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# Impact of germination on nutritional and physicochemical properties of adlay seed (*Coixlachryma-jobi* L.)

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ABSTRACT

Adlay has garnered a great deal of research attentions in recent years as a highly nutritious food material and herbal medicine. This study characterized the changes of nutritional and physicochemical properties of adlay seeds during a 60-h germination. The results showed that the 60-h germination brought about a 3.4-fold increase in γ-aminobutyric acid (GABA) and 3.6-fold increase in coixol compared to ungerminated adlay seeds, while the triolein content slightly decreased. Some high molecular proteins were hydrolyzed into smaller proteins, peptides and amino acids after germination. Scanning electron micrographs (SEM) showed that the germination process destroyed the continuous matrix structure of adlay flour and created pits and holes on the surface of some starch granules. Germination resulted to changes in the pasting and gelatinization properties of adlay flour. The results of present study suggest that germination efficiently enhances the nutritional compounds while altering the physicochemical characteristics of adlay seeds.



## 1. Introduction

Adlay (*Coix lachryma-jobi* L. var. *ma-yuen* Stapf), an annual crop, has been widely cultivated for centuries in China and Japan and has long been consumed as both food and medicine. Many health-beneficial components have been found in adlay, including protein (Watanabe, Kato, & Ayugase, 2012), polysaccharide (Lu et al., 2013; Yao, Zhu, Gao, & Ren, 2015), coixenolide, coixol (Chung et al., 2011), and oil (Yu, Gao, Zeng, & Liu, 2011). Modern research has demonstrated that adlay seeds exhibited numerous health benefits, including the abilities to prevent the formation of tumors (Chang, Huang, & Hung, 2003), reduce inflammation (Chen, Chung, Chiang, & Lin, 2011), and aid in immune system regulation (Lin & Tsai, 2008).

Germination is an inexpensive and effective processing technology that improves the nutritional quality of cereals and legumes. Geminated products are widely consumed across the globe. Germination is a complex process in which endogenous seed enzymes are activated resulting in significant changes in the biochemical and physical properties of the seeds (Hao, Wu, Li, Wang, & Liu,

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2016). Starches, fibers, and proteins can be hydrolyzed into small molecules by activating hydrolytic enzymes such as amylases and proteases, which makes the cereal kernels easily cooked and improves their texture (Chung, Cho, Park, Kweon, & Lim, 2012; Wu, Yang, Touré, Jin, & Xu, 2013). Moreover, germination greatly enhances the content of various bioactive compounds through these enzymatic actions while at the same time reducing antinutrient levels in the seeds. Recent studies have shown that germination enhances the content of GABA, phenolic compounds and γ-oryzanol in rice (Cáceres, Martínez-Villaluenga, Amigo, & Frias, 2014; Kim et al., 2012). In short: Germinated seeds are nutritionally superior to their original seeds.

The nutritional and physicochemical properties of cereal are very important when they are used as ingredients in the development of functional foods. Germinated adlay products have been available in the market of some Asian countries for many years. Previous research has demonstrated that germination is a convenient and effective natural process to enrich phenolic contents and their antioxidant activities. However, there is little information available regarding the nutritional and physicochemical properties of germinated adlay seeds. Comprehensive assessments of changes in these properties are necessary for the optimization of the nutrients and texture of adlay-based foods. Therefore, this study aimed to investigate the dynamic changes of nutrients and physicochemical properties of adlay seeds during germination. The contents of crude proteins, fats, starches, triolein, GABA, and coixol in adlay at different germination stages were measured. The changes in certain physicochemical properties were also estimated. We hope that the results presented here lend some insight into the germination-induced changes in adlay seeds and provide useful information for germinated adlay products development and utilization.

## 2. Materials and methods

### 2.1. Materials

The adlay we used in this study was obtained from Xinlong Co., Ltd. (Guizhou, China) in September 2015, and the cultivar was the most commonly consumed in the local area. The adlay seeds were sealed in plastic bags and stored at 4–8 °C until use. Trioleoylglycerol (triolein), 6-methoxybenzoxazolone (coixol) and 2,4-dinitrofluorobenzene (FDNB) were purchased from Sigma Chemical Co.(St. Louis, MO, USA). HPLC-grade acetonitrile and dichlormethane were obtained from J&K Scientific Ltd. (Beijing, China). All other chemicals and regents were purchased from Sinopharm Chemical Reagent Co., Ltd. (Suzhou, China) and were of analytical grade.

