Food Chemistry 119 (2010) 957–963

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Effect of fermentation on antioxidant properties of some cereals and pseudo cereals

Tijana M. Đorđević *, Slavica S. Šiler-Marinković, Suzana I. Dimitrijević-Branković

Division of Biochemical Engineering and Biotechnology, Faculty of Technology and Metallurgy, Karnegijeva 4, University of Belgrade, 11000 Belgrade, Serbia

ARTICLE INFO

Article history:
Received 19 December 2008
Received in revised form 23 July 2009
Accepted 29 July 2009

Keywords:
Cereals
Fermentation
Total phenolic content (TPC)
Antioxidant activity
DPPH
FRAP
TBA test

ABSTRACT

The influence of fermentation by two types of microorganisms (lactic acid bacteria *Lactobacillus rhamnosus*, and yeast *Saccharomyces cerevisiae*) on antioxidant activities and total phenolics of 4 cereals, namely buckwheat, wheat germ, barley and rye, was determined and compared with those of their unfermented counterparts. The total phenolic content (TPC), determined by the Folin–Ciocalteu method, increased upon fermentation. Antioxidant activities (AOA) were assessed using 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging capacity, ferric ion-reducing antioxidant power (FRAP) and thiobarbituric acid (TBA) methods. The presence of those microorganisms was more or less important for enhanced levels of antioxidant activity. Thus fermentation offers a tool to further increase the bioactive potential of cereal products.

1. Introduction

Although synthetic antioxidants such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) and tert-butylhydroquinone (TBHQ), as well as propyl gallate (PG), have widely been used in retarding lipid oxidation, their safety has recently been questioned due to toxicity and possible carcinogenicity (Shahidi, 2000). Thus, development of safer natural antioxidants from extracts of plant materials that can replace synthetic antioxidants has been of interest (Branen, 1975). Food antioxidants such as amino acids, peptides, proteins, flavonoids and other phenolic compounds might play a significant role as physiological and dietary antioxidants, thereby augmenting the body's natural resistance to oxidative damage (Shahidi, 2000).

Significant levels of antioxidants have been detected in cereals and cereal-based products (Baublis, Decker, & Clydesdale, 2000; Emmons, Peterson, & Paul, 1999). Cereals and pseudo cereals are an important source of macronutrients. Cereal grains provide significant quantities of energy, protein and selected micronutrients in the animal and human diet. Chemical composition and bioavailability of nutrients varies between species and varieties of grains and may be affected by the forms of processing as feed and food (Senter, Horvat, & Forbus, 1983). Cereals also contain a wide range of chemical classes with antioxidant activity (Adom & Liu, 2002). Cereal grains are rich in phenolic acids and saponins, while phytoestrogens and flavonoids are present in small quantities (Senter et al., 1983). Wheat extracts have shown potential antioxidant properties as wheat phenolics appear to serve as powerful antioxidants through radical scavenging and/or metal chelation (Liyana-Pathirana, Dexter, & Shahidi, 2006; Liyana-Pathirana & Shahidi, 2006), while barley contains substantial amounts of phenolic antioxidants that effectively scavenge peroxyl, DPPH, and hydroxyl radicals, and effectively control oxidation of LDL cholesterol, thereby having a great potential in the development of nutraceuticals rich in antioxidants (Madhujith & Shahidi, 2006, 2007). It has been suggested that antioxidants may contribute to the health benefits of cereal-based foods by reducing the incidence of aging-related chronic diseases including heart diseases and some types of cancer (Miller, Rigelhof, Marquart, Prakash, & Kanter, 2000).

On the other hand, cereals are known to contain certain amounts of antinutritive components, such as salts of phytic acid (myoinositol hexakisphosphates, phytates), which are not very soluble and of very limited digestibility (Guenter, 1997), as well as hemicelluloses that are associated with cellulose and pectic substances and comprise several nonstarch, noncellulosic polysaccharides, including xylans (arabinoxylans and 4-0-methyl glucuronoxylans), galactomannans, glucomannans, ß-D-glucans (3- and 4-linked), ß-D-glucan-callose (3-linked), and xyloglucans (4-linked ß-D-glucans with attached side chains) (Chesson, 1987). The ß-glucans and arabinoxylans have been recognized as antinutritive factors in cereals (Bedford, 1995).

Throughout history, fermentation has been used to improve product properties. Previous studies have shown that microorganisms start to modify plant constituents during fermentation

* Corresponding author. Address: Bulevar JNA 1E, 11223 Belgrade, Serbia. Tel.: +38 165 6000 250; fax: +38 111 3940 860.
E-mail address: tijana.djordjevic@hotmail.com (T.M. Đorđević).

0308-8146/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2009.07.049

(Katina, Liukkonen et al., 2007). Many biochemical changes occur during fermentation, leading to altered ratio of nutritive and antinutritive components of plants, which affect product properties such as bioactivity and digestibility (Heiniö et al., 2003; Katina, Laitila et al., 2007). Therefore the objective of this work was to assay the influence of different types of fermentation (yeast fermentation and lactic acid fermentation) on antioxidative activity and total phenolic content in selected cereals.

2. Materials and methods

2.1. Materials

The cereal samples used in this study included buckwheat (*Fagopyrum esculentum*) manufactured by Organic Biopharm, China, and unpeeled wheat (*Triticum aestivum*), peeled rye (*Secale cereale*) and peeled barley (*Hordeum vulgare*) manufactured by KLAS, Sarajevo. The compounds 1,1-diphenyl-2-picrylhydrazyl (DPPH), thiobarbituric acid (TBA) and gallic acid were purchased from Sigma–Aldrich Chemie GmbH (Taufkirchen, Germany), Folin–Ciocalteu reagent was purchased from Merck & Co., Inc. (New York, USA), and all other chemicals and solvents were the highest commercial grades purchased from Lachema Ltd. (Brno, Czech Republic) and Fluka Chemie GmbH (Buchs, Switzerland), and used without additional purification. Microorganisms used in this study (*Lactobacillus rhamnosus* A71 and *Saccharomyces cerevisiae*) were from a collection of the Laboratory of Microbiology of the Faculty of Technology and Metallurgy, Belgrade. The MRS broth and Tryptic soy broth (TSB) growth media were purchased from Merck & Co., Inc. (New York, USA).

