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ORIGINAL ARTICLE

Evaluation of γ- aminobutyric acid, phytate and antioxidant activity of tempeh-like fermented oats (*Avena sativa* L.) prepared with different filamentous fungi

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Abstract Tempeh is a popular traditional fermented food in Asia. Many tempeh-like foods are made from cereal grains. However, the information of γ-aminobutyric acid (GABA) accumulation in those tempeh-like cereal grains during fermentation is lacking. Meanwhile, little information is available on the anti-nutrient contents and antioxidant activity of tempeh-like fermented oats. The aim of the present work was to study the changes of GABA, phytate, natural antioxidants and antioxidant activity of tempeh-like fermented oats. As fermentation time progressed, the GABA, total phenolics content (TPC) and flavonoids increased rapidly. The *Aspergillus oryzae*-fermented oats had the highest GABA, whereas *Rhizopus oryzae*-fermented oats had the highest TPC. Phytate, an anti-nutrient component, was dramatically reduced in the fermented oats, especially those by *A. oryzae* (reduced by about 63 %). The antioxidant activities of fermented oats were also significantly enhanced after 72 h fermentation ($p<0.05$). This study demonstrated that oats fermented by generally recognized as safe (GRAS) fungi can be recommended as tempeh-like functional foods with higher GABA, more natural antioxidants and lower phytate compared with native oats.

Keywords GABA · Phytate · TPC · Flavonoids · Antioxidant activity · Filamentous fungi

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Introduction

Recently, interest and demand has been increasing in the use of dietary supplements to improve health, which has promoted the research and development of functional foods. γ-Aminobutyric acid (GABA), a major inhibitory neurotransmitter in the brain and in the spinal cord of mammals, is a four-carbon non-protein amino acid widely distributed in foods, such as vegetables and fruits (Manyam et al. 1981). This amino acid functions in the regulation of blood pressure, heart rate and alleviation of pain and anxiety (Mody et al. 1994). Given these physiologic functions, the consumer demand for GABA-enriched functional foods is increasing. Many methods have been used to produce GABA-enriched foods, including the anaerobic or cyclic treatments of tea leaves or shoots to obtain GABA-enriched green tea (Ohmori et al. 1987) and the soaking of rice germ in water to enrich its GABA content (Saikusa et al. 1994).

The nutritional characteristics can be influenced by processing method (Tiwari and Awasthi 2012). Fermentation, as a common processing method, has been used for centuries to improve the nutritional value of foods. The capacity of microorganisms to synthesize GABA has also been studied to produce functional fermented foods. The GABA in soymilk and wheat increases significantly when fermented by lactic acid bacteria (Tsai et al. 2006; Rizzello et al. 2008). Kim et al. (2009) found that black raspberry juice contains high GABA levels when fermentation by *Lactobacillus brevis* GABA100. However, most studies have focused on lactic acid bacteria for producing GABA. Filamentous

fungi, which are generally recognized as safe (GRAS), have received little attention.

Compared with lactic acid bacteria, filamentous fungi have some advantages in producing functional fermented foods. During fermentation, these fungi can produce many different enzymes with high activities, such as β-glucosidase, esterase, phytase and xylanase (Koseki et al. 2010; Fujita et al. 2003). These enzymes can hydrolyze insoluble-bound nutrients and release free nutrient substances (Koseki et al. 2010), e.g. polyphenols. Polyphenolic compounds widely distribute in food materials and also have a number of biological effects, including antioxidant and anti-inflammatory properties (Trouillas et al. 2003). A previous study has found that, compared with non-fermented soybeans, the TPC of soybean fermented with various filamentous fungi increased significantly (Lin et al. 2006). Moreover, antinutrient compounds in food materials would also be hydrolyzed during fermentation, such as phytate (Fujita et al. 2003). Phytate can strongly bind minerals such as iron, magnesium, calcium and zinc (Bohn et al. 2004). However, no enzymes can degrade phytate in the human gastrointestinal tract. Therefore, a diet rich in phytate leads to considerable malabsorption of dietary minerals (Bohn et al. 2004). Feng et al. (2007) studied barley tempeh fermentation by *R. oligosporus* and yeast and found that *R. oligosporus* dramatically reduces the phytate content, whereas yeasts do not.