### 2.2. Germination process

The brown adlay seeds were surface sterilized with 0.1% (V/V) sodium hypochlorite (seeds: NaOCl ratio, 1:5 w/v) for 30 min and then rinsed with distilled water before being soaked for 12 h at 25 °C. After draining the soaking water, the hydrated seeds were washed and germinated by being layered over a wet filter paper at 25 °C and 95% relative humidity in the dark. Seeds were germinated for 0, 12, 24, 36, 48 and 60 h and denoted as G0, G12, G24, G36, G48 and G60, respectively. The seeds taken out at 0 h were considered as raw seeds. Samples were freeze-dried, milled through an 80-mesh sieve, and stored at 4 °C for further analysis.

### 2.3. Determination of crude proteins, starches and fats

The crude protein content was measured via the Kjeldahl method (FOSS-8200 apparatus, Denmark) with a factor of 6.25 to convert nitrogen to crude protein content. The crude fat content was measured via the Soxhlet extraction method using petroleum ether. The total starch of the germinated flour was determined using the Total Starch Assay Kit (AA/AMG) (Megazyme International, Wicklow, Ireland).

### 2.4. Determination of triolein content

Triolein (trioleoylglycerol) was extracted by blending 0.3 g of adlay flour with 20 mL of acetonitrile/dichlormethane (65:35, v/v) for 2 h. The extracts were centrifuged at 10,000*g* for 10 min, and the supernatants were filtered through a 0.45-mm filter. High performance liquid chromatography (HPLC) was performed to determine the triolein content on Agilent 1100 HPLC system (Agilent Technologies Inc., Palo Alto, CA, USA) equipped with an ELSD detector and SunFire C18 column (4.6 × 250 mm i.d., 5 μm, Waters, Milford, MA, USA). The chromatographic separation was performed using acetonitrile-dichlormethane (65:35, v/v) at 0.5 mL/min. The flow rate of nebulizer gas was 1.2 L/min, and the drift tube temperature was set to 70 °C.

### 2.5. Determination of GABA content

GABA content was determined by the method of Lü, Zhang, Meng, Wang, and Guo (2010). Briefly, GABA was extracted by blending the adlay flour (1 g) with 15 mL of 70% ethanol for 2 h. The obtained mixture was then centrifuged at 4000*g* for 15 min. The supernatant was collected, and the residue pellet was re-extracted twice. All the supernatants were collected and dried by a rotary evaporator. The dried extracts were then re-dissolved in 5 mL of water. A 1 mL of the re-dissolved solution, 1 mL of 0.5 mol/L $NaHCO_3$ (pH = 9.0), and 1 mL of FDNB (1%) reagent were mixed in a 10-mL volumetric flask and reacted in dark at 60 °C for 1 h. The volume of the mixture was brought up to 10 mL with phosphate buffer (0.02 mol/L, pH = 7.0). The derivative was quantitatively analyzed by HPLC with an Inertsil ODS-SP column. The mobile phase was developed using 50% (v/v) acetonitrile (A) and phosphate buffer (0.02 mol/L, pH = 7.0) (B) at 1.2 mL/min. The gradient elution profile was as follows: 16–100% A at 0–20 min; 100–16% A at 20–26 min; 16% A at 26–30 min. The UV detection was set at a wavelength of 360 nm, and the injection volume of each sample was 20 μL.

### 2.6. Determination of coixol (6-methoxybenzoxazolone) content

The coixol was extracted by subjecting 1.0 g of adlay flour three times with 25 mL of acetone. After the solvent was completely evaporated by rotary evaporation, the residue was redissolved in 10 mL of methanol. Then, the solution was filtered through a 0.45-μm filter, and the filtrate was quantitatively analyzed using Shimadzu LC-20AT high-performance liquid chromatography (HPLC) system with a Inertsil ODS-SP column (250 mm × 4.6 mm) column at 25 °C. The chromatographic separation was performed using 0.075% $H_3PO_4$ in 25% acetonitrile at 1 mL/min, and the detection wavelength was 232 nm.

### 2.7. Scanning Electron Microscopy (SEM)

Powdered adlay flours were micro examined by a scanning electron microscope (Quanta-200, FEI Company, Netherlands) at an accelerating potential of 5 kV with ×2400 magnification. Freeze-dried, finely ground samples were mounted on an aluminum stub using a double-sided stick tape and were coated with a thin film of gold (10 nm) for observation.