QBS
Vol. 36, No. 1, pp. 51-55 (2017)
https://doi.org/10.22283/qbs.2017.36.1.51
pISSN 2288-1344 eISSN 2508-7185 **Original Article**

Quantitative Bio-Science
Official Journal of the Institute of Natural Science at Keimyung University
Available Online at https://qbs.kmu.ac.kr:442/

Free Radical Scavenging Effect of Added Ferulic Acid on Wheat Flour Products

Perry K.W. Ng[1], Bong-Kyung Koh[2,*]

[1]Department of Food Science and Human Nutrition, Michigan State University, East Lansing, MI 48824, USA
[2]Department of Foods and Nutrition, Keimyung University, Daegu 42601, Korea
(Received February 17, 2017; Revised May 9, 2017; Accepted May 10, 2017)

ABSTRACT

The free radical scavenging effects of baked and extruded wheat flour products were determined. Ferulic acid was selected as a model phenolic compound and mixed with wheat flour at a level of 250 μg/g based on the flour weight. Free radical scavenging activity (FRSA) against the 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) was determined. In the baking process, the most predominant change in activity was observed during fermentation, where increases in FRSA were observed in both fermented doughs with and without added ferulic acid. In contrast, the FRSA of the baked bread crumb decreased, despite the addition of ferulic acid. Additionally, FRSA differed with the cooking method. Cookies showed the highest FRSA because of the Maillard products. In bread, there was a large increase in FRSA following the addition of ferulic acid, and bread crumb was the most effective in retaining the free radical activity of the added ferulic acid.

Key words : Baked and extruded wheat flour products, Ferulic acid, Free radical scavenging effect

Introduction

Free radicals are factors that activate chemical reactions in the body, causing numerous degenerative diseases. Antioxidants such as vitamins C and E, β-carotene, flavonoids, and polyphenols that are found in grains, vegetables, and fruit may scavenge free radicals, prevent various diseases, and promote good health [1]. Wheat can be a major dietary source of antioxidants, and several studies have analyzed the antioxidant potential of wheat [2-10]. However, most active antioxidant components are in the bran; thus, attempts have been made to fortify wheat flour products with antioxidants [11-18].

Most studies of antioxidant activity have focused on the fresh tissues of plants. A few studies have examined the effects of different cooking and processing methods on antioxidant properties [11,12,14-16,19,20-24]. Processed fruits and vegetables are considered to have lower nutritional values than fresh produce because of the loss of vitamin C during processing. However, various antioxidant components show different stabilities against heat and oxidation. Dewanto et al. [20] reported that heat processing of sweet corn decreased its vitamin C content, but increased free ferulic acid, increasing the total antioxidant activity of the corn. The present study focused on changes in the antioxidant effects of wheat flour products before and after cooking. Ferulic acid was selected as a model antioxidant and was added to baked and extruded wheat flour products. Changes in free radical scavenging activity (FRSA) and free ferulic acid contents before and after cooking were then examined.

* Correspondence should be addressed to Dr. Bong Kyung Koh, Department of Food and Nutrition, Keimyung University, 1095 Dalgubeol-daero, Dalseo-Gu, Daegu 42601, Korea. Tel: +82-53-580-5876, Fax: +82-53-580-5885, E-mail: kohfood@kmu.ac.kr

Materials and Methods

1. Materials

Commercially milled untreated soft and hard wheat flours were selected. The hard wheat flour for bread-baking was obtained from Mennel Milling Co. (Fostoria, OH, USA) and contained 12.84% protein (N × 5.74) and 11.90% moisture. Soft wheat pastry flour for crackers and cookies was obtained from the Mennel Milling Co. (Fostoria, OH, USA). It contained 8.18% protein and 11.6% moisture. Ferulic acid and DPPH (1,1-diphenyl-2-picryhydrazyl) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Ingredients of bread, cookies, crackers, and extrudates are shown at Table 1.

