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CHANGES IN NUTRITIVE VALUE AND IN VITRO DIGESTIBILITY OF PROTEINS FROM NAKED OATS DURING GERMINATION

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ABSTRACT: *Changes in the content, nutritive value, and in vitro protein digestibility of proteins were investigated during germination of naked oats. Compared with raw groats, an increase in crude protein from oat groats was continuously observed during germination. The nutritive value of proteins and in vitro protein digestibility of oats increased during germination as compared with raw oats. However, the choice of germination time might be of great importance and germination for 48h under highly controlled conditions would be sufficient to improve the nutritive value and digestibility of naked oats in the present study.*

KEYWORDS: Nutritive value, Protein, Naked oats, Germination

INTRODUCTION

Oat (*Avena sativa*) has received increased interest because of its excellent health-related properties, such as high contents of soluble dietary fiber, protein, carbohydrate, oil, and other compounds that were often enriched in the outer part of the cereal grains such as several vitamins, minerals (Petkov *et al.*, 2001), and abundant antioxidant compounds (Peterson 2001). However, oat groat protein has poor solubility at neutral and slightly acidic pH and functional properties (Peterson 2001) even though its content was higher as compared with other cereals and some processing methods could result in the decrease of antioxidant compounds in oats (Bryngelsson *et al.*, 2002), which affects oat product quality and commercial utilization for human consumption. Therefore, how to improve palatability, bioavailability, and to enhance nutritional value and health function of oats has been a hotpot in this field.

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 (Briggs *et al.*, 1981), which can result in the changes in protein content and size distribution, as well as protein properties without any chemical modifications. The germination process was also one of methods used to improve the functionality of oat seed protein (Kaukovirta-Norja *et al.*, 2004). 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). It is reported that long germination periods have a negative effect on the organoleptic properties of

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legume seeds (Nnanna *et al*., 1990; Bau *et al.*, 2000; Uwaegbute *et al.*, 2000). 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. However, information on these aspects is very limited in oats, especially naked oat cultivars (*Avena nuda* L.) from China.

With regard to the changes in phenolic compounds and antioxidant activity as well as free amino acids including γ–Aminobutyric acid in oats during germination were analyzed in our previous studies (Xu *et al*., 2009; Xu *et al*., 2010). As a follow-up to a joint study, the objective of the present study was to further investigate the effect of a highly controlled germination process on the nutritive value and in vitro protein digestibility of naked oat proteins, which that are required to produce high quality food based on naked oats.

MATERIALS AND METHODS

Oat 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.
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. Oat groats were steeped and germinated in demonized water under controlled conditions in an incubator. The oat groats (500 g) were steeped with 1000 mL 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 process. After steeping, the remaining oat groats were drained and germinated for 72 h in a controlled environment 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. All samples were milled with a micro plant grinding machine set at a fine setting of 0.5 mm. This was carried out just prior to the different analysis. Raw groats were also freeze dried and used as reference samples in all performed analysis.

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.

Determination of Protein Content: Total nitrogen was determined by the method of Kjeldahl (AOAC Official Methods of Analysis, 1990). Crude protein content was calculated by N×6.25.

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Amino Acid Analysis: Amino acid composition was determined following the method originally described with some modifications (Rizzello *et al.*, 2008). Each sample was hydrolyzed in test tubes under vacuum with triple-glass-distilled constant boiling 6.0 M HCl for 24 h at 110±0.5 °C. The HCl were removed with nitrogen and the resulting precipitates were reconstituted with 0.2 M sodium citrate loading buffer solution (pH 2.2) to a final volume of 10 mL, followed by centrifugation at 10 000 g for 15 min at 4 °C. After centrifugation, the supernatant was filtered through a 0.45 μm of nylon syringe filter (Filtrex Technology, Singapore) prior to analysis and analyzed by a Biochrom 30 series Amino Acid Analyzer with a Na–cation–exchange column (8 μm, 4.6mm × 200 mm). The injection volume was 20 μL, the duration of a single run was 50 min. Amino acids were postcolumn derivatized with ninhydrin reagent and detected by absorbance at 570 nm. The acid hydrolysates and amino acid standard solution were analyzed under the same conditions, and all of the above experiments were replicated three times. Identification of amino acids was performed by comparisons to the retention time and UV spectra of authentic standards from Sigma. To determine the tryptophan, sample was hydrolyzed with 5 M NaOH including 5% $SnCl_2$ for 20 h at 110±0.5 °C.