2.2. Extraction and sample preparation

2.2.1. Preparation of microbiological cultures

Microbiological culture of *Lactobacillus rhamnosus* A71 was kept in lyophilized form and activated in 10 ml of MRS broth using 1% inoculums, and microbiological culture of *Saccharomyces cerevisiae* was kept on agar plates at 4 °C and activated in 10 ml of tryptic soy broth using 1% inoculums. Incubation for 24 h was conducted at 37 °C for *L. rhamnosus* and 30 °C for *S. cerevisiae*. After three passages, fresh microbiological cultures so obtained were used for inoculation of milled cereal samples.

2.2.2. Preparation of cereal extracts

Samples of each cereal were prepared in triplicate. Cereal grains (100 g) were submerge in distillate water for 24 h, then filtrated and milled with 400 ml of distilled water. Cereal mashes were then sterilised in an autoclave for 1 h and cooled. The first sample of each cereal was inoculated with *L. rhamnosus* (5 ml of inoculum), the second one was inoculated with *S. cerevisiae* (5 ml of inoculum), and the third sample was the control without any inoculation. All three samples of each plant were allowed to ferment at 30 °C for 24 h. The samples were extracted with 70% (v/v) ethanol (700 ml) for 3 h on the magnetic paddle (Heidolph MR 3001, Sigma–Aldrich Chemie GmbH, Taufkirchen, Germany), and then centrifuged at 3060*g* (4500 rpm) for 10 min using the SIGMA 2-16 Versatile Centrifuge (MBI, Dorval, Canada). Extracts were poured into a lab dish, and residues were re-extracted with 70% (v/v) ethanol (300 ml) for another 3 h using the magnetic paddle, and centrifuged at 3060*g* (4500 rpm) for 10 min. The extracts were combined. Volumes of extracts were approximately 1200 ml, and kept in a refrigerator until drying.

Before drying samples were concentrated to 100 ml using the Büchi rotavapor R 210/215 (Büchi Labortechnik AG, Flawil, Switzerland) (temperature 50 °C, pressure 50–150 mbar). Concentrated extracts were dried using the Büchi Mini Spray Dryer B-290 (Büchi Labortechnik AG, Flawil, Switzerland) after dissolving in water to obtain less than 20% (v/v) ethanol concentration, which is the working condition for this unit. Inlet temperature and pump were adjusted to 120–125 °C and 15–20%, respectively, leading outlet temperature of 60–63 °C.

Dried samples were kept in hermetically sealed dishes in a freezer until further analysis.

2.2.3. Determination of total phenolics content

The content of total phenolics in extracts was determined by a modified Folin–Ciocalteu method (Singleton & Rossi, 1956). Briefly, 100 μl of each extract were shaken for 1 min with 500 μl of Folin–Ciocalteu reagent and 6 ml of distilled water. After the mixture was shaken, 2 ml of 15% Na_2CO_3 were added and the mixture was shaken once again for 0.5 min. Finally, the solution was brought up to 10 ml by adding distilled water. After 2 h, the absorbance was read on the UV/visible spectrophotometer (Ultrospec 3300 pro, Amersham Bioscience, Sweden) at 750 nm (25 °C) using glass cuvettes against a blank (100 μl of distilled water instead test samples). The TPC was assessed by plotting the gallic acid calibration curve (from 1 to 1500 μg/ml) and expressed as milligrams of gallic acid equivalents (GAE) per gram of dried extract. The equation for the gallic acid calibration curve was $Y = 1.34577 \cdot X - 0.07823$ (where X = concentration of gallic acid equivalents (GAE) expressed as milligrams of GAE per gram of dried extract; Y = measured absorbance), and the correlation coefficient was $R^2 = 0.9897$.

2.2.4. Determination of DPPH radical scavenging activity

Antioxidant activity of the dried ethanol extract was measured on the basis of scavenging activities of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical (Cuendet, Hostettmann, & Potterat, 1997). In a lab dishes containing 50 μl of test samples of various concentrations were added: 3.95 ml of methanol and 1 ml 0.2 mM of DPPH methanol solution. After 30 min of incubation in the dark at room temperature, the absorbance was measured against a blank (methanol) at 517 nm using the UV/visible spectrophotometer (Ultrospec 3300 pro, Amersham Bioscience, Sweden). Inhibition of DPPH radical was calculated as a percentage (%) using the formula:

$$\text{Percentage inhibition } (\%) = [(A_{\text{control}} - A_{\text{sample}})/A_{\text{control}}] \times 100$$

where, A_{control} is the absorbance of the control reaction (containing all reagents except test compound), and A_{sample} is the absorbance of the test compound. IC_{50} values (concentration of sample required to scavenge 50% of free radicals) were calculated from the regression equation, prepared from the concentration of the samples and percentage inhibition of free radical formation (percentage inhibition DPPH was assayed). Synthetic antioxidant L-ascorbic acid was used as a positive control and all tests were carried out in triplicates.