In Asia, *A. oryzae* var. *effuses*, *A. oryzae* and *R. oryzae* are GRAS and commonly used in the preparation of many foods, such as sauce, tempeh. Tempeh is a popular Indonesian traditional food, prepared by inoculating soybeans with a living mould (mostly *R.* spp.) (Nout and Kiers 2005). The fungal mycelium binds the soybeans together into a compact cake during fermentation (Feng et al. 2005). Cereal grains are very important in human health and nutrition (Kaur et al. 2012) and can be used to produce cereal based functional food (Das et al. 2011). However, to our knowledge, information on GABA accumulation in tempeh-like fermented cereal grains is lacking. Oat (*Avena sativa* L.), an important cereal, has been recognized as a kind of healthy food for some time. The bran of oat is rich in antioxidants, such as avenanthramides and phenolic compounds having strong antioxidant activity in vitro (Peterson et al. 2002), while little information is available on the changes of total phenolics content (TPC) in tempeh-like fermented oats during fermentation. Moreover, oats are rich in phytate, which limits the development of oat-based functional foods. Therefore, the objective of the present work was mainly to study the GABA, phytate, natural antioxidants and antioxidant activity of tempeh-like fermented oats prepared with different filamentous fungi (*A. oryzae* var. *effuses*, *A. oryzae* and *R. oryzae*).

Materials and methods

Chemicals

Gallic acid, rutin, GABA, phytic acid dipotassium salt, *o*-phthaldialdehyde, 2, 2-azobis-2-methylpropion- amidine dihydrochloride (AAPH), 6-hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid (trolox), fluorescein sodium salt and mercaptoethanol were purchased from Sigma-Aldrich. The Folin–Ciocalteu reagent was purchased from Merck & Co., Inc. The other reagents used were all of analytical grade.

Microorganism

The *A. oryzae* var. *effuses* 3.2825, *A. oryzae* 3.5232 and *R. oryzae* 3.2751used in the current study were purchased from the Institute of Microbiology, Chinese Academy of Sciences and activated in potato dextrose agar (PDA). After three passages in PDA medium, the fungi were cultivated at 25 °C for 7 days to produce spores. Spore suspensions were prepared by suspending the spores from a PDA culture in 20 mL normal saline. All of these three spore suspensions were adjusted to 1.0×10^7/mL. The spore suspensions were maintained at 4 °C for further use.

Fermentation of oats

Oats (genotype, G4; harvested in 2010, Hebei, China) were purchased from the Chinese Academy of Agricultural Sciences and stored at 4 °C prior to use. The oats were soaked in water for 8 h and then ground using a HK-06A high-speed grinder (Changsha, Hunan, China), the moisture content of ground oats was adjusted to about 35±5 % and sterilized at 121 °C for 15 min. The fermentations were performed by inoculation with a spore suspension in a 500 mL Erlenmeyer flask containing 50 g of smashed oats (10^6 spores/g of oats). The final moisture content was adjusted to about 45±5 % with normal saline. Oats was incubated at 25 °C under static conditions. During the cultivation period, the oats were stirred after 24, 36, 48 and 60 h of cultivation. Similarly treated oats, except for the spore inoculation, were used as the control.

Determination of GABA

Samples were prepared according to a previous method with some modifications (Bai et al. 2009). Native and fermented oats were defatted initially and then extracted using 4 % (v/v) acetic acid with ultrasonication for 180 min to extract GABA sufficiently and then centrifuged at 3,572×g for 30 min. The supernate was collected and treated with ethanol to remove the macromolecular polymers and then

centrifuged at 12,640×g for 20 min. The purified supernate was evaporated at 45 °C under reduced pressure to volatilize the acetic acid and ethanol. The residues were dissolved in distilled water and centrifuged at 3,212×g for 10 min.

