International Food Research Journal 23(5): 1980-1987 (2016)
Journal homepage: *http://www.ifrj.upm.edu.my*

Bioactive compounds, antioxidant and enzyme activities in germination of oats (*Avena sativa* L.)

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Article history

Received: 15 October 2015
Received in revised form: 28 December 2015
Accepted: 13 January 2016

Keywords

Bioactive compounds
Antioxidant activity
Germination
Oats
Phenolics

Abstract

By this study, nutritional compositions, phenolic and γ-aminobutyric acid (GABA) contents, and enzyme activities after germination of oats (*Avena sativa* L.) were evaluated. Protein content significantly increased whilst ash, fat contents and total starch negligibly changed. The total phenolics increased by 0.5 mg ferulic acid equivalents g^{-1} dry weight in the methanolic extract. In the water extract it was promoted 0.03 mg ferulic acid equivalents g^{-1}. The GABA content increased > 30% after 5 days of germination. Catechin, eligallocatechin and luteonin were found in only ungerminated seeds, whereas gallic acid, epicatechin, epigallocatechin gallate, ferulic acid were detected only in germinated seeds. Ellagic acid was observed in both ungerminated and germinated oats. The increase of > 30% inhibitory activity of α-amylase and reduction of > 25% of α-glucosidase inhibition proposed that germinated oats is a promising source for safe dietary supplements, despite further elaboration is needed.

Introduction

Oats (*Avena sativa* L.) is a cereal that is commonly consumed as whole grains and known to provide essential nutrients to human. Many studies have shown the important physiological effects of oats (Skoglund, 2008). Avenanthramides, a unique group of N-cinnamoylanthrannilic acid derivatives presenting in oats but not in other cereals are known to reduce oxidation of low density lipoprotein cholesterol in animals and humans (Liu *et al*., 2011).

Germination is a process involved in incorporating those events that commence with the uptake of water by a quiescent dry seed and terminate with the elongation of the embryonic axis (Bewley, 1997). At the time of germinating, huge amounts of nutrients are prepared for the growth of sprout. The birth of the sprout activates all the dormant enzymes in the grains in order to supply the sprout with the best nutrition. As a result, the available nutrients in the grains greatly increase. Increased nutrients in germinated grains may include phenolics, dietary fiber, inositols, ferulic acid, phytic acid, tocotrienols, GABA, magnesium, potassium, zinc, γ-oryzanol, and prolylendopeptidase inhibitors. In oats, several studies were conducted on investigating the chemical components of oats after germination. Whilhelmson *et al*. (2001) reported that the content of β-glucan strongly reduced after germination. The amount of avenanthramides in oats also increased in germinated seeds (Bryngelsson *et al*., 2003). Skoglund (2008) examined the content of avenanthramide and activities of the avenantheramide-synthesizing enzyme hydrocycinnamoyl-CoA: hydroxyanthranilate N-hydroxycinnamoyl transferase (HHT) and the evenanthramide-oxidizing enzyme phenoloxidase (PO) in three different oat cultivars.

Phenolic compounds in oats contain quinones of benzoic acid, cinnamic acid, flavones, flavonols, flavanones, anthocyanidins, aminophenols and precursors of these compounds (Collins, 1986). Oksman-Caldentey *et al*. (2001) reported that after germination, most of the phenolics were bound (58%), in ester form (25%) or in glucosidic form (15%), whereas only 2.03% of phenolic acids were free. The major free acids were caffeic, syringic, ferulic, and sinapic acids. Phenols in oats significantly influenced oat flavor and quality.

The softer kernel structure of germinated oat opens new possibilities to use whole kernels in human and animal nutrition (Kaukovirta-Norja *et al*., 2004). However, only a minor part of the great potential of germinated/malted oat has been currently utilized, requiring the further elaboration of chemical composition of ungerminated and germinated oat. The research was conducted to evaluate changes in

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nutritional compositions, phenolics, antioxidants, enzyme activities including inhibition of α-amylase and α-glucosidase, and GABA in ungerminated and germinated oat.