2.2.5. FRAP method

In the FRAP method the yellow Fe^{3+}-TPTZ complex is reduced to the blue Fe^{2+}-TPTZ complex by electron-donating substances under acidic conditions. Any electron donating substance with a half reaction of lower redox potential than Fe^{3+}/Fe^{2+}-TPTZ will drive the reaction and the formation of the blue complex forward. To prepare the FRAP reagent, a mixture of 300 mmol/l acetate buffer pH 3.6 (containing 6.4 ml 2 M sodium acetate solution and 93.6 ml 2 M acetic acid solution diluted in a volumetric flask (1 l)), 10 mmol/l TPTZ (in 40 mmol/l HCl) and 20 mmol/l ferric chloride (10:1:1, v:v:v) was made. 150 μl of ethanol plant extract were mixed with 4.5 ml of FRAP reagent. The absorbance readings were started after 5 min and they were performed at 593 nm using the UV/visible spectrophotometer (Ultrospec 3300 pro, Amersham Bioscience, Sweden). The blank consisted of FRAP reagent. The final

absorbance of each sample was compared with those obtained from the standard curve made from ferric sulphate ($FeSO_4 \times 7H_2O$) (200–1000 μmol/l). The results were expressed in nmol Fe^{2+}/mg dried extracts (Szőllősi & Szőllősi Varga, 2002).

2.2.6. Thiobarbituric acid test (TBA)

Thiobarbituric acid tests were performed according to the method of Afanas'ev, Dorozhko, Brodskii, Kostyuk, and Potapovitch (1989) to determine the TBA reactive substance (TBARS) from lipid peroxidation. Lipid peroxidation was measured in the liposome rimifon Lipotech 10 (0.3 g lecithin/ml). The mixture contained 20 μl $FeSO_4$ (0.075 M), 50 μl liposomes, 10 μl of test samples of various concentrations (1–10% w/v), 20 μl L-ascorbic acid (0.1 M) and 3.9 ml phosphate buffer pH 7.4 (containing 5 ml of 0.2 mol/l monopotassium phosphate solution and 3.91 ml of 0.2 mol/l sodium hydroxide solution diluted in a volumetric flask (20 ml)). The mixture maintained at 37 °C for 1 h in thermostat and then mixed with 0.2 ml ethylenediaminetetraacetic acid (EDTA) (0.1 M) and 1.5 ml TBA reagent (3 g thiobarbituric acid, 120 g trichloroacetic acid and 10.4 ml perchloric acid in 800 ml demineralised water). After heating at 100 °C for 15 min, and centrifugation at 1107*g* (3000 rpm) for 10 min using the SIGMA 2-16 Versatile Centrifuge (MBI, Dorval, Canada), the absorbance of the supernatant was measured at 532 nm against the blank (distilled water) using the UV/visible spectrophotometer (Ultrospec 3300 pro, Amersham Bioscience, Sweden). Inhibition of lipid peroxidation was calculated as percentage (%) by the formula:

$$\text{Percentage inhibition } (\%) = [(A_{\text{control}} - A_{\text{sample}})/A_{\text{control}}] \times 100$$

where, A_{control} is the absorbance of the control reaction (containing all reagents and distilled water against test compound), and A_{sample} is the absorbance of the test compound.

2.2.7. Statistical analyses

All analyses for antioxidative activity determination and also test for the determination of the total phenolic content (TPC) were run in triplicate. The mean value and standard deviation were calculated using the Microsoft Excel software (Microsoft Corporation, Redmond, WA). Analysis of variance (ANOVA) was used to evaluate the significant difference among various treatments with the criterion of $P < 0.05$.

3. Results and discussion

3.1. Total phenolics and antioxidant activities of plant materials

The antioxidant activities and total phenolics of 4 examined native (pseudo) cereals are shown in Table 1.

All plants showed a significant amount of total phenolics and effective antioxidant activities (Table 1). Buckwheat had the highest amount of total phenolics, with the highest DPPH radical scavenging activity and capacity for Fe^{3+} reduction, but it had the lowest lipid peroxidation inhibition ability. These data suggest that it might be more critical, in delaying lipid peroxidation, to suppress the initiation of radical chain reaction than to terminate it by quenching or removing the radicals generated during propagation of the radical chain reaction (Yu, Haley, Perret, & Harris, 2002). Other plants had similar TPC or AOA.

Fermentation led to increase in phenol content, measured by TPC method, and more or less of antioxidant activities, measured by all three methods. Higher antioxidative activity was observed in samples fermented with *L. rhamnosus*, compared with samples fermented with *S. cerevisiae* (Table 1). Bound phenolics, whose importance in the total phenolic content (TPC) was recognized in one of the early studies by Naczk and Shahidi (1989), may be released by alkali, acid or enzymatic treatment of samples prior to extraction (Krygier, Sosulski, & Hodge, 1982a,b; Bartolome & Gomez-Cordoves, 1999), which can be used to explain fermentation-induced increase in the TPC leading to higher antioxidative potential of fermented cereal extracts.

3.2. Total phenolic content

As shown in Table 1, total phenolic compound contents in the examined cereals and pseudo cereal were the highest in buckwheat, 50.7 mg GAE/g dry extract. A similar phenolic content in buckwheat had been reported by Velioglu, Mazza, Gao, and Oomah (1998). Lower total phenolic compound contents were present in wheat and barley (16.2 and 16.4 mg GAE/g dry extract, respectively) and the lowest in rye 13.2 mg GAE/g dry extract. The data reported by Zhou and Yu (2004) had shown that the contents of TP in wheat and barley grain were affected by the extraction solvents in the following descending order: acetone > ethanol > methanol, which can be used to explain lower amounts of phenols present in this plants (Table 1). According to Zielinski and Troszynska (2000), extraction duration and extraction method greatly affect the TPC in rye, so it is very difficult to compare the results in our work with those reported by others. Generally, it is difficult to compare our data with other literature data due to different methods used for extraction and AOA determination, and different interpretation of data applied by other authors.