Then the centrifugal supernatant was filtered through a 0.45 μm membrane filter. The filtered supernate was analyzed with a Shimadzu HPLC (Model LC-10 ATvp Pumps and DGU-12A Degasser) equipped with a diode array detector (Model SPD-M10 Avp) (Shimadzu, Kyoto, Japan). The assay was performed on a Shim-Pack VP-ODS column (250 mm× 4.6 mm i.d., particle size 5 μm) with a guard column (Shim-pack G VP-ODS, 10 mm×4.6 mm i.d., particle size 5 μm) (Shimadzu, Kyoto, Japan), according to a previous method with minor modifications (Liu et al. 2011). The standard GABA solution and the samples were determined by pre-column derivatization of *o*-phthaldialdehyde-GABA (OPA-GABA) from *o*-phthaldialdehyde. Sodium acetate buffer (pH 7.3, 0.02 M) containing 20 % acetonitrile was used as the mobile phase at a flow rate of 0.8 mL/min during the entire run. Up to 20 μL of each sample were injected and detected at 338 nm at a column temperature of 40 °C. All sample solutions were injected in triplicate. GABA was expressed as μg g^{-1} oats

Determination of phytate

The phytate concentration during fermentation was assayed by colorimetric method (Latta and Eskin 1980). Samples were extracted using 2.4 % HC1 (0.65 M) with ultrasonication at 25 °C for 180 min to extract the total phytate. Then, the extract was eluted through an AG1-X8 anion exchange resin to remove the inorganic phosphorus, as well as other interfering compounds. The phytate content was measured using the Wade reagent and calculated from the calibration curve of phytate dipotassium salt standard solutions and expressed as mg g^{-1} oats. All measurements were done in triplicate.

Preparation of ethanol extracts

The native and fermented oats were extracted using 80 % ethanol with ultrasonication at 45 °C for 30 min. After cooling to room temperature, the slurries were centrifuged at 2,862×g for 15 min and then the supernate was collected. The residue was extracted once more under the same conditions and the supernates were pooled. The solution was then evaporated at 45 °C under reduced pressure to obtain the ethanol extracts used for the determinations of TPC, flavonoid and antioxidant activity (sample fermentation for 72 h).

Determination of TPC

The TPC in all ethanol extracts was determined according to the Folin–Ciocalteu method (Singleton and Rossi 1956). Ethanol extract was initially dissolved in 80 % ethanol. Then, about 1 mL of dissolved sample was mixed with 1 mL of Folin–Ciocalteu reagent. After 1 min of incubation at room temperature, 1.5 mL of 20 % Na_2CO_3 solution was added to the mixtures, followed by 7.5 mL of distilled water. The mixtures were then kept in a constant-temperature water bath at 70 °C for 10 min. After cooling to room temperature, the absorbance was recorded at 765 nm. TPC was expressed as gallic acid equivalent (GAE) from the calibration curve of a gallic acid standard solution and expressed as mg GAE g^{-1} oats. All samples were performed in triplicate.

Determination of flavonoids

The total flavonoids in the ethanol extracts were determined using a colorimetric method with minor modifications (Dewanto et al. 2002). Initially, ethanol extract was dissolved in 80 % ethanol. About 1 mL of dissolved sample was placed in a 10 mL volumetric flask. Distilled water was added to obtain a total volume of 5 mL and then 0.3 mL of $NaNO_2$ was added. About 0.3 mL of $AlCl_3$ was added after 5 min and the mixture was allowed to stand for another 6 min. About 2 mL of 1 M NaOH was added and the total volume was increased to 10 mL with distilled water. The solution was mixed well and allowed to stand for 30 min. The absorbance was recorded against a blank at 510 nm. The flavonoid content was determined as the rutin equivalent from the calibration curve of rutin standard solutions and expressed as mg rutin g^{-1} oats. All measurements were done in triplicate.

Determination of antioxidant activity

Cyclic voltammetry assay

The assay was performed by the protocol outlined previously with some modification (Chevion et al. 2000). Model CHI 620 C electrochemical analyzer (CHENHUA, Shanghai, China), using a three-electrode system, was used for the cyclic voltammetry assay in the present work. A glassy carbon electrode is working electrode. An Ag/AgCl electrode was used as reference; and a platinum foil served as the auxiliary electrode. The glassy carbon electrode was polished on alumina powder before each scanning. The 0.2 M Britton–Robinson buffered solutions (BRS) was used as supporting electrolytes, containing 0.3 M KCl, at pH= 7.24. All ethanol extracts were prepared with methanol as solvent and diluted into appropriate concentration by BRS. Prior to each run, the dissolved oxygen in sample solutions were removed by N_2 for about 15 min. Voltammetric scans were carried out from -0.4 V versus Ag/AgCl to 1.2 V at room temperature with a scan rate of 400 mV/s. The area under the anodic current wave (Q) was obtained by EC Application Software.