Amino Acid Chemical Score (CS) and Essential Amino Acid Index (EAAI): The CS is the percentage of the most deficient essential amino acid in the protein as compared to the requirement pattern and the EAAI is based on the ratios of the amounts of essential amino acids in a protein relative to their amount in whole egg protein (Sikka & Johari 1979).

Determination of in Vitro Protein Digestibility: In vitro protein digestibility was estimated by enzymatic method of Wang et al. (2008) with some modifications. Firstly, 500 mg dried protein was mixed with 20 mL of 0.5 mg/mL pepsin dissolved in 0.l M HCl and incubated for 2 h at 37 °C in a water bath, then terminated by adjusting pH to 7 with 1 M NaOH solution. The mixtures were added 10 mg trypsin, stired and incubated for an additional 2 h at 37 °C. For the nitrogen release analysis of digestion, the trichloroacetic acid (TCA)-precipitation method was used. The nitrogen content of the corresponding precipitates was measured by the micro-Kjeldahl method. The in vitro protein digestibility was defined as follows: in vitro protein digestibility (%) =100 × $(N_0-N_1)/N_0$, where N_0 represents the TCA-precipitated nitrogen content in protein samples before the digestion (mg), N_1 represents the TCA-precipitated nitrogen content after pepsin and trypsin digestion (mg).

Statistical Analysis: All experiments were conducted three times independently and the experimental data were expressed as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) and Duncan's multiple range tests were carried out to determine significant differences ($p < 0.05$) between the means by DPS (version 3.01). Correlation coefficient and regression analyzes were determined by DPS (version 3.01) and EXCEL program.

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RESULTS

Changes in the Content of Crude 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.

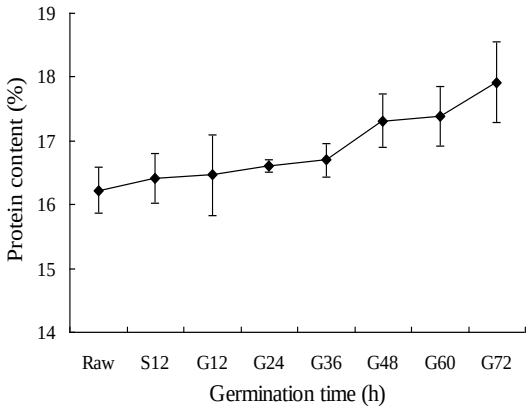

Figure 1. The levels of crude protein in oats at different stages of germination

Changes in Amino Acid Compositions of Protein: The levels of the different amino acids, total amino acids (TAA), essential amino acids (EAA) as well as nonessential amino acids (NEAA) of protein from oat groats were all influenced by the germination process and the results are shown in Table 1. The content of TAA of protein from raw groats was 479.41 mg/g protein, it decreased during steeping and the first 12 hours of germination, and then it first increased to 567.74 mg/g protein at G48 stage, and then decreased gradually to 533.91 mg/g protein by the end of germination. The most abundant amino acid was glutamic acid, followed by leucine, and the lowest was tryptophan in protein from raw groats, which was no change during germination of oats. Similar to TAA, the content of EAA decreased by 6.75% during steeping, and then it first increased by 34.94% at G48 stage and then decreased during germination. In all EAA, the extent of increasing tryptophan (52.36%) was the largest, followed by threonine (50.53%) and leucine (50.16%), the lowest for phenylalanine (21.58%) during germination. The changes of NEAA in protein from oats during germination had a similar trend to that of EAA, except for difference in the extent of the change.

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Table 1. Amino acid compositions of protein in oats at different germination stages