Fermentation affected the bioactive constituent (Table 1). In buckwheat extracts TPC increased from 50.7 mg GAE/g dry extract in native unfermented sample to 53.2 mg GAE/g dry extract in extract fermented with *S. cerevisiae* and 59.4 mg GAE/g dry extract in extract fermented with *L. rhamnosus*. In wheat, TPC varied from 16.2 mg GAE/g dry extract to 18.4 and 20.7 mg GAE/g dry extract, respectively in those three samples, and in barley the respective values were 16.4, 18.5 and 20.1 mg GAE/g dry extract. Rye showed the lowest TPC of 13.3 mg GAE/g dry extract in the unfermented sample, 16.2 mg GAE/g d.e. in extract fermented with *S. cerevisiae* and 18.4 mg GAE/g d.e. in extract fermented with *L. rhamnosus*. These results can be explained by the fact that levels of bioactive compounds can be modified during fermentation by the metabolic activity of microbes. Also fermentation-induced structural breakdown of cereal cell walls may occur, leading to the liberation and/or synthesis of various bioactive compounds (Katina, Liukkonen et al., 2007). During fermentation, enzymes such as amylases, xylanases and proteases derived from the grain and microbes contribute to the modification of grain composition (Katina, Laitila et al., 2007; Loponen, Mikola, Katina, Sontag-Strohm, & Salovaara, 2004), and, as mentioned, bound phenolics may be released by enzymatic treatment of samples prior to extraction (Bartolome & Gomez-Cordoves, 1999; Krygier et al., 1982a,b). The type of fermentation clearly determined the degree of modification of the levels of most bioactive compounds in the examined cereals. This might be due to differences in the pH of different fermentations, knowing that optimum pH influences the liberation of cell wall degrading enzymes originating from the cereal kernel (Boskov-Hansen et al., 2002). Other authors have also demonstrated that fermentation has a positive influence on TPC and antioxidative activity of cereals, but the degree of influence depended on microorganism species (Kariluoto et al., 2006). More detailed studies of the changes in microbe populations and the activities of relevant enzymes during fermentation of cereals are required to determine the exact mechanisms causing improved nutritional value of the fermented cereals, especially knowing that the Folin–Ciocalteu method, which was used to determine the total amount of phenolic compounds, is nonspecific and has limitations when used in fermentation studies. In particular, knowing that additional nonphenolic

Table 1
Total phenolics and antioxidant activities of native and fermented plant materials.

Sample name		TPC[A] (mg GAE/g dried extract)*	DPPH[B] (IC_{50}) (μg/ml)*	FRAP[C] (nmol Fe^{2+}/mg dried extract)*	TBARS inhibition[D] (%)*
Buckwheat (Fagopyrum esculentum)	Native	50.7 ± 0.04[a]	76.7	49.43 ± 0.49[a]	45.6 ± 0.55[a]
	Fermented with *L. rhamnosus*	59.4 ± 0.06[b]	63.4	51.54 ± 0.65[a]	50.2 ± 0.45[b]
	Fermented with *S. cerevisiae*	53.2 ± 0.02[c]	66.3	49.76 ± 0.62[a]	49.1 ± 0.85[a]
Barley (Hordeum vulgare)	Native	16.4 ± 0.04[a]	>200	15.56 ± 0.67[a]	50.8 ± 0.75[a]
	Fermented with *L. rhamnosus*	20.1 ± 0.08[b]	>200	20.0 ± 0.54[b]	60.9 ± 0.75[b]
	Fermented with *S. cerevisiae*	18.5 ± 0.09[c]	>200	19.83 ± 0.51[b]	52.4 ± 0.50[a]
Wheat (Triticum durum)	Native	16.2 ± 0.07[a]	>200	12.15 ± 0.60[a]	55.2 ± 0.35[a]
	Fermented with *L. rhamnosus*	20.7 ± 0.06[b]	>200	15.11 ± 0.57[a]	61.7 ± 0.75[b]
	Fermented with *S. cerevisiae*	18.4 ± 0.08[c]	>200	12.25 ± 0.62[a]	56.5 ± 0.70[a]
Rye (Secale cereale)	Native	13.2 ± 0.06[a]	>200	8.94 ± 0.86[a]	57.6 ± 0.45[a]
	Fermented with *L. rhamnosus*	18.4 ± 0.06[b]	196.3	13.94 ± 0.91[a]	62.4 ± 0.60[a]
	Fermented with *S. cerevisiae*	16.2 ± 0.04[c]	>200	10.68 ± 0.83[a]	60.0 ± 0.65[a]

[A] Total phenolics content (TPC) by Folin–Ciocalteu method.
[B] DPPH radical scavenging activity.
[C] Ferric reducing ability of plasma (FRAP).
[D] Thiobarbituric acid method (TAB).
* Values with different superscripts (a, b, c) within each individual cereal species were significant different ($P = 0.05$).

organic substances that react with the Folin–Ciocalteu reagent include adenine, adenosine, alanine, aniline, aminobenzoic acid, ascorbic acid, benzaldehyde, creatinine, cysteine, cytidine, cytosine, dimethyaniline, diphenylamine, EDTA, fructose, guanine, guanosine, glycine, histamine, histidine, indole, methylamine, nitriloacetic acid, oleic acid, phenylthiourea, proteins, pyridoxine, sucrose, sulfanilic acid, thiourea, thymine, thymidine, trimethylamine, tryptophan, uracil, uric acid, and xanthinethe, the amounts of amino acids and organic acids formed during fermentation can give elevated apparent phenolic concentrations (Prior, Xianli, & Schaich, 2005).

3.3. DPPH radical scavenging activity

The DPPH radical has been widely used for assessment of radical scavenging activity because of the ease and convenience of the method. The scavenging effect of plant extracts using the highest sample concentration (200 μg/ml), as shown in Table 2, was weak for the wheat extract, with only 31% DPPH radical inhibition. In other investigations, wheat extracts also demonstrated weak activity in scavenging the DPPH radical at this sample concentration (Yu, Perret, Davy, Wilson, & Melby, 2002). Stronger scavenging effects on the DPPH radical were found for barley and rye (36.6% and 45%, respectively) (Table 2). The results of this work, showing significant DPPH radical inhibition ability for barley, are consistent with data reported by others, apart from differences in data interpretations. Madhujith and Shahidi (2006) demonstrated that barley contained substantial amounts of phenolic antioxidants that effectively scavenged free radicals, especially peroxyl, DPPH, and hydroxyl radicals. The strongest scavenging effects on the DPPH radical were found for buckwheat extract (82.5%) (Table 2). The DPPH radical scavenging effect observed in this work is in agreement with literature data reported by Sun and Ho (2005).

