Job's tear had the lower protein, lipid, and amylose contents, these components apparently affected gelatinization behavior and viscosity. In addition, the protein component of Job's tear might be a factor related to the lower swelling volume of black Job's tear that, in turn, affected peak viscosity and pasting temperature (Martin and Fitzgerald, 2002). The degermed flours of both cultivars showed higher pasting viscosity profiles but lower pasting temperatures than those of the whole grain flours. This was a result of the lower lipid content for the degermed flour.

The Job's tear starches had the higher peak viscosities and lower trough viscosities, final viscosities, and pasting temperatures compared with the flours ($p < 0.05$). This indicated that the interactions with the lipid and protein components affected starch gelatinization and the pasting properties of starch in the flours. Therefore, a high level of thermal treatment should be employed to cook Job's tear flours, especially for those made from the black cultivar.

**Gelatinization and Retrogradation of Starch**

The gelatinization parameters of Job's tear starches are shown in Table 4. The $T_p$, $T_c$, and $T_{c-}$ $T_o$ of the WS were significantly higher than those of the BS ($p < 0.05$). Furthermore, the $T_p$ values of the WS and BS were the same as their pasting temperatures. The $\Delta H$ of the WS was slightly greater than that of the BS, correlating with their relative crystallinities. A starch with a higher degree of crystallinity maintains structural stability; consequently,

**Table 3. Pasting properties of whole grain, degermed flours and starches of Job's tear**

| Sample | Pasting parameter (RVU) | | | | | Pasting temperature (°C) |
|---|---|---|---|---|---|---|
| | Peak viscosity | Trough viscosity | Breakdown | Final viscosity | Setback | |
| WGWF[1] | 54.25[c] | 50.50[b] | 3.75[e] | 64.31[c] | 13.80[d] | 77.02[c] |
| WGBF[2] | 19.75[e] | 18.42[d] | 1.33[f] | 41.08[d] | 22.67[c] | 93.12[a] |
| DGWF[3] | 86.31[b] | 74.28[a] | 12.03[c] | 103.13[a] | 29.03[b] | 75.95[d] |
| DGBF[4] | 48.56[d] | 43.20[c] | 5.36[d] | 79.50[b] | 36.30[a] | 88.87[b] |
| WS[5] | 101.61[a] | -4.28[f] | 105.89[a] | -1.17[f] | 3.11[f] | 73.30[e] |
| BS[6] | 70.92[b] | -0.86[e] | 71.78[b] | 7.06[e] | 7.92[e] | 71.33[f] |

[a, b, c, d, e] *Different letters within the same column indicate a significant difference ($p < 0.05$).*

[1] *whole grain white Job's tear flour*

[2] *whole grain black Job's tear flour*

[3] *degermed white Job's tear flour*

[4] *degermed black Job's tear flour*

[5] *white Job's tear starch*

[6] *black Job's tear starch*

the granule is more resistant toward gelatinization and thus exhibits a higher transition temperature. Song and Jane (2000) reported that a starch with a longer branch chain-length developed into large crystallites, which required a higher temperature to gelatinize. Therefore, the slightly higher relative crystallinity and proportion of longer branch chain-length of the WS was probably responsible for its higher gelatinization temperature compared with the BS.

The retrogradation temperatures of white and black Job's tear starch were in the ranges of 39.2 - 59.5 and 40.0 - 52.9°C respectively (Table 4), which are similar to starch from most sources. However, the thermal transition of retrogradation of both starches was observed on day 39 at 4°C, which is considerably slower than other A-type cereal starches, such as wheat, barley, triticle, rice, and waxy rice, at the same storage temperature (Ao and Jane, 2007; Lin *et al*., 2001). The Job's tear starches also had a considerably lower setback value. This was probably related to the low amylose content together with a higher proportion of short-branch-chain of amylopectin.

## Conclusions

The germ of Job's tear is entrapped in the endosperm beneath the crease and its size is relatively large. When the germ was removed, the protein content remained high, indicating degermed Job's tear flour is a good source of protein, especially that from black Job's tear flour. The antioxidant activities, which were DPPH radical scavenging and reducing power, and coixenolide contents for both cultivars and types of flour were not different. However, the TPC of the black husk Job's tear was slightly higher for both types of flour as compared with the white ones. The pasting profile of the white Job's tear flours was higher but the pasting temperature was lower. In contrast, the gelatinization temperature of the white Job's tear starch was higher. The granular size of Job's tear starch from both cultivars was small with the average size of 12 $\mu$m. The average chain length distribution of its amylopectin was relatively short compared with other cereals. These structural features contributed to the low paste viscosity of Job's tear starch and its slow retrogradation.

**Table 4. Gelatinization and retrogradation properties of Job's tear starches**

| Starch | Thermal transition parameters | | | | |
|---|---|---|---|---|---|
| | $T_o$ (°C) | $T_p$ (°C) | $T_c$ (°C) | $T_c$-$T_o$ (°C) | ΔH(J/g) |
| **Gelatinization** | | | | | |
| white Job's tear | 68.28 ± 0.13 | 73.98 ± 0.77[a] | 80.94 ± 1.70[a] | 12.66 ± 1.60[a] | 15.00 ± 0.24 |
| black Job's tear | 66.82 ± 0.22 | 70.98 ± 0.09[b] | 76.74 ± 0.29[b] | 9.92 ± 0.09[b] | 14.22 ± 0.65 |
| **Retrogadation at 4°C for 7, 14, 21, and 28 days** | | | | | |
| white Job's tear | nd | nd | nd | nd | nd |
| black Job's tear | nd | nd | nd | nd | nd |
| **Retrogadation at 4°C for 39 days** | | | | | |
| white Job's tear | 39.02 ± 0.60 | 47.02 ± 1.22[a] | 59.48 ± 1.70[a] | 20.79 ± 1.42[a] | 8.20 ± 1.57 |
| black Job's tear | 40.20 ± 0.20 | 45.75 ± 0.11[a] | 52.96 ± 2.41[b] | 12.76 ± 4.09[b] | 10.17 ± 3.33 |

[a, b] *Different letters within the same column indicate a significant difference ($p < 0.05$).*
*nd = not detectable*

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