Oxygen Radical Absorbance Capacity (ORAC) assay

A fluorescein stock solution (7.98×10^{-4} mM) was prepared in phosphate buffer (75 mM, pH 7.4). AAPH was dissolved in phosphate buffer to a final concentration of 173 mM. The black 96 wells microtiter plates (Nunc, Thermo Fisher Scientific, Roskilde, Denmark) was used to perform the assay. The ethanol extract solution (25 μl), dissolved in methanol, mixed with fluorescein solution (100 μl) was incubated at 37 °C for 10 min. Adding 75 μl of AAPH solution in the mixture to initiate the assay. The fluorescence was read every minute for about 120 min using a fluorescence filter with an excitation wavelength of 485 nm and an emission wavelength of 515 nm with a Multidetection microplate reader, SpetraMax M2^e. The trolox equivalents were calculated from a standard curve.

Statistical analysis

All experiments were repeated three times. Data are expressed as the mean ± standard deviation for each measurement (n=3). The data were also analyzed by one-way ANOVA. Tukey's procedure was used to determine the significant of the differences (p<0.05). Analysis was done with SPSS 13.0 (SPSS, Inc., Chicago, IL, USA). All figures were created by Microsoft Office 2010.

Results and discussion

GABA content of the tempeh-like fermented oats

The GABA content of the native oats was 57.1 μg g^{-1} oats (Fig. 1a), higher than that reported by Coda et al. (2010) (27 μg g^{-1}). The discrepancy between the present work and that of Coda et al. could be explained as follows: (1) In the present work, whole oats were used, whereas in the work by Coda et al., oat flour without bran was used. Iimure et al. (2009) found that barley bran contains some GABA. Therefore, native oat bran may also have some GABA. (2) The oats used in the present work were soaked in water at room temperature for 8 h. A previous study found that the GABA in rice germ could be increased by soaking in water (Saikusa et al. 1994). Thus, the soaking process may also cause an increase in the GABA content of the native oats.

The effect of fungal fermentation on the GABA content of the oats is shown in Fig. 1a. The GABA content increased significantly, especially in the *A. oryzae*-fermented oats (p<0.05). The GABA content of the fermented oats ranged from 59.0 to 435.2 μg g^{-1} oats, which depended on the starter organisms and fermentation time. During the early stage of fermentation (≤36 h), the GABA content of the three fermented oats had no statistical difference (p>0.05). As shown in Fig. 1a, the 72 h fermentation of *A. oryzae* var. *effuses*, *A. oryzae* and *R. oryzae* induced the accumulation of GABA by about 6, 8 and 2 times higher (330.9 μg g^{-1} oats, 435.2 μg g^{-1} oats and 125.6 μg g^{-1} oats) than that of the native oats (57.1 μg g^{-1} oats), respectively.

The blood pressure lowering effect of GABA in experimental animals and humans has already been reported. A study found that hypertensive patients benefit from oral GABA at 10 mg daily for 12 weeks (Inoue et al. 2003). A previous study also showed that daily oral administration of 26.4 mg of GABA from rice germ is effective in treating neurologic disorders (Okada et al. 2000). The amount of GABA produced in the fermented oats in the present study was sufficient to provide these functional benefits.

GABA is synthesized by the decarboxylation of glutamic acid, which is catalyzed by glutamate decarboxylase (GAD) contained in GABA-producing microorganisms (Hayakawa et al. 1997). When these microorganisms are used in fermentation, the GAD produced by the microorganisms catalyzes the decarboxylation of glutamic acid into GABA. In the present work, the *A. oryzae*-fermented oats had the highest GABA after 72 h of fermentation, which may be caused by *A. oryzae*, which produces more GAD and/or has the highest GAD activity. Future studies should include a test to determine the amount and the activity of GAD in these filamentous fungi.