Fermentation with *L. rhamnosus* had a positive influence on DPPH inhibitory effect in each cereal species (Table 2). For buckwheat, scavenging effect of the DPPH radical increased from 82.5% in the unfermented sample to 86.0% in the sample fermented with *L. rhamnosus*, leading to IC_{50} values of 76.7 and 63.4 μg/ml,

Table 2
DPPH radical scavenging activity for native and fermented plants.

Sample name		Inhibition of DPPH radical (%)*				
		s. c.[A] 10 μg/ml	s. c. 20 μg/ml	s. c. 50 μg/ml	s. c. 100 μg/ml	s. c. 200 μg/ml
Buckwheat (Fagopyrum esculentum)	Native	11.6 ± 0.55[a]	21.6 ± 0.50[a]	34.8 ± 0.65[a]	63.3 ± 0.50[a]	82.5 ± 0.45[a]
	Fermented with *L. rhamnosus*	14.7 ± 0.65[a]	23.5 ± 0.70[a]	42.4 ± 0.55[b]	70.1 ± 0.95[b]	86.0 ± 0.45[b]
	Fermented with *S. cerevisiae*	12.6 ± 0.95[a]	22.0 ± 0.85[a]	42.4 ± 0.55[b]	65.4 ± 0.65[a]	85.3 ± 0.55[a]
Barley (Hordeum vulgare)	Native	6.5 ± 0.45[a]	12.5 ± 0.65[a]	17.9 ± 0.75[a]	28.0 ± 0.75[a]	36.6 ± 0.95[a]
	Fermented with *L. rhamnosus*	15.5 ± 0.80[b]	16.1 ± 0.75[a]	23.2 ± 0.45[b]	35.4 ± 0.65[b]	42.9 ± 0.90[b]
	Fermented with *S. cerevisiae*	12.5 ± 0.55[b]	14.3 ± 0.65[a]	22.0 ± 0.80[a]	28.0 ± 0.95[a]	41.7 ± 0.65[a]
Wheat (Triticum durum)	Native	6.7 ± 0.55[a]	12.2 ± 0.60[a]	15.6 ± 0.55[a]	23.4 ± 0.50[a]	31.0 ± 0.65[a]
	Fermented with *L. rhamnosus*	10.1 ± 0.60[a]	15.9 ± 0.85[a]	18.5 ± 0.65[a]	28.2 ± 0.55[b]	35.9 ± 0.55[b]
	Fermented with *S. cerevisiae*	8.2 ± 0.70[a]	13.4 ± 0.60[a]	16.9 ± 0.65[a]	27.4 ± 0.55[b]	34.3 ± 0.45[a]
Rye (Secale cereale)	Native	10.6 ± 0.65[a]	18.9 ± 0.65[a]	25.8 ± 0.55[a]	32.7 ± 0.60[a]	45.0 ± 0.80[a]
	Fermented with *L. rhamnosus*	15.1 ± 0.65[b]	24.6 ± 0.50[b]	31.5 ± 0.55[b]	39.2 ± 0.65[b]	50.4 ± 0.65[b]
	Fermented with *S. cerevisiae*	13.6 ± 0.65[a]	22.2 ± 0.65[a]	30.0 ± 0.95[b]	35.2 ± 0.55[a]	48.8 ± 0.65[a]

[A] Sample concentration.
* Values with different superscripts (a, b) within each individual cereal species were significant different ($P = 0.05$).

respectively (Table 2, Table 1). Similar increase in scavenging of the DPPH radical after fermentations with *L. rhamnosus* was also noticed in rye (45.0% and 50.4%, respectively), barley (36.6% and 42.9%, respectively) and wheat (31.0% and 35.9%, respectively). Fermentation with *S. cerevisiae* had no significant influence on DPPH inhibitory effect in the cereal species examined (Table 2). The inhibition percentages in samples fermented with *S. cerevisiae* were 85.3% for buckwheat (with IC_{50} value 66.3 μg/ml), 48.8% for rye, 41.7% for barley and 34.3% for wheat. These results are in agreement with literature data reported by Moore et al. (2007), who noticed some increase in DPPH radical scavenging activity of wheat after yeast fermentation, using some yeast species, while this ability decreased after other types of yeast fermentation, indicating that yeast preparations themselves might have different DPPH scavenging capacities.

No correlation existed between TPC and DPPH radical scavenging activity in cereals (Table 1). The cereals with higher TPC values were not necessarily better in DPPH inhibition. According to Brand-Williams, Cuvelier, and Berset (1995), ferulic acid, the main phenolic acid in cereal grains, showed a weak antiradical effect in experiments with the DPPH radical, which may explain the discrepancies. Besides, although the Folin–Ciocalteu method is widely used to determine total phenolic contents in botanical and biological samples, it has its own limitations. Other reducing agents, such as L-ascorbic acid and sulphur dioxide, may also react with the Folin–Ciocalteu reagent and contribute to total absorbance, which generally results in overestimated levels of total phenolic contents. In addition, individual phenolic compounds may have different reactivities toward the Folin–Ciocalteu reagent, which could result in potential errors in total phenolic content measurements (Yu, Perret et al., 2002).

Generally, there are several explanations of the ambiguous relationship between the antioxidant activity and total phenolics: (1) Total phenolic content did not include all antioxidants, such as ascorbic acid, carotenoids and tocopherols; (2) The synergism between antioxidants in the mixture made the antioxidant activity not only dependent on antioxidant concentration but on the structure and interactions among antioxidants as well. That is why samples with similar concentrations of total phenolics may vary remarkably in their antioxidant activity; (3) Different methods used for measuring antioxidant activity based on different mechanisms may lead to different observations (Sun & Ho, 2005).