Materials and Methods

Oat materials

Commercial oat grains (*Avena sativa* L.) were provided by the Sanitarium Ltd. Co., Australia in 2011.

Germinated oat preparation

An amount of 50 g of oats were emerged in 0.1% NaOCl for 30 min for sterilization, and then rinsed many times by water. The treated oats were steeped in distilled water, at room temperature (28 to 30°C) for 24 h. The steeped oat kernels were distributed on double layers of cotton cloth and placed in a plastic tray, which was then covered by other double layers of cotton cloth. During germination, the relative humidity was maintained above 90% by spraying distilled water each 4 h for 5 days. The germinated seeds were freeze-dried to approximately 10% of moisture content and stored at -20°C for further analysis.

Determination of nutritional compositions

The proximate analysis (fats, protein, moisture, starch and ash) of oat samples including ungerminated and germinated seeds was determined by using methods described in AOAC (1995). The nitrogen value, which is the precursor for protein of a substance, was determined by micro–Kjeldahl method (Guebel *et al.*, 1991). The moisture and ash contents were determined using the weight difference method while determination of crude lipid content was conducted using Soxhlet type of the direct solvent extraction method. The solvent used was petroleum ether (boiling range from 40 to 60°C). Total starch was measured by the Megazyme assay based on two methods including the AOAC method 996.11 and the AACC method 76.13 (AOAC, 1995). The values were expressed in percentage in dry weight basis.

Extraction progress

An amount of 2 g of sample was ground using a mortar and pestle for 15 min. The powder was added with 5 ml of 100% methanol and stirred vigorously using vortex mixer for 15 min, and then centrifuged at 4000 rpm for 10 min. The supernatant was transferred to a new tube, and then added 20 ml of methanol to the pellet and continued to extract twice. The final volume of the phenolics in methanol was approximately 15 ml, filtered through a 0.2 µm filter and adjusted the volume to 20 ml with methanol. After methanol extraction, the residue was dried at 30°C for 30 min, and then added 5 ml of water to extract other hydrophilic phenolics. The extracts were kept at -20°C for further analysis.

Determination of total phenolics

The total phenolic content of samples was determined following the Folin-Cicalteau assay described by Xu *et al*. (2009) with some modifications. An amount of 0.5 ml of the extract and an equal amount of Folin-Ciocalteu reagent were added to a falcon tube, and then swirled for 3 min, followed by added 10 ml of 7.5% sodium carbonate solution and 5 ml distilled water and shaken the tubes thoroughly. Then they were incubated for 1 h at room temperature, and then centrifuged at 4000 rpm for 5 min. The solution was examined the absorbance of 750 nm and calculated total phenolic content relevant to the ferulic acid equivalents (FAE) per gram dry weight (DW), of which the standard curve was established from different concentrations of 0 µg ml^{-1} to 100 µg ml^{-1}.

Determination of γ-aminobutyric acid (GABA)

An amount of 0.5 g of oat powder was added to a 15 ml falcon tube, and then poured 1 ml of ethanol 70% and vortexed for 1 min. The extraction process was centrifuged at 4000 rpm for 30 min. The supernatant was collected and the pellet was extracted again with ethanol 70% (v/v). Subsequently, an aliquot of 1 ml of the supernatant was transferred to a new tube, and 0.6 ml Borax buffer (pH 8.0) and 2 ml 2-Hydroxynaphthaldehyde (0.3% w/v in methanol) were added. The tube was placed at 80°C using water bath for 10 min. After cooling, the content was filtered with 0.2 µm filters to HPLC vials for HPLC analysis. GABA content was determined using a HPLC System (Agilent 1200, Japan). An aliquot of 5 µl of the extract was injected on a RP C18 column with a gradient system including solvent A (0.1% formic acid) and solvent B (100% acetonitrile) are as follows: 0 to 5 min (35 to 40%), 5 to 10 min (40 to 50%), and 10 to 20 min (55 to 35%) with a flow rate of 1 ml min^{-1}. The UV detection by PI array was at 330 nm (Varanyanond *et al*., 2005). GABA standard was purchased and the standard curve from 0-100 µg ml^{-1} was applied for quantification of GABA.