Amino acids	Amino acid content (mg/g protein)							
	Raw groats	S12 [a]	G12	G24	G36	G48	G60	G72
Glycine	21.86	20.45	21.56	22.92	24.32	23.10	21.66	22.12
Alanine	19.21	18.91	18.95	19.32	19.34	19.31	19.20	19.32
Valine	27.64	26.34	26.11	28.67	32.83	38.64	37.24	33.11
Isoleucine	23.93	20.95	23.99	27.04	25.76	32.90	34.22	33.31
Leucine	38.18	35.42	40.65	46.10	51.48	57.33	52.45	46.21
Aspartic acid	32.78	30.44	30.91	31.12	32.28	31.77	31.98	32.06
Glutamic acid	109.72	106.75	105.8	109.66	112.54	117.32	109.39	110.16
Arginine	28.74	27.94	28.32	28.53	29.45	29.58	29.75	29.70
Lysine	24.29	22.86	23.89	26.32	28.27	26.30	26.11	24.32
Histidine	11.79	11.80	11.90	12.78	13.62	13.35	13.32	13.75
Phenylalanine	23.73	22.34	23.94	24.85	26.26	28.85	25.92	25.57
Tyrosine	19.08	17.10	19.30	21.73	23.81	24.80	24.26	23.41
Threonine	23.75	21.14	25.36	27.96	30.13	32.83	35.75	36.04
Serine	18.33	17.25	18.18	19.24	20.80	20.10	22.20	21.48
Tryptophane	5.92	5.21	6.62	7.30	8.94	9.02	8.71	8.11
Cysteine	10.02	11.08	10.81	12.23	14.96	15.71	14.60	13.76
Methionine	12.46	13.50	12.39	14.63	15.18	16.89	15.54	13.84
Proline	27.98	27.96	28.41	29.12	29.82	29.94	27.96	27.64
EAA[a]	179.90	167.76	182.95	202.87	218.85	242.76	235.94	220.51
NEAA	299.51	289.68	294.14	306.65	320.94	324.98	314.22	313.40
TAA	479.41	457.44	477.09	509.52	539.79	567.74	550.16	533.91

[a] *Abbreviations of amino acids (EAA, essential amino acids; NEAA, nonessential amino acids; TAA, total amino acids).*

Amino Acid Chemical Score and EAAI: According to the results of amino acid composition of protein (Table 1) and FAO/WHO provisional pattern of protein (FAO/WHO, 1973), amino acid score (AAS) indicated that the most deficient amino acid was found to be lysine for raw oats (no shown for AAS), which was well supported by the results of earlier workers (Robbins *et al.*, 1971; Zarkadas 1982). Although the content and AAS of lysine are being increased except at steeping and G12 stages, it was the most deficient amino acid during the whole germination. The changes of the ratio of essential amino acids to nonessential amino acids (E/N), essential amino acids to protein (E/P) and total amino acids (E/T), the "chemical score percent egg", "chemical score

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percent FAO/WHO" as well as EAAI of oats during germination are shown in Table 2. It can be seen from the figure that these parameters decreased during steeping; they first increased and then decreased during germination. However, there were differences in the extent of change and/or time reaching the peak for different parameters. For example, the ratio of E/N, E/P, and E/T was the highest between stages G48 and G60, while the CS and EAAI of oats reached the maximum at stages G36 and G48, respectively.

Table 2. E/N, E/P, E/T ratios, chemical score (%), EAAI (%), and in vitro protein digestibility (%) of oat groats at different germination stages [a]

Samples	E/N	E/P	E/T	CS (egg)	CS(FAO/WHO)	EAAI	In vitro digestibility
Raw groat	0.60	0.18	0.38	36.25	44.98	35.21	58.39
S12	0.58	0.17	0.37	34.12	42.33	32.83	58.72
G12	0.62	0.18	0.38	35.66	44.24	35.80	60.25
G24	0.66	0.20	0.40	39.28	48.74	39.70	64.38
G36	0.68	0.22	0.41	42.19	52.35	42.83	65.01
G48	0.75	0.24	0.43	39.25	48.70	47.51	69.58
G60	0.75	0.24	0.43	38.97	48.35	46.17	70.21
G72	0.70	0.22	0.41	36.30	45.04	43.15	70.04

[a] *EAAI (essential amino acid index) is based on the ratios of the amounts of essential amino acids in a protein relative to their amount in whole egg protein. Chemical score is the percentage of the most deficient essential amino acid in the protein as compared to the requirement pattern. E/N, ratio of essential amino acids to nonessential amino acids. E/T, ratio of essential amino acids to total amino acids. E/P, ratio of essential amino acids to protein.*

Changes in Vitro Protein Digestibility: The results of in vitro digestibility assay are also shown in Table 2. The results showed that the in vitro protein digestibility of oats increased with the prolonging of germination time. Compared with raw groats, in vitro protein digestibility was no obvious change basically and it only increased by 0.6% during steeping, and it was increasingly during germination and increased by 19.16%, while it seems to have reached a plateau at G48 stage. Thereafter, in vitro protein digestibility did not change much, which indicates that the influence of germination on in vitro protein digestibility of oats is also limited.