3.4. FRAP method

Ferric reducing antioxidant power (FRAP) of the examined cereals, as shown in Table 1, correlated with total phenolic content. The highest FRAP value, expressed in nmol of Fe^{2+}/mg dry extract, was found in buckwheat (49.43 nmol Fe^{2+}/mg dry extract), followed by lower FRAP in barley and wheat (15.56 and 12.15 nmol Fe^{2+}/mg dry extract, respectively), and the lowest ferric reducing antioxidant ability was found to be in rye (8.94 nmol Fe^{2+}/mg dry extract).

Difficulty in the interpretation at comparison of data is even more obvious with the FRAP method. In our work, the results were expressed in nmol of Fe^{2+}/mg dry extract, however other authors reported their results in nmol or mmol Fe^{2+} in mg or g of grains or flour (Xand & Chang, 2007). Besides, extraction solvents and methods of sample preparation used in other studies were different, and both were shown to have influence on FRAP (Xand & Chang, 2007).

It is interesting that rye, which had significant DPPH radical inhibitory activity, showed the lowest ferric-reducing power (Table 1). It appears that care should be taken when using free radicals as a basis for antioxidant activity tests because the measured antioxidant activity of a biological sample depends on the free radical or oxidant that is being used in assay (Cao, Sofic, & Prior, 1996). Different free radicals should be used and antioxidant score calculated as done by Cao et al. (1996) or should be preferably used the FRAP assay, which is the only assay that directly measures antioxidants or reductants in a sample as done by Halvorsen et al. (2002).

Fermentation with the microorganisms used in our study was not found to have any significant effect on ferric reducing antioxidant power of the cereals examined (Table 1). Only in barley, samples fermented with *L. rhamnosus* showed a higher increase in FRAP than unfermented samples (20.00 nmol Fe^{2+}/mg dry extract compared with 15.56 nmol Fe^{2+}/mg dry extract), while the difference is insignificant in wheat, rye and buckwheat (Table 1). Differences in FRAP values between samples fermented with *S. cerevisiae* and unfermented samples were also insignificant (Table 1). There are insufficient literature data for comparing fermentation influences on FRAP but, as our experiment used an incubation period of 24 h, the data derived are compatible with those reported by Hubert, Berger, Nepveu, Paul, and Daydé (2008), who indicated that the initial ferric-reducing power was maintained for up to 6 h of incubation period, followed by a significant decrease until 48 h of incubation. Further research is needed to evidence the influence of incubation time on of ferric-reducing power for the examined plants.

3.5. Thiobarbituric acid test (TBA)

As shown in Table 1, there is a lack of correlation between TPC and the ability of lipid peroxidation inhibition in cereals. The cereals with higher TPC values were not necessarily better inhibitors of lipid peroxidation. An explanation of this may be in the complex

Table 3
Ability of lipid peroxidation inhibition for native and fermented plants.

Sample name		Inhibition of lipid peroxidation (%)*		
		s. c.[A] 25 μg/ml	s. c. 125 μg/ml	s. c. 250 μg/ml
Buckwheat (Fagopyrum esculentum)	Native	37.4 ± 0.65[a]	40.8 ± 0.65[a]	45.6 ± 0.55[a]
	Fermented with *L. rhamnosus*	41.3 ± 0.50[b]	46.9 ± 0.85[b]	50.2 ± 0.70[b]
	Fermented with *S. cerevisiae*	39.5 ± 0.65[a]	44.5 ± 0.55[b]	49.1 ± 0.95[a]
Barley (Hordeum vulgare)	Native	43.1 ± 0.65[a]	46.3 ± 0.50[a]	50.8 ± 0.75[a]
	Fermented with *L. rhamnosus*	48.4 ± 0.50[b]	52.6 ± 0.75[b]	60.9 ± 0.90[b]
	fermented with *S. cerevisiae*	45.5 ± 0.80[a]	48.1 ± 0.60[a]	52.4 ± 0.50[a]
Wheat (Triticum durum)	Native	43.0 ± 0.55[a]	51.0 ± 0.45[a]	55.2 ± 0.35[a]
	Fermented with *L. rhamnosus*	52.3 ± 0.65[b]	55.2 ± 0.65[b]	61.7 ± 0.70[b]
	Fermented with *S. cerevisiae*	46.6 ± 0.75[a]	53.1 ± 0.65[a]	56.5 ± 0.65[a]
Rye (Secale cereale)	Native	50.5 ± 0.55[a]	53.1 ± 0.95[a]	57.6 ± 0.45[a]
	Fermented with *L. rhamnosus*	55.8 ± 0.50[b]	59.2 ± 0.95[b]	62.4 ± 0.75[b]
	Fermented with *S. cerevisiae*	53.2 ± 0.60[a]	56.8 ± 0.95[a]	60.0 ± 0.60[a]

[A] Sample concentration.
* Values with different superscripts (a, b) within each individual cereal species were significant different ($P = 0.05$).

mechanism of lipid peroxidation inhibition, which includes not only uncompounded phenols, but also high-molecular polyphenols and other nonphenolic antioxidants. According to results, rye had the greatest capacity for inhibition of lipid peroxidation of all cereals examined (57.6%), followed by wheat and barley (55.2% and 50.8%, respectively), while the weakest results in TBA tests were for buckwheat (45.6%) (Table 1). Besides, wheat extracts, for instance, demonstrated a high ability to inhibit lipid peroxidation in liposomes, but showed the lowest ability to directly react with and quench DPPH radical (Table 1). As already mentioned, these data may suggest that it might be more critical to suppress the initiation of radical chain reaction than to terminate it by removing the radicals generated during propagation of radical chain reaction (Yu, Haley et al., 2002).