Determination of phenolic components

Phenolic compounds of ungerminated and germinated oats were separated in RP C18 HPLC column using Agilent 1200 HPLC system (Japan)

including GG1361 Pump, G2258A Autosampler and G1315C Detector. The rate 1 ml min^{-1} was set for the pump and 5 µl was injected. The gradients of two mobile phase solvents consisting of acetonitrile (A) and 0.1% acetic acid (B) were mixed as 0 to 30 min (0 to 50% of B), 30 to 50 min (50 to 85% of B), and 50 to 60 min (85 to 100% of B) (Bekkara *et al.*, 1998). The phenolic standards included gallic acid, catechin, epigallocatechin gallate, epicatechin, ferullic acid, epigallocatechin, ellagic acid, and luteolin. For quantification, a curve was established at concentrations from 0 µg ml^{-1} to 100 µg ml^{-1}.

Antioxidant activity - DPPH assay

DPPH assay was employed for evaluating antioxidant activity of oat samples with some modifications from the method described by Tai *et al.* (2010). A mixture of each 100 µl of the extracts and 3.9 ml 0.075 mM DPPH solution was prepared in a 10 ml falcon tube. The reaction was left in the dark for 30 min and measured at 517 nm. Ascorbic acid of the concentrations of 0 µg ml^{-1} to 100 µg ml^{-1} was used as a positive control. The antioxidant activity was calculated by the following formula:

$$\% \text{ radical scavenging} = [(Abs_{control} - Abs_{sample}) / Abs_{control}]x100$$

where: Abs is Absorbance; control: 100 µL of methanol was used instead of the sample.
Results of radical scavenging percentage were converted to IC_{50} concentration (mg ml^{-1}) where 50% inhibition of the DPPH radical is obtained.

α-amylase inhibition

The measurement of α-amylase inhibition was followed the method previously described by Silva *et al.* (2004). The extract (100 µl) and the enzyme α-amylase solution (100 µl) were added to 15 ml falcon tubes. The tubes were incubated at 37°C for 5 min, and then were added with 250 µl of 1% starch solution. The tubes were continuously incubated for 5 min at 37°C. Afterward, dinitrosalicylic acid (200 µl) was immediately added and then they were placed in a boiling water bath for around 15 min to stop the reaction. After cooling, the samples were diluted with 2 ml distilled water and measured at 540 nm. The α-amylase inhibition was calculated by the following formula:

$$\% \text{ α-amylase inhibition} = [(Abs_{control} - Abs_{sample}) / Abs_{control}]x100$$

where Abs is absorbance; control: 100 µl the water was used instead of extracts

α-glucosidase inhibition

The activity to inhibit α-glucosidase was followed an assay described by Yao *et al.* (2010) with some modifications. An aliquot of 50 µl of the extracts was pipetted in 2 ml tubes containing 100 µl α-glucosidase solution (1U ml^{-1}). After 10 min of incubation at 37°C, 50 µl of 4-nitrophenyl-α-D-glucopyranoside 5 mM was added to the tubes and they were continuously incubated for 30 min. Subsequently, an aliquot of 1 ml Na_2CO_3 was added to stop the reaction. The samples were measured the absorbance at 400 nm. The alpha-glucosidase inhibition was calculated by the following formula:

$$\% \text{ α-glucosidase inhibition} = [1-(Abs_{sample} - Abs_{blank}) / Abs_{control}]x100$$

where: Abs is absorbance; control was the mixture without extract, and blank was the mixture without α-glucosidase.

Statistical analysis

All experiments were carried out in triplicate of each sample. Difference of means between un-germinated and germinated oats was assessed by paired-test with a confidential level of 95% using SPSS 16.0 software. The means were expressed in mean ± standard deviation (SD).