DISCUSSION

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. 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. Also, the amino acid composition, nutritive value as well as in vitro digestibility of protein in oats were strongly influenced by germination. The level of essential amino acid especially the deficient amino acid lysine was increased and nutritive value as well as in vitro digestibility of protein was improved during germination of oats, which was supported by

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some previous studies (Alonso *et al*., 2000; Wu *et al*., 1980; Rodríguez *et al*., 2007; Ghavidel & Prakash 2007). However, a relatively long time of germination might lead to the decrease of nutritive value and in vitro protein digestibility of oats, which was similar to that reported by Urbano et al. (2005) in peas. These differences between some previous reports and our results on the changes in content and nutritive value of protein as well as protease activity from plant seeds during germination might attributed to plant species and varieties, and experimental conditions such as germination time and temperature including analytical methods (Koehler *et al*., 2007; Hübner *et al*., 2010; Elmaki *et al*., 1999; Urbano *et al*., 2005). As showed by the results, we conclude that germination for 48 h would be sufficient to significantly improve the nutritive utilisation of protein from naked oats.

CONCLUSIONS

Conclusively, the current study indicates that germination can led to so many different changes in the content, nutritive value, and in vitro protein digestibility of proteins from 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 to improve the nutritive value and digestibility of naked oats. 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, further studies are needed to perform to optimize the germination process improving nutritive value and more assays need to be further performed with more cultivars (oats and others).

REFERENCES

Alonso, R., Aguirre, A. and Marzo, F. (2000). Effects of extrusion and traditional processing methods on antinutrients and in vitro digestibility of protein and starch in faba and kidney beans. Food Chemistry, 68: 159–165.

AOAC Official Methods of Analysis. (1990). Association of Official Analytical Chemists, (15 Ed.). Washington, DC.

Bau, H.M., Villaume, C.H. and Méjean L. (2000). Effects of soybean (*Glycine max*) germination on biologically active components, nutritional values of seeds, and biological characteristics in rats. Nahrung/Food, 44: 2–6.

Briggs, D.E., Hough, J.S., Stevens, R. and Young, T.W. (1981). Malting and brewing science (2 Ed.). Chapman & Hall: London, U. K.

Bryngelsson, S., Dimberg, L.H. and Kamal-Eldin, A. (2002). Effects of commercial processing on levels of antioxidants in oats (*Avena sativa* L.). Journal of Agricultural and Food Chemistry, 50: 1890–1896.

Elmaki, H.B., Babiker, E.E. and El Tinay, A.H. (1999). Changes in chemical composition, grain malting, starch and tannin contents and protein digestibility during germination of sorghum cultivars. Food Chemistry, 64: 331–336.

FAO/WHO. 1973. "Energy and Protein Requirements", Report of a Joint FAO/WHO Nutrition Report, Series No. 52, FAO, Rome.

ISSN 2056-5798(Print), ISSN 2056-5801(online)

Ghavidel, R.A. and Prakash, J. (2007). The impact of germination and dehulling on nutrients, antinutrients, in vitro iron and calcium bioavailability and in vitro starch and protein digestibility of some legume seeds. LWT-Food Science and Technology, 40: 1292–1299.

Hübner, F. and Arendt, E.K. (2010). Studies on the influence of germination conditions on protein breakdown in buckwheat and oats. Journal of Institute of Brewing, 116: 3–13.

Kaukovirta-Norja, A., Wilhemson, A. and Poutanen, K. (2004). Germination: a means to improve the functionality of oat. Agricultural and Food Sciences, 13: 100–112.

Klose, C., Schehl, B.D., and Arendt, E.K. (2009). Fundamental study on protein changes taking place during malting of oats. Journal of Cereal Sciences, 49: 83–91.

Koehler, P., Hartmann, G., Wieser, H., Rychlik, M., 2007. Changes of folates, dietary fiber, and proteins in wheat as affected by germination. Journal of Agricultural and Food Chemistry 55, 4678–4683.

Nnanna, I.A., Dixon-Phillips, R., McWatters, K.H. and Hung, Y.C. (1990). Effect of germination on the physical, chemical, and sensory characteristics of cowpea products: flour, paste, and akara. Journal of Agricultural and Food Chemistry, 38: 812–816.

Peterson, D.M. (2001). Oat antioxidants. Journal of Cereal Sciences, 33: 115–129.