Phytate content of the tempeh-like fermented oats

In plant seeds, phytate is the principal storage form of phosphate. Diets rich in phytate lead to malabsorption of dietary minerals, decreased protein solubility and proteolytic digestibility because of the anti-nutritional property of phytate (Bohn et al. 2004; Kumar et al. 2010). Major efforts have been exerted to reduce the amount of phytate in foods using different processes, e.g., soaking, germination, malting and fermentation (Kumar et al. 2010). Fermentation is the most common method used to reduce the phytate content of food materials. Food products, such as tempeh, koji and soy sauce, which are produced by the fermentation of soybeans with *R. oligosporus* and *A. oryzae*, have been shown to produce both intracellular and extracellular phytate-degrading activity (Fujita et al. 2003). The study by Feng et al. (2007) found that *R. oligosporus* remarkably reduces the phytate content in tempeh.

In the present work, three filamentous fungi were used to ferment oats. As shown in Fig. 1b, the amount of phytate decreased significantly after 72 h of fermentation, especially in the *A. oryzae*–fermented oats (p<0.05). In the early stage of fermentation (≤24 h), the amount of phytate in the fermented oats had no significant difference with that of the native oats (p>0.05). After 72 h of fermentation with *A. oryzae* var. *effuses*, *A. oryzae* and *R. oryzae*, the amount of

