Changes in Protease Activity and Proteins in Naked Oats (*Avena nuda* L.) during Germination

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ABSTRACT—*Changes in protease activity, the content and composition of proteins were investigated during germination of naked oats. Compared with raw groats, an increase in protease activity from oat groats was continuously observed, and it increased by 252% and reached the maximum at stage G60; the crude protein and prolamin increased basically in the content, and they increased by 10.35%, 18.33% at the end of germination respectively; while the levels of albumin, globulin, glutelin, soluble and insoluble protein first increased and then decreased during germination. The protease activity correlated highly with the content of different protein fractions but globulin during germination.*

Keywords—Naked oat, Protein, Protease activity, Germination

1. INTRODUCTION

Germination has been widely used for centuries to soften the kernel structure, to decrease antinutritional compounds and to improve its nutritional value in beans (Alonso *et al*., 2000) and some cereal seeds (Wu & Wall 1980; Koehler *et al*., 2007). During germination, endogenous enzymes are produced or activated, which may degrade macromolecular to small molecular substances, and produce some active substances such as phenolics, γ–aminobutyric acid and so on (Xu *et al*., 2009; Xu *et al*., 2010; Shi *et al*., 2010). One of the most important physical-chemical changes that occur during germination is the degradation of the protein and their conversion into soluble peptides and amino acids to provide substrates for the plant's development, which can result in the changes in protein content and size distribution. During germination of oats, the content of crude protein increased and protein was degraded to increase the soluble protein content and free amino acids (Tian *et al*., 2010; Wu 1983; Klose *et al*., 2009). However, germination was also a very active and complex metabolic process that may decrease nutritive value of pulses (Nnanna *et al*., 1990; Rozan *et al*., 2000; Urbano *et al*., 2005). Wilhelmson *et al*. (2001) reported that the chemical composition of malted oat seeds has concern with the conditions and the level of germination. Therefore, it is necessary to evaluate what happens to the changes in nutrients, phytochemicals and related properties affected by these changes in oats during germination, especially the changes of proteins. However, information on these aspects is very limited in oats, especially naked oat cultivars (*Avena nuda* L.) from China.

The objective of the present study was to further investigate the effect of a highly controlled germination process on the protease activity, the chemical composition and content of proteins, which that are required to produce high quality food based on naked oats.

2. MATERIALS AND METHODS

2.1 Plant materials

Baiyan II, a naked oat cultivars (*Avena nuda* L.), was used in the study. The cultivar was grown in 2012 in bases for growing organic oat, Shanxi, China. The harvested oat groats were dried to about 10% moisture and then stored until time of steeping and germination.

2.2 Germination

Oat groats were surface sterilized using a 1% solution of sodium hypochlorite for 30 s, and then they were washed three times with demonized water before steeping. The oat groats were steeped with demonized water for 12 h at 25 ^{o}C, aeration for 1 h every 4 h, and a sampling (S12) was carried out at the end of steeping. After steeping, the remaining oats were drained and germinated for 72 h at 25 ^{o}C and 95% relative humidity, and six samplings were carried out (G12, G24, G36, G48, G60, and G72), which took at 12, 24, 36, 48, 60 and 72 h during the germination process. After sampling, samples were immediately freeze dried and stored at –40 ^{o}C until time of analysis.

2.3 Determination of protease activity

The protease activity was measured according to the method of Harvey and Oaks (1974) with some modifications. Two grams of milled oats were mixed, on an ice bath, with 10 mL of citric acid-disodium hydrogen phosphate buffer (0.02 M, pH 6.0) containing 5 mM *β*–mercaptoethanol and 2.5 mM disodium ethylenediamine tetraacetic acid (EDTA) and shaken with a laboratory rotary shaker at 250 rpm for 20 min at 4 ^{o}C. Then the homogenate was centrifuged at 10 000g for 20 min at 4 ^{o}C, and the supernatant was the crude protease extracts. The reaction mixture consisted of 2 mL of crude enzyme liquid and 2 mL of substrate (20 g/L casein) was incubated for 60 min at 40 ^{o}C and then terminated for 5 min at 90 ^{o}C, followed by cooling and centrifugation (5 000 g, 15min) at 4 °C. The absorbance was measured at 275 nm and the activity of protease was defined as the μg of L-tyrosine produced at 40 ^{o}C per minute per gram on a dry weight basis (DW).