Petkov, K., Biel, W., Kowieska, A. and Jaskowska, I. (2001). The composition and nutritive value of naked oat grain (*Avena sativa var. nuda*). Journal of Animal and Feed Science, 10: 303–307.

Rizzello, C.G., Cassone, A., Di Cagno, R. and Gobbetti, M. (2008). Synthesis of angiotensin I-converting enzyme (ACE)–inhibitory peptides and γ-aminobutyric acid (GABA) during sourdough fermentation by selected lactic acid bacteria. Journal of Agricultural and Food Chemistry, 56: 6936–6943.

Robbins, G.S., Pomeranz, Y. and Briggle, L.W. (1971). Amino acid composition of oat groats. Journal of Agricultural and Food Chemistry, 19: 536–539.

Rodríguez, C., Frías, J., Vidal-Valverde, C. and Hernández, A. (2007). Total chemically available (free and intrachain) lysine and furosine in pea, bean, and lentil sprouts. Journal of Agricultural and Food Chemistry, 55: 10275–10280.

Rozan, P., Kuo, Y. and Lambein, F. (2000). Free amino acids present in commercially available seedlings sold for human consumption. A potential hazard for consumers. Journal of Agricultural and Food Chemistry, 48: 716–723.

Shi, H., Nam, P.K. and Ma, Y. (2010). Comprehensive profiling of isoflavones, phytosterols, tocopherols, minerals, crude protein, lipid, and sugar during soybean (*Glycine max*) germination. Journal of Agricultural and Food Chemistry, 58: 4970–4976.

Sikka, K.C. and Johari, R.P. (1979). Comparative nutritive value and amino acid content of different varieties of sorghum and effect of lysine fortification. Journal of Agricultural and Food Chemistry, 27: 962–965.

Tian, B., Xie, B., Shi, J., Wu, J., Cai, Y. and Xu, T. (2010). Physicochemical changes of oat seeds during germination. Food Chemistry, 119: 1195–1200.

Urbano, G., Aranda, P., Vílchez, A., Aranda, C., Cabrera, L. and Porres J.M. (2005). Effects of germination on the composition and nutritive value of proteins in *Pisum sativum* L. Food Chemistry, 93: 671–679.

Urbano, G., López-Jurado, M., Frejnagel, S., Gómez-Villalvaa, E., Porres, J.M. and Frías, J. (2005). Nutritional assessment of raw and germinated pea (*Pisum sativum* L.) protein and carbohydrate by in vitro and in vivo techniques. Nutrition, 21: 230–239.

ISSN 2056-5798(Print), ISSN 2056-5801(online)

Uwaegbute, A.C., Iroegbu, C.U. and Eke, O. (2000). Chemical and sensory evaluation of germinated cowpeas (*Vigna unguiculayta*) and their products. Food Chemistry, 68: 141–146.

Wang, X-S., Tang, C-H., Yang, X-Q. and Gao, W-R. (2008). Characterization, amino acid composition and in vitro digestibility of hemp (*Cannabis sativa* L.) proteins. Food Chemistry, 107: 11–18.

Wilhelmson, A., Oksman-Caldentey, K-M., Laitila, A., Suortti, T., Kaukovirta-Norja, A. and Poutanen, K. (2001). Development of a germination process for producing high β-glucan, whole grain food ingredients from oat. Cereal Chemistry, 78: 715–720.

Wu, Y.V. 1983. Effect of germination on oats and oat protein. Cereal Chemistry, 60: 418–420.

Wu, Y.V. and Wall, J.S. (1980). Lysine content of protein increased by germination of normal and high-lysine sorghum. Journal of Agricultural and Food Chemistry, 28: 455–458.

Xu, J.G., Tian, C.R., Hu, Q.P., Luo, J.Y., Wang, X.D. and Tian, X.D. (2009). Dynamic changes in phenolic compounds and antioxidant activity in oats (*Avena nuda* L.) during steeping and germination. Journal of Agricultural and Food Chemistry, 57: 10392–10398.

Xu, J.G., Hu, Q.P., Duan, J.L. and Tian, C.R. (2010). Dynamic changes in γ-aminobutyric acid and glutamate decarboxylase activity in oats (*Avenel nuda* L.) during steeping and germination. Journal of Agricultural and Food Chemistry, 58: 9759–9763.

Zarkadas, C.G. (1982). A comparison of the amino acid composition of two commercial oats groats. Cereal Chemistry, 59: 323–327

ISSN 2056-5798(Print), ISSN 2056-5801(online)