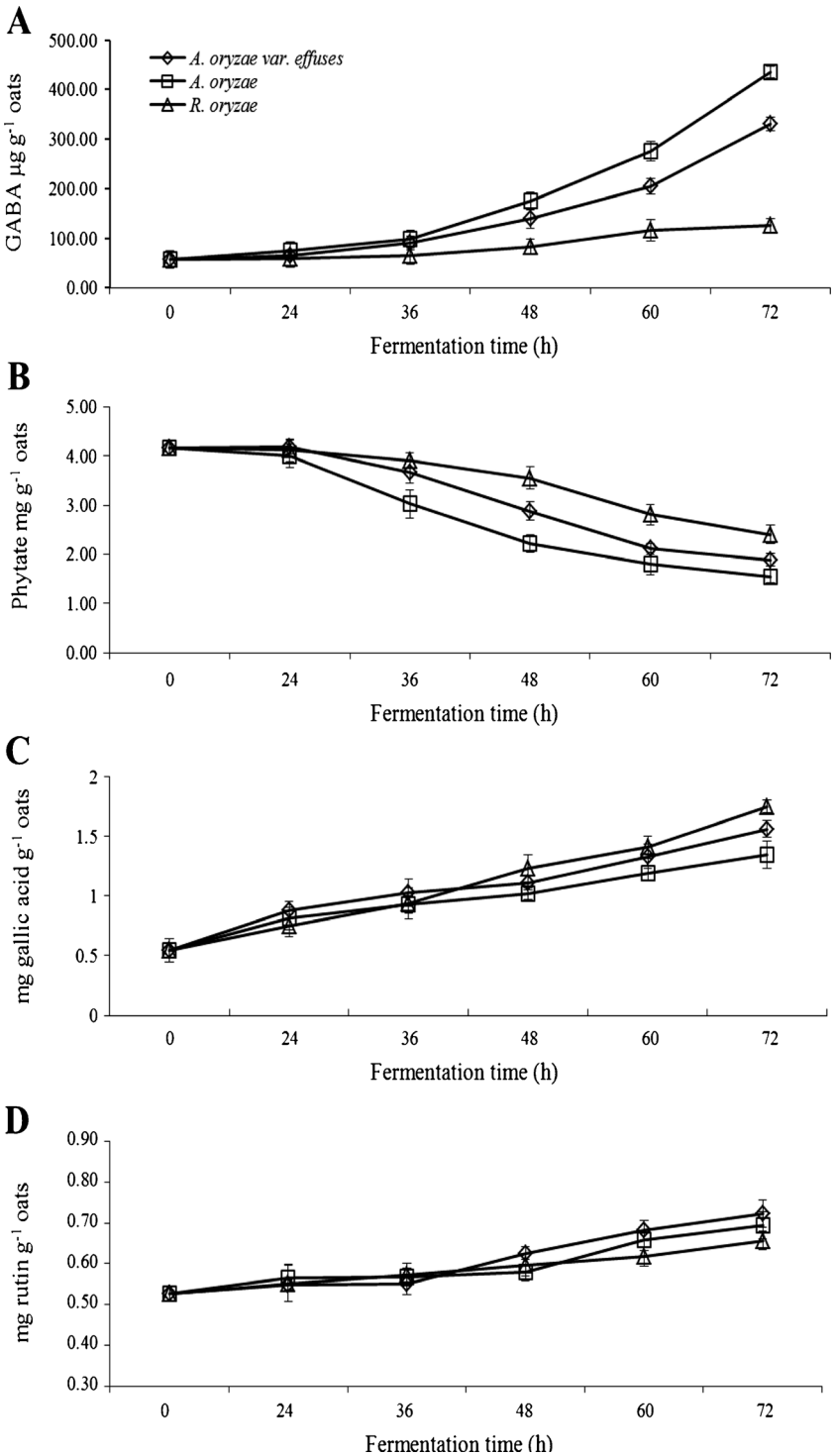

Fig. 1 Changes in contents of GABA (γ-aminobutyric acid) (**a**), Phytate (**b**), Total phenolics (**c**) and Flavonoid (**d**) of oats fermented by *Aspergillus oryzae* var. *effuses, Aspergillus oryzae* and *Rhizopus oryzae* during incubation periods (n=3)

phytate varied from 4.2 to 1.9 mg g^{-1} oats, 1.6 mg g^{-1} oats and 2.4 mg g^{-1} oats, respectively. The result indicates that the three filamentous fungi effectively decrease the amount of phytate in the whole oats and they can be used to produce plant-based food with lower phytate.

Although oats also have phytase activity, endogenous phytate activity is minimal because the oats were pretreated by steaming. Phytase produced by fungi may be responsible for the decrease in phytate. Two types of *A. oryzae* phytase had been found during industrial koji making (Fujita et al. 2003). Figure 2b indicates that the amounts and/or activities of phytases produced by the three fungi may be different from each other. Among the three fungi, *A. oryzae* may produce the highest amount and the most active phytase, whereas *R. oryzae* has the lowest phytase expression. Further experiments are needed to elucidate the specific

relationship between phytate reduction and the phytase produced by these filamentous fungi.

TPC of the tempeh-like fermented oats

A previous study found that the TPC of soybeans fermented using various filamentous fungi is significantly increased compared with non-fermented soybeans (Lin et al. 2006). Recently, Bhanja et al. (2009) also found that the TPC of wheat fermented with two filamentous fungi is increased remarkably. In the present paper, three GRAS filamentous fungi were employed as starter organisms. The fermentation markedly increased the TPC of all fermented oats ($p<0.05$) (Fig.1c). The TPC of the 80 % ethanolic extract from the oats fermented with these fungi ranged between 0.8 and 1.8 mg GAE g^{-1} oats, also depending on the starter organisms and fermentation time. All these results were higher than those of non-fermented oats (0.5 mg GAE g^{-1} oats). In the early phase of fermentation (≤ 36 h), the TPC of the three fungi showed no significant difference ($p>0.05$). As fermentation time progressed, the difference of the TPC among the three fungi gradually became significantly. After 72 h of fermentation, the *R. oryzae*–fermented oats had the highest TPC ($p<0.05$), followed by that of *A. oryzae* var. *effuses* and by *A. oryzae*.

The three GRAS fungi applied in the present study are commonly used in the food processing industry in Asia. They can produce various enzymes during fermentation, e.g., α-amylase, β-glucosidase and xylanase (Bhanja et al. 2009). These enzymes can decompose the cereal cell walls and release insoluble-bound nutrient substances (Koseki et al. 2010). Daniels and Martin (1968) found that most oat phenols are insoluble-bound in oat bran. The increase in TPC in the fermented oat may be due to the activity of these enzymes, which can hydrolyze bound phenols during fermentation. However, the ratio of TPC increase during fermentation was dependent on fungal strain and fermentation time. Both of these two factors influence the amounts and/or activities of the enzymes. In Fig. 1c, the data clearly reveals that the increased TPC ratios are dependent on the fungal strain and fermentation time. Previously, Bhanja et al. (2009) also found that the TPC of fermented wheat depends on the starter organism and fermentation time. They pointed out that the xylanase and β-glucosidase produced by filamentous fungi are mainly responsible for the release of phenolics.