The results presented in Tables 1 and 3 indicate that fermentation with *L. rhamnosus* affected the capacity for inhibition of lipid peroxidation in cereals, while fermentation with *S. cerevisiae* had no significant effect on that capacity. The percentages of lipid peroxidation inhibition in liposomes were 57.6% in the unfermented sample of rye, 60.0% for the rye sample fermented with *S. cerevisiae* and 62.4% for the rye sample fermented with *L. rhamnosus*. For wheat, those values were respectively 55.2%, 56.5% and 61.7%, for barley 50.8%, 52.4% and 60.9%, respectively, and for buckwheat 45.6%, 49.1% and 50.2%, respectively. There are insuficient literature data for comparing the influence of fermentation on lipid peroxidation inhibition ability. What is evident is that the antioxidant activity of natural antioxidants is very much system-dependent and that a wide range of activities can be observed depending on lipid systems used as substrates (Frankel, Huang, & Aeschbach, 1997).

4. Conclusions

This study indicates that cereals, used widely for human consumption, exhibit significant free radical scavenging activities, ferric-reducing power, capacity for inhibition of lipid peroxidation and total phenolic contents. These factors suggest that cereal-based foods alone could contain important dietary antioxidants and therefore warrant further research to determine whether these dietary antioxidants could be beneficial to human health. Several significant differences were found among the cereals regarding these characteristics, which warrant further study, especially in terms of their effects on human health.

The use of fermentation as a separate processes can enhance the levels of many bioactive compounds in cereals and can be used to improve product properties by changing the ratio of nutritive and antinutritive components of plants. The type of fermentation clearly had effect on potentially bioactive constituents of cereals, by determining the degree of modification of most bioactive compounds. However, more detailed studies of microorganism population changes and activities of relevant enzymes during fermentation of cereals are required in order to establish precise mechanisms causing fermented cereals to improve their nutritional value. More research is needed to adequately clarify the composition of extracts of fermented cereals, to identify the antioxidant compounds in those extracts, and to evaluate the potential use of cereal products as natural antioxidants and, consequently, as food supplements. In addition, identification of both biological (e.g., digestion) and food processing conditions that impact the distribution, stability and activity of cereal antioxidants is needed in order to be able to produce food with maximum health benefits. Thus, natural bioactivity of wholemeal cereals can be further increased by using tailored bioprocess to produce nutritionally superior cereal meal which can be used as a quality improver in breads, breakfast cereals and snack foods. In conclusion, it seems advantageous to select microorganism starter cultures for fermentation of cereals based on their positive correlation with total antioxidant capacity.

References

Adom, K., & Liu, R. (2002). Antioxidant activity of grains. *Journal of Agricultural and Food Chemistry, 50*, 6182–6187.

Afanas'ev, I. B., Dorozhko, A. I., Brodskii, A. V., Kostyuk, V. A., & Potapovitch, A. I. (1989). Chelating and free radical scavenging mechanisms of inhibitory action of rutin and quercetin in lipid peroxidation. *Biochemical Pharmacology, 38*(11), 1763–1769.

Bartolome, B., & Gomez-Cordoves, C. (1999). Barley spent grain: release of hydroxycinnamic acids (ferulic and p-coumaric acids) by commercial enzyme preparations. *Journal of the Science of Food and Agriculture, 79*, 435–439.

Baublis, A., Decker, E. A., & Clydesdale, F. M. (2000). Antioxidant effects of aqueous extracts from wheat-based ready-to-eat breakfast cereals. *Food Chemistry, 68*, 1–6.

Bedford, M. R. (1995). Mechanism of action and potential environmental benefits from the use of feed enzymes. *Animal Feed Science and Technology, 53*, 145–155.

Boskov-Hansen, H., Andersen, M. F., Nielsen, L. M., Back-Knudsen, K. E., Meyer, A. S., Christensen, L. P., et al. (2002). Changes in dietary fibre, phenolic acids and activity of endogenous enzymes during rye bread making. *European Food Research and Technology, 214*, 33–42.

Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *Lebensmittel-Wissenschaft und Technologie, 28*, 25–30.

Branen, A. L. (1975). Toxicology and biochemistry of butylated hydroxyanisole and butylated hydroxytoluene. *Journal of the American Oil Chemists Society, 52*(2), 59–63.

Cao, G., Sofic, E., & Prior, R. L. (1996). Antioxidant capacity of tea and common vegetables. *Journal of Agricultural and Food Chemistry, 44*, 3426–3431.

Chesson, A. (1987). Supplementary enzymes to improve the utilization of pig and poultry diets. In D. C. Wiseman, P. C. Garnsworthy, & J. Wiseman (Eds.), *Recent advances in animal nutrition* (pp. 71–89). Butterworths, London: UK.

Cuendet, M., Hostettmann, K., & Potterat, O. (1997). Iridoid glucosides with free radical scavenging properties from *Fagraea blumei*. *Helvetica Chimica Acta, 80*, 1144–1152.

Emmons, C. L., Peterson, D. M., & Paul, G. L. (1999). Antioxidant capacity of oat (*Avena sativa* L.) extracts. 2. In vitro antioxidant activity and contents of phenolic and tocol antioxidants. *Journal of Agricultural and Food Chemistry, 47*, 4894–4898.

Frankel, E. N., Huang, S. W., & Aeschbach, R. (1997). Antioxidant activity of green teas in different lipid systems. *Journal of the American Oil Chemists Society, 74*, 1309–1315.

Guenter, W. (1997). Phytases in cereals and hemicelluloses in canola (rapeseed) meal and lupins. In R. R. Marquardt & Z. Han (Eds.), *Enzymes in poultry and swine nutrition* (pp. 99–114). Ottawa, Canada: International Development Research Center.

Halvorsen, B. L., Holte, K., Myhrstad, M. C. W., Barikmo, I., Hvattum, E., Fagertun Remberg, S., et al. (2002). A systematic screening of total antioxidants in dietary plants. *Journal of Nutrition, 132*, 461–471.

Heiniö, R.-L., Katina, K., Wilhelmson, A., Myllymäki, O., Rajamäki, T., Latva-Kala, K., et al. (2003). Relationship between sensory perception and flavour-active volatile compounds of germinated, sourdough fermented and native rye following the extrusion process. *Lebensmittel-Wissenschaft und Technologie, 36*, 533–545.