Results

Content of proximate compositions

The chemical components including moisture, crude ash, crude protein, fat content and total starch of ungerminated and germinated oats are shown in Table 1. It is observed that after germination, the values of the examined categories increased, however only percentage of moisture and protein significantly promoted. The values of ash, fat and starch were greater as compared with those of the ungerminated oat but they were not marked difference.

Total phenolic and GABA contents

Phenolic compounds are consisting of free and bound phenolics (Suominen *et al.*, 2003). Total phenolics (mg of ferulic acid equivalent g^{-1} of sample) in oats and their changes after germination are showed in Table 2. In the methanolic extract, total phenolics strongly increased after germination (from 0.33 to 0.81 mg FAE g^{-1} DW), whereas that of the water extract slightly increased (from 0.60 to 0.63 mg FAE g^{-1} DW), and it was not significantly different as

Table 1. Comparison of chemical components of ungerminated and germinated oats

	Moisture (%)	Ash (%)	Protein (%)	Fat (%)	Starch (%)
Ungerminated	8.33±0.22^{b}	1.64±0.03^{ns}	10.76±0.14^{b}	6.92±0.33^{ns}	41.29±2.67^{ns}
Germinated	11.74±0.16^{a}	7.16±0.88^{ns}	14.96±0.31^{a}	9.36±1.39^{ns}	46.74±3.60^{ns}

Means ±SD within columns followed by the different letters are significant difference at p<0.05; ns: not significant difference

Table 2. Changes of phenolic contents (mg FAE g^{-1} DW) and GABA contents (µg g^{-1} DW) of ungerminated and germinated oats

Oat	Total phenolics (mg FAE g^{-1} DW)		GABA content (µg g^{-1} DW)
	Methanolic extract	Water extract	
Ungerminated	0.33±0.009^{b}	0.60±0.009^{ns}	0.08±0.005^{b}
Germinated	0.81±0.009^{a}	0.63±0.009^{ns}	0.11±0.002^{a}

Means ±SD (standard deviation) within columns followed by the different letter are significant difference at p<0.05; ns: not significant difference

Table 3. Antioxidant activity of ungerminated and germinated oat

	Antioxidant activity (%)		IC_{50} (mg ml^{-1})	
	Methanolic extract	Water extract	Methanolic extract	Water extract
Ungerminated	4.88±0.86^{ns}	5.21±0.52^{ns}	0.34±0.05^{b}	0.57±0.07^{ns}
Germinated	0.37±0.04^{ns}	7.75±1.32^{ns}	11.12±1.11^{a}	0.43±1.11^{ns}

Means ±SD (standard deviation) within columns followed by the different letter are significant difference at p<0.05; ns: not significant difference

compared with that of ungerminated oat. The GABA content significantly increased after germination, from 0.076 µg g^{-1} DW to 0.109 µg g^{-1} DW. On the other hand, the increase GABA after germination was >30% (Table 2).

Antioxidant activity

Table 3 shows that the antioxidant activity varied between the methanolic and water extracts. Of them, the water extract exhibited stronger antioxidant activity (5.21%) as compared with the value of 4.88% of the methanolic extract. After germination, the antioxidant activity of the water extract increased to 7.75% whereas that of the methanolic extract strongly reduced (0.37%) and both of them were not significantly different as compared with that of the ungerminated oat. As for the IC_{50} value, the methanolic extract was the strongest (0.34 mg ml^{-1}), whereas that of the methanolic extract after germination was the lowest (11.12 mg ml^{-1}) (Table 3).

Enzymatic inhibitory activities

The enzyme activities in ungerminated and germinated oat are shown in Table 4. The alpha-amylase inhibition strongly increased in the methanolic extract (from 37.06 to 53.43 %) and it negligibly promoted in the water extract (from 54.03 to 56.85 %). In contrary, the alpha-glucosidase inhibitory activity is slightly reduced in germinated seeds (2.06%) as compared to that of ungerminated oat seeds (2.77%). It is observed that only the inhibitory activities of the alpha-amylase in the methanol extract was significantly different after germination (Table 4).