Flavonoids content of the tempe-like fermented oats

Flavonoids, currently of scientific and therapeutic interest, are a class of phenolic compounds. They have been proposed to act as beneficial agents in many disease states, including neurodegenerative disorders, cardiovascular disease and cancer (Spencer et al. 2004). Similar to phenolics, the flavonoid content of oats fermented by *A. oryzae* var. *effuses*, *A. oryzae* and *R. oryzae* increased remarkably compared with that of native oats ($p<0.05$) (Fig. 1d). At the initial phase (≤36 h), the flavonoids of the native oats and the three fermented oats were not significantly different ($p>0.05$). After 72 h, the flavonoid content of the oats fermented by *A. oryzae* var. *effuses* was much higher than that of *R. oryzae*-fermented oats ($p<0.05$) and had no statistical difference with that of oats fermented by *A. oryzae* ($p>0.05$).

In plants, many flavonoids are usually found in conjugated form with cellulose, protein and other polymerides through hydroxyl groups and they become insoluble (Juan and Chou 2010). Therefore, the increase in the flavonoids of the fermented oats may also be due to the effects of the enzymes produced by fungi during fermentation. These enzymes catalyze the release of flavonoids from the insoluble-bound state during fermentation, which may lead to an increase in the content of those compounds (Fig. 1d). Juan and Chou (2010) observed a similar phenomenon in black soybeans fermented with *Bacillus subtilis* BCRC 14715.

Determination of the antioxidant activity of native and fermented oats

In cyclic voltammetry (CV), the working electrode potential is scanned in two inverse directions. These two inverse scans form an area in a current–potential plot. The area under the anodic current wave represents the total antioxidant capacity of antioxidant. In the present study, the values of areas under anodic current (up to 1,200 mV), Q_{1200}, were used to determine the antioxidant capacities of all samples. As shown in Fig. 2a, the antioxidant capacities of various tempeh-like fermented oats were significantly increased after 72 h fermentation in comparison with non-fermented oat ($p<0.05$). Among all tempeh-like fermented oats, the *R. oryzae*-fermented oat had the highest Q_{1200}. Thus, the *R. oryzae*-fermented oat had the strongest antioxidant capacity. There is no statistical difference between antioxidant capacities of the *R. oryzae*-fermented oat and the *A. oryzae* var. *effuses*-fermented oat ($p>0.05$). However, the *A. oryzae*-fermented oat had the weakest antioxidant capacity ($p<0.05$), compared with the other two tempeh-like fermented oats. Taking all samples into account, there was a good correlation between TPC and Q_{1200} values ($r=0.998$, $p<0.01$). This result indicates that the predominant components of antioxidant activity may be derived from phenolic compounds in oat.

Oxygen radical absorbance capacity (ORAC) assay was another method employed in the present work to determine the antioxidant capacities of the samples. The results are presented in Fig. 2b, expressed as μmol trolox g^{-1} extracts, which shows quite similar results compared to those