2.4 Extraction of protein

Two-gram milled oat samples were extracted with 50 mL Tris-HCl buffer (0.05 M, pH 7.8) for 30 min at room temperature by an ultrasonic homogenizer, and then the homogenates were centrifuged at 10 000 g for 15 min at 4 ^{o}C. After centrifugation, the supernatants were removed and extraction was repeated two times at the same conditions. Then adjusting the pH value of the combined supernatants to about 4.5, the resulting precipitates after centrifugation were resuspended in 50 mL of Tris-HCl buffer and analyzed for soluble protein. The residues for determination of insoluble protein were extracted with 50 mL 0.2% NaOH and then extracts were treated under the same conditions as the soluble protein.

Oat protein fractions were extracted according to the Osborne procedure with some modifications. Namely, milled samples were sequentially extracted with distilled water, salt solution (2.0% NaCl), ethanol (75%, v/v), and dilute alkali (0.02 M NaOH) to yield albumin, globulin, prolamin, and glutelin, respectively. Each fraction was extracted at a 1:10 (w/v) solid-to-solvent ratio. Each extraction was carried out at room temperature for 30 min by an ultrasonic homogenizer and centrifuged afterwards at 4 000 g for 10 min.

2.5 Determination of protein content

Total nitrogen was determined by the method of Kjeldahl (AOAC 1990). Crude protein content was calculated by N×6.25. The content of protein fractions was also determined by the method of Lowry *et al*. (1951) with bovine serum albumin as the standard.

2.6 Statistical analysis

All results are expressed as mean ± SD (n=3). One-way analysis of variance (ANOVA) and Duncan's test were performed with significant level being considered at $P < 0.05$.

3. RESULTS AND DISCUSSION

3.1 Changes in protease activity

The protease activity of the oat groats at different germination stages are shown in Figure 1. Compared with raw groats, the levels of protease activity increased with the processing of steeping and germination. The protease activity increased approximately 17% during steeping and the first 12 hours of germination (G12). Thereafter, a rapid increase ($p < 0.05$) in the protease activity was detected, and it increased by 252% and reached the maximum at stage G60 compared with raw groats, and then the protease activity began to decrease. But no difference in the protease activity was found from stages G36 to G72.

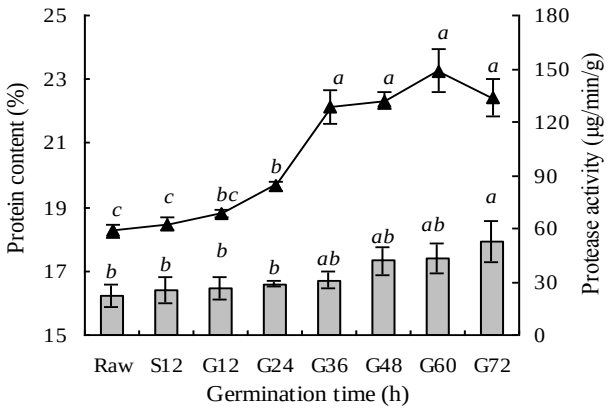

Figure 1: The levels of protease activity (line) and crude protein (bar) in oats at different stages of germination

3.2 Changes in the content of crude protein, soluble, and insoluble protein

The crude protein increased gradually during germination of oats (Figure 1). Nevertheless, the content of the crude protein did not change significantly ($p > 0.05$) from stages S12 to G60, and it increased ($p < 0.05$) by 10.35% at the end of germination compared with raw groats. The levels of soluble and insoluble protein at different germination stages are shown in Figure 2. Insoluble protein first increased by 28.41% and reached the maximum at stage G48, and then it decreased by 9.88% at the end of germination. Similar to insoluble protein, the content of soluble protein first increased significantly ($p < 0.05$) by 59.41% and reached the maximum at stage G48, and then it decreased by 30.09%. In addition, there was no distinct difference in soluble protein during steeping and the first 24 hours of germination (G12), though soluble protein decreased slightly at S12 and G24 stages as compared with raw groats, which was different from the changes of insoluble protein.