Changes of phenolic components after germination

The existence and quantification of eight common phenolics including gallic acid, catechin, epigallocatechin gallate, epicatechin, ferulic acid, epigallocatechin, ellagic acid, and luteolin were determined. There were 3 compounds detected in ungerminated seeds but no trace of them was found in the germinated oats, including eligallocatechin, catechin, and luteonin. Of which, eligallocatechin and luteonin were found in both methanolic and water extract, whereas catechin was detected only in the water extract of ungerminated oats (Table 5).

There were 4 phenolics were identified only after germinated consisting of gallic acid, epicatechin, apigallocatechin gallate, and ferulic acid. Only ellagic acid was detected in both ungerminated and germinated oats from the methanolic layer but its quantity reduced from 20.9 to 12.1 µg g^{-1} DW after germination (Table 5). In general, total amount of these individual phenolic acids reduced when the oat seeds were germinated.

Discussion

Germination requires an amount of water to

Table 4. Enzyme activities of ungerminated and germinated oats

	α-amylase inhibition (%)		α-glucosidase inhibition (%)	
	Methanolic extract	Water extract	Methanolic extract	Water extract
Ungerminated	37.06±0.27^{b}	54.03±5.30^{ns}	-	2.77±0.32^{ns}
Germinated	53.43±1.14^{a}	56.85±0.54^{ns}	-	2.06±0.04^{ns}

Means ±SD within columns followed by the different letter are significant difference at $p<0.05$
ns: not significant difference
-: trials are not conducted.

Table 5. Components and concentrations (μg g^{-1} DW) of phenolics in samples

Phenolic Standards	Methanolic extract		Water extract	
	Ungerminated	Germinated	Ungerminated	Germinated
Gallic acid	-	-	-	4.4
Eligallocatechin	26.2	-	20.6	-
Catechin	-	-	7.9	-
Ellagic acid	20.9	12.1	-	-
Epigallocatechin gallate	-	-	-	3.3
Epicatechin	-	24.6	-	-
Ferullic acid	-	5.1	-	-
Luteonin	10.3	-	5.7	-
Total	57.5	41.8	34.2	7.7

Values with - are not detected

activate the enzymes, which cause dramatically changes in the grain components to generate the essential elements for plant development (Skoglund, 2008). The absorption of water in oat seeds after germination may explain for the increase of moisture (8.33% to 11.74%). During germination process, the activities of enzymes produced some non-protein nitrogen substances (Moongngarm and Saetung, 2010). Therefore the percentage of protein increased from 10.76 to 14.96% (Table 1). As the chemical compositions are converted by many enzyme processes during germination, the percentages of ash, fat and starch are therefore increased despite their values are not significantly different as compared with the ungerminated seeds.

A previous research conducted by Kovačova and Malinova (2007) to determine the phenolic contents in 21 oats cultivars and reported that the quantities were between 1.11 to 5.53 mg FAE g^{-1} DW. The total phenolics of the studied oats was 0.93 mg FAE g^{-1} DW and the content increased about 0.50 mg FAE g^{-1} DW after germination (Table 2). Commonly, the phenolics in the methanolic extract is the bounded phenolics whereas that associated with the water extract is the free phenolics (Kovačova and Malinova, 2007), which indicates that only the bounded phenolics were strongly promoted after germination whereas that of free phenolics were negligibly increased.