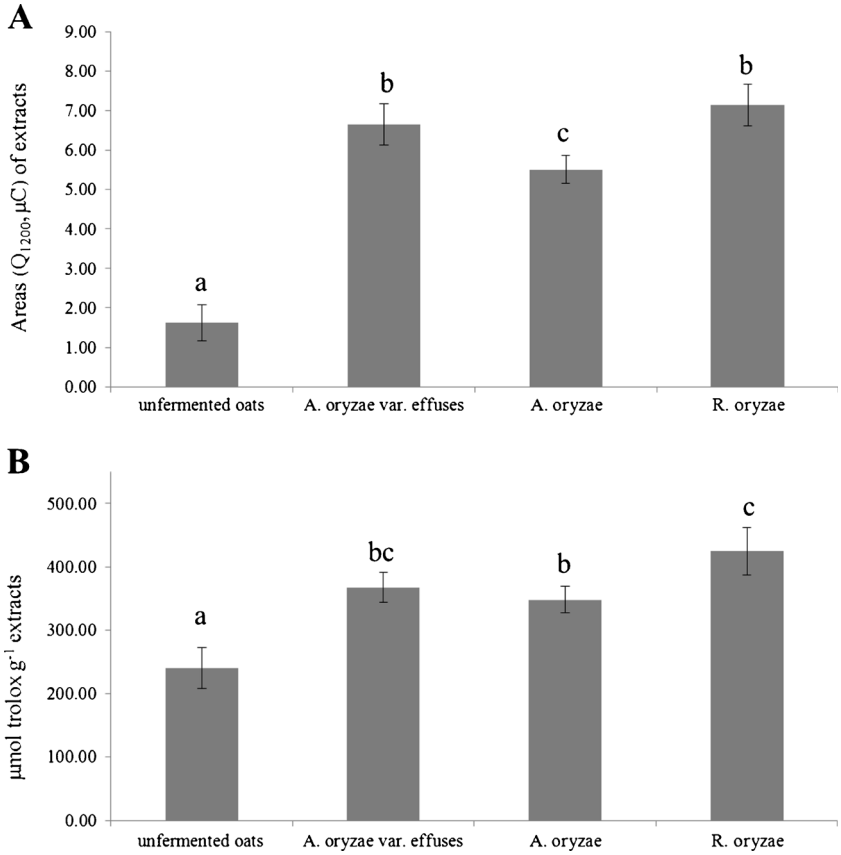

Fig. 2 The results of antioxidant activity of ethanol extracts from native and temph-like fermented oats (fermentation for 72 h) (*Avena sativa L.*). **a** The areas under anodic current waves (Q_{1200},μC) of the ethanol extracts from native and fermented oats, **b** The results of ORAC (Oxygen Radical Absorbance Capacity) of the ethanol extracts from native and fermented oats. Means (*bar value*) with different letters are significantly different ($p<0.05$) ($n=3$)

obtained in CV assay. The antioxidant capacities of the various tempeh-like fermented oats were dramatically enhanced in comparison with unfermented oat ($p<0.05$). The strongest antioxidant capacity was observed in *R. oryzae*-fermented oat ($p<0.05$). However, there is no significant difference between the antioxidant capacities of the *R. oryzae*-fermented oat and the *A. oryzae* var. *effuses*-fermented oat ($p>0.05$). Similar to the CV assay, TPC and ORAC values were also have highly correlated ($r=0.987$, $p<0.05$).

Previous studies have found that phenolic compounds exhibited good antioxidant activities (Shahidi et al. 1992). Bhanja et al. (2009) have reported that the antioxidant activities of wheat kojis prepared with two filamentous fungi were significantly enhanced. They also found that there was a good correlation between the TPC and antioxidant activities of wheat kojis (Bhanja et al. 2009). In the present work, tempeh-like fermented oats were prepared with three filamentous fungi (*A. oryzae* var. *effuses*, *A. oryzae* and *R. oryzae*). The antioxidant activities of these tempeh-like fermented oats were all increased ($p<0.05$). It was also observed that the TPC of these tempeh-like fermented oats were significantly increased ($p<0.05$). The TPC and antioxidant activities determined by ORAC assay and CV assay were highly correlated providing strong evidence that the enhancements of antioxidant activity of various tempeh-like fermented oats were due to the increase of TPC after fermentation.

Conclusions

A. oryzae var. *effuses*, *A. oryzae* and *R. oryzae* are very effective in enhancing the GABA content, TPC and flavonoid content and in reducing the phytate content of tempeh-like fermented oats. Furthermore, the antioxidant activity of the fermented oats improved significantly after 72 h fermentation. The oats fermented by *A. oryzae* had the highest amount of GABA and the lowest amount of phytate compared with those fermented by the other two filamentous fungi. However, the *R. oryzae*-fermented oats had the highest amount of TPC and the strongest antioxidant activity. In conclusion, fungal fermentation is a promising method for producing GABA- and antioxidants-enriched oat-based tempeh-like food with higher antioxidant activity and lower phytate compared with native oats. Further studies are needed to determine the activity of GAD, phytase and other relevant enzymes in the three filamentous fungi during fermentation.

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