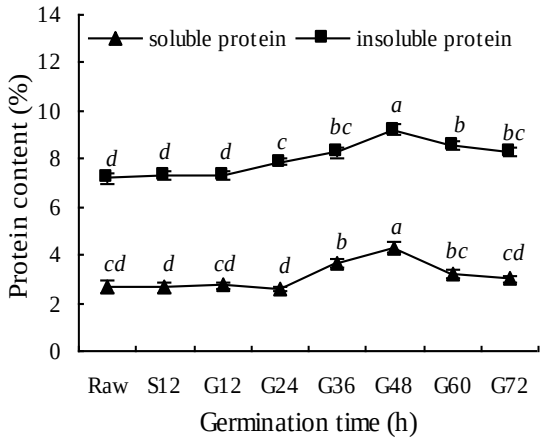

Figure 2: The content of soluble and insoluble proteins in oats at different germination stages

3.3 Changes in the content of protein fractions

In general, cereal proteins have been classified based on their solubility (Osborne fractionation) albumin, globulin, prolamin and glutelin fractions. The levels of the different protein fractions in oats during steeping and germination are shown in Table 1. Unlike wheat and some other cereals, the highest content was globulin, followed by glutelin and prolamin, the lowest albumin in oat seeds, which have been obtained by some authors (Shewry 1995; Ma & Harwalkar 1984). However, there were some differences in the content of individual protein fractions because of the differences in the extraction solvent and its concentration (Robert *et al.*,1985). The levels of protein fractions in oats were all influenced by the germination process. Compared with raw groats, albumin content decreased significantly by 19.18%, 10.75% at stage S12 and G12, respectively. Thereafter, a rapid increase ($p < 0.05$) in albumin content was detected, and it increased ($p < 0.05$) by 53.57% and reached the maximum at stage G60 compared with raw groats (Table 1), and then albumin content decreased. There was a slight decrease in globulin content during steeping, then it increased by 7.25% and reached the maximum at stage G36, and then it decreased and was lower than that of raw groats at the last germination stage. Prolamin first increased, and then it decreased by 4.84% and reached the minimum at stage G24. Subsequently, prolamin increased continuously and significantly by 18.33% and reached the maximum at the end of germination stage. Glutelin first increased by 14.63% at G24 stage and then decreased by 24.19% by the end of germination compared with raw groats, its content from stages G48 to G72 were lower than that of raw groats.

Table 1: Content (mg/g DW) of different protein fractions in oats at different germination stages

	Albumin	Globulin	Prolamin	Glutelin
Raw groat	15.53±0.54 d	59.35±0.22 d	22.75±0.88 de	35.55±2.52 ab
S12[b]	12.55±0.52 e	58.65±0.35 d	24.56±0.64 cd	36.06±2.33 ab
G12[c]	13.86±0.44 de	62.62±0.46 ab	23.88±0.50 cd	38.28±2.04 a
G24	18.22±0.83 bc	62.01±0.43 bc	21.65±0.72 e	40.75±3.45 a
G36	17.54±0.61 c	63.65±0.78 a	24.33±0.41 cd	37.22±1.88 ab
G48	23.62±0.93 a	60.88±0.63 c	24.85±0.56 bc	31.54±2.17 bc

G60	23.85±0.65 a	58.44±0.60 d	26.55±0.75 ab	27.28±1.80 c
G72	19.91±0.50 b	58.08±0.34 d	26.92±0.84 a	26.95±1.44 c

Values are represented as mean ± standard deviation of triplicates; Different small letters within the same column indicate statistically significant differences between the means of different germination stages at P < 0.05.