By this study, it was observed that the amount of γ-Aminobutyric acid (GABA) increased >30% after germination. GABA is a nonprotein amino acid that is widely found in many plants, animals, and microorganism (Li *et al*., 2010). It was report that GABA is a major inhibitory neurotransmitter in the central nervous system in animal (Robert, 1974) and gives beneficial effects for human and other animals' health by decreasing blood pressure (Yoshimura *et al*., 2010), preventing chronic alcohol-related diseases (Oh *et al*., 2003), and inhibiting cancer proliferation (Oh and Oh, 2004). Therefore, accumulation of GABA in the plants and some cereals including brown rice (Komatsuzaki *et al.*, 2007), tea (Jeng *et al.*, 2007), wheat bran (Nogata and Nagamine, 2009) and soybean (Bouche and Fromm, 2004) has been studied. Stress from steeping and germination was also reported to be effective for stimulating the production of GABA in brown rice (Shu *et al*., 2008), foxtail millet (Bai *et al.*, 2009), barley (Chung *et al*., 2009), and buckwheat (Lin *et al.*, 2008). Xu *et al.* (2010) studied the contents of GABA and glutamic acid in two different naked oats (*Avena nud*a L.) and reported that in controlled conditions, the amount of GABA increased by steeping and germination. In contrast, the quantity of glutamic acid decreased. There are several studies to examine the content of GABA and reported that it was significantly increased after each of 12 h after germination (Banchuen *et al*., 2009; Jiraporn *et al.*, 2010). The contents of GABA in oats in this study were 0.076 and 0.109 μg g^{-1} in ungerminated and germinated seeds, respectively.

Youn *et al*. (2011) noted that the amount of GABA in soft white winter and dark northern spring wheats were 0.008 mg g^{-1} and 0.012 mg g^{-1}, respectively. Therefore, the amount of GABA in oats was in much higher quantity than that of wheat. In fact, GABA is generated by metabolism of glutamic acid thanks to the activity of enzyme glutamate decarboxylase and it is proposed to have a strong relationship with the ratio of germination (Komatsuzaki *et al*., 2007).

Despite the fact that the antioxidant activity was increased in a stronger magnitude of the water extract as compared to that of the methanolic extract, total phenolics in the methanolic extract was higher than that of water extract (Tables 2,3). It is suggested that the antioxidant activity determined in this study is proportional to the free phenolics in the water extract and further antioxidant activities such as scavenging activities of nitric oxide and peroxynitrite, Trolox quivalent antioxidant capacity (TEAC)/ABTS radical cation decolorization assay, total radical-trapping antioxidant parameter (TRAP), ferric reducing-antioxidant power (FRAP), superoxide radical scavenging activity (SOD), hydroxyl radical scavenging activity, hydroxyl radical averting capacity (HORAC), oxygen radical absorbance capacity (ORAC), reducing power (RP), phosphomolybdenum method, ferric thiocyanate (FTC) method, thiobartiburic acid (TBA) method, DMPD (N,N-demethyl-p-phenylene diamine dihydrochloride) method, β-carotene linoleic acid, xanthine oxidase method, cupric iron reducing antioxidant capacity (CUPRAC) method, as described in Alam *et al*. (2013).

α-amylase is one of the main enzymes in human that is responsible for the breakdown of starch to more simple sugars. Thus the inhibitors of this enzyme can delay the carbohydrate digestion and reduce the rate of glucose absorption (Nickavar *et al*., 2008). α-glucosidase plays a crucial role in hydrolyzing non-reducing sugar to glucose (Stanley *et al*., 2011). However, the activity of this enzyme becomes the dangerous enemy of diabetic patients. The sugars are quickly digested and transferred to blood system, so it increases the glucose level. Grains of cereals are considered the potential sources of α-glucosidase inhibitors (Kim *et al*., 2011). Such inhibitors have ability to block the alpha-glucosidase activity, and then reduce the level of glucose released, thus delay carbohydrate digestion and glucose absorption. Although the α-amylase inhibition of the germinated seeds was not significant different from that of ungerminated seeds, their activity in the methanolic extract increased >30%. In addition, the α-glucosidase inhibition also reduced by 25.6% after germination.

Conclusions

The study highlighted a considerable change of phytochemicals including protein, phenolics, and GABA and biological activities in term of antioxidant activity and enzymatic activity after germination of oats. Findings of the study reveal that the germinated oats appear to be a promising source for safe dietary supplement, but it apparently needs further elaboration.

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