Hubert, J., Berger, M., Nepveu, F., Paul, F., & Daydé, J. (2008). Effects of fermentation on the phytochemical composition and antioxidant properties of soy germ. *Food Chemistry, 109*, 709–721.

Kariluoto, S., Aittamaa, M., Korhola, M., Salovaara, H., Vahteristo, L., & Piironen, V. (2006). Effects of yeasts and bacteria on the levels of folates in rye sourdoughs. *International Journal of Food Microbiology, 106*(2), 137–143.

Katina, K., Laitila, A., Juvonen, R., Liukkonen, K-H., Kariluoto, S., Piironen, V., et al. (2007). Bran fermentation as a means to enhance technological properties and bioactivity of rye. *Food Microbiology, 24*, 175–186.

Katina, K., Liukkonen, K.-H., Kaukovirta-Norja, A., Adlercreutz, H., Heinonen, S.-M., Lampi, A.-M., et al. (2007). Fermentation-induced changes in the nutritional value of native or germinated rye. *Journal of Cereal Science, 46*, 348–355.

Krygier, K., Sosulski, F., & Hodge, L. (1982a). Free, esterified, and insoluble bound phenolic acids. 1. Extraction and purification procedure. *Journal of Agricultural and Food Chemistry, 30*, 330–334.

Krygier, K., Sosulski, F., & Hogge, L. (1982b). Free, esterified and insoluble bound phenolic acids. 2. Composition of phenolic acids in rapeseed flour and hulls. *Journal of Agricultural and Food Chemistry, 30*, 334–336.

Liyana-Pathirana, C. M., Dexter, J., & Shahidi, F. (2006). Antioxidant properties of wheat as affected by pearling. *Journal of Agricultural and Food Chemistry, 54*, 6177–6184.

Liyana-Pathirana, C. M., & Shahidi, F. (2006). Antioxidant properties of commercial soft and hard winter wheats (*Triticum aestivum* L.) and their milling fractions. *Journal of the Science of Food and Agriculture, 86*, 477–485.

Loponen, J., Mikola, M., Katina, K., Sontag-Strohm, T., & Salovaara, H. (2004). Degradation of HMW glutenins during wheat sourdough fermentations. *Cereal Chemistry, 81*, 87–90.

Madhujith, T., & Shahidi, F. (2006). Optimization of the extraction of antioxidative constituents of six barley cultivars and their antioxidant properties. *Journal of Agricultural and Food Chemistry, 54*, 8048–8057.

Madhujith, T., & Shahidi, F. (2007). Antioxidative and antiproliferative properties of selected barley (*Hordeum vulgarae* L.) cultivars and their potential for inhibition of low-density lipoprotein (LDL) cholesterol oxidation. *Journal of Agricultural and Food Chemistry, 55*, 5018–5024.

Miller, H. E., Rigelhof, F., Marquart, L., Prakash, A., & Kanter, M. (2000). Whole-grain products and antioxidants. *Cereal Food World, 45*, 59–63.

Moore, J., Cheng, Z., Hao, J., Guo, G., Liu, J. G., Lin, C., et al. (2007). Effects of solid-state yeast treatment on the antioxidant properties and protein and fiber compositions of common hard wheat bran. *Journal of Agricultural and Food Chemistry, 55*, 10173–10182.

Naczk, M., & Shahidi, F. (1989). The effect of methanol-ammonia-water treatment on the content of phenolic acids of canola. *Food Chemistry, 31*, 159–164.

Prior, R. L., Xianli, W., & Schaich, K. (2005). Standardized methods for the determination of antioxidant capacity and phenolics in food and dietary supplements. *Journal of Agricultural and Food Chemistry, 53*, 4290–4302.

Senter, S. D., Horvat, R. J., & Forbus, W. R. (1983). Comparative GLCMS analysis of phenolic acids of selected tree nuts. *Journal of Food Science, 48*, 798–803.

Shahidi, F. (2000). Antioxidants in food and food antioxidants. *Nahrung, 44*, 158–163.

Singleton, V. L., & Rossi, J. A. (1956). Colorimetry of total phenolics with phosphomolybdic–phosphotungstic acid reagent. *American Journal of Enology and Viticulture, 16*, 144–158.

Sun, T., & Ho, C-T. (2005). Antioxidant activities of buckwheat extracts. *Food Chemistry, 90*, 743–749.

Szőllősi, R., & Szőllősi Varga, I. (2002). Total antioxidant power in some species of Labiate (Adaptation of FRAP method). *Acta Biologica Szegediensis, 46*(3–4), 125–127.

Velioglu, Y. S., Mazza, G., Gao, L., & Oomah, B. D. (1998). Antioxidant activity and total phenolics in selected fruits, vegetables and grain products. *Journal of Agricultural and Food Chemistry, 46*, 4113–4117.

Xand, B. J., & Chang, S. K. C. (2007). A comparative study on phenolic profiles and antioxidant activity of legumes as affected by extraction solvents. *Journal of Food Science, 72*(2), 159–166.

Yu, L., Haley, S., Perret, J., & Harris, M. (2002). Antioxidant properties of hard winter wheat extracts. *Food Chemistry, 78*, 457–461.

Yu, L., Perret, J., Davy, B., Wilson, J., & Melby, C. L. (2002). Antioxidant Properties of Cereal Products. *Journal of Food Science, 67*(7), 2600–2603.

Zhou, K., & Yu, L. (2004). Effects of extraction solvent on wheat bran antioxidant activity estimation. *Lebensmittel-Wissenschaft und Technologie, 37*, 717–721.

Zielinski, H., & Troszynska, A. (2000). Antioxidant capacity of raw and hydrothermal processed cereal grains. *Polish Journal of Food and Nutrition Sciences, 9*(50), 79–83.