3.4 Correlation analysis

To further investigate the their interrelationship, the correlations among protease activity and the content of different protein fractions were established in oat groats during germination, and correlation coefficients (R) are shown in Table 2. Soluble protein consists chiefly of albumin and globulin while insoluble protein is mainly composed of prolamin and glutelin. Correlation analysis showed that albumin may be responsible for the increase of soluble protein, and while the change in insoluble protein content was determined by the degree of prolamin increasing and of glutelin decreasing. The protease activity correlated highly with the content of different protein fractions, but it was irrelevant to globulin, however, there was significantly negative correlation ($p < 0.05$) between glutelin content and the protease activity, which indicates that the protease activity had a great influence on the changes in the content of different protein fractions.

Table 2: Correlation analysis between protease activity and different protein fractions [a]

	SP [b]	IP [b]	Albumin	Globulin	Prolamin	Glutelin
IP [b]	0.8698 [c]					
Albumin	0.6824 [d]	0.4113				
Globulin	0.1931	0.0006	-0.1835			
Prolamin	0.3754	0.5058	0.4779	-0.5417		
Glutelin	-0.3847	-0.5688	-0.6679	0.7103 [d]	-0.9012 [c]	
Protease activity	0.7077 [d]	0.9023 [c]	0.8699 [c]	-0.0950	0.7062 [d]	-0.7081 [d]

[a] Correlation coefficient *R*. [b] Abbreviations of soluble and insoluble proteins (SP, soluble protein; IP, insoluble protein). [c] Significant ($p < 0.01$). [d] Significant ($p < 0.05$).

4. DISCUSSION

In the current study, the levels of protease activity increased during germination of oats, which was supported by previous researches (Klose *et al*., 2009; Mikola & Jones 2010; Hübner & Arendt 2010) that the highest increase in proteolytic activities could be detected during the first three days of germination, but not after that. The crude protein increased gradually during germination, which was in agreement with previous report (Tian *et al*., 2010). However, because the crude protein content in food is commonly determined by measuring the total nitrogen content and multiplying it with an appropriate factor, its content may not be equal to the natural protein especially in the case of germination, which was confirmed by the following work. Namely, soluble and insoluble protein first increased and then decreased during germination. Urbano *et al*. (2005) reported the protein in peas was significantly decreased with the processing of germination, while Alonso *et al*. (2000) reported the increase in protein from faba and kidney beans during germination. In the present study, the slight decrease in the level of soluble protein during germination was similar to that reported by Elmaki *et al*. (1999), which may result from its loss during steeping of seeds and also, utilization for the growth and development of the embryo (Wu *et al*., 1980), while the increase of soluble protein may result from synthesization and degradation of other proteins. Some reported studies demonstrated that insoluble protein decreased during germination of plant seeds (Urbano *et al*., 2005; Wanasundara *et al*., 1999), which was in disagreement with the present study. The explanation for this result in present study is probably that insoluble protein is being degraded while being produced. In addition, the insoluble protein can be easily obtained from oats because of looser kernel structure during the later period of germination, which may also be another cause of the increase of insoluble protein. A significant increase in albumin fraction was expected because of the fact that this fraction contains the majority of the metabolically enzymes while glutelin fraction decreased significantly in the later stage of germination, which supported the result obtained by Klose *et al*. (2009), however, there were some differences from changes in the content of prolamin and globulin fractions. In fact, each of protein fractions extracted according to the Osborne procedure was likely composed of a group of different molecular weight proteins. Consequently, the increase in level of one protein fraction does not simply mean that the degradation of the protein fraction did not take place during germination.

5. CONCLUSION

Conclusively, the current study indicates that germination can improve the protease activity, at same time which led to so many different changes in proteins oat grains during germination. However, the choice of germination time might be

of great importance and germination for 48 h under highly controlled conditions would be sufficient. Besides, because the physiological and biochemical reaction resulting from steeping and germination of cereal seeds is extremely complex process affected by several factors such as the time, temperature, and so on.

6. ACKNOWLEDGEMENT

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