Table 1. Ingredients of wheat flour-based products

Products	Ingredients
Bread	Hard wheat flour, Yeast, Shortening, Salt, Sugar, Water
Cookie	Soft wheat flour, White & brown sugar, Non-fat dry milk, Salt, Sodium bicarbonate, Ammonium bicarbonate, Fructose, Water
Cracker	Soft wheat flour, Yeast, Sodium bicarbonate, Shortening, Salt, Water
Extrudate	Hard wheat flour, Water

2. Sequential preparation of dough and bread

Hard wheat flour dough was prepared following the optimized straight-dough bread-making formula of the American Association of Cereal Chemists (AACC) Method 10-10B [25] without the addition of ascorbic acid. The formula for the control dough calculated based on wheat flour weight (baker's %) was as follows: flour, 100%; water, 65%; sugar, 6%; salt, 1.5%; shortening, 3%; yeast, 1%. Ferulic acids were added at a concentration of 250 μg/g of flour. This level of added ferulic acid was determined to induce rapid breakdown of hard wheat flour dough by mixograph.

The dough was divided into three portions. The first portion was immediately lyophilized and the second was fermented and immediately lyophilized. The third portion was fermented, baked, and immediately lyophilized. The lyophilized un-fermented dough, fermented dough, and bread crumb were analyzed immediately after grinding and sieving through a 60-mesh screen.

3. Preparation of cookies and crackers

The preparation and baking of the cookie dough followed AACC methods 10-54 [25] using soft wheat pastry flour. The crackers were prepared in a modified one-stage fermentation method [26] using soft wheat pastry flour as shown at Table 1. Ferulic acid was mixed with the flour at a level of 250 μg/g based on the flour weight of the baked product formula. After baking, the cookies and crackers were cooled for 2 hr at ambient temperature, sealed in plastic bags, and frozen until analysis. The samples were analyzed immediately after grinding and sieving through a 60-mesh screen. All analyses were conducted within three days after baking.

4. Extrusion cooking

Hard wheat flour extrudates were produced using a laboratory-scale co-rotating and intermeshing twin-screw extruder (MP19TC-25, APV Baker Inc., Grand Rapids, MI, USA). The barrel diameter and its length to diameter ratio were 19.0 mm and 25 : 1, respectively. The flour and 250 μg/g of ferulic acid were dry-mixed in a mixer (N50, Hobart, Northyork, ON, Canada) for 15 min. Process variables were selected to provide optimum wheat flour expansion and minimize inactivation of ferulic acid based on ferulic acid's melting point (174°C). The total process moisture was controlled to 30% and barrel temperature profiles were maintained at 40, 60, 80, 110, and 125°C for zones 1, 2, 3, 4, and 5, respectively. The extruded products were immediately placed in a forced-air drier (Blue M Electric, Blue Island, IL, USA) and dried at 50°C for 3 hr and stored in a freezer. Analyses were completed within three days after extrusion; the samples were ground and sieved through a 60-mesh screen and immediately analyzed.

5. Radical scavenging activity using the 1,1-diphenyl-2-picryhydrazyl (DPPH) method

Antioxidant activity was determined using the DPPH method as described by Yu et al. [8] with some modifications. The freshly ground sample (1 g) was dispersed in 10 mL of methanol and sonicated for 2 hr with occasional vortexing without light and air. The extract was then filtered through filter paper (Whatman paper, #1). The methanol extract (1 mL) and 1 mL of a freshly prepared 1 mM DPPH methanolic solution were thoroughly mixed and stored in the dark for 2 hr. The absorbance of the reaction mixture was read at 517 nm with a spectrophotometer (UltrospecIII, Pharmacia-LKB, Uppsala, Sweden). The blank was prepared by replacing the extract with methanol (1 mL). Radical scavenging activity was calculated as follows:

% radical scavenging activity = [(absorbance blank − absorbance sample)/absorbance blank] × 100.

Scavenging activity was calculated based on the product moisture content.

6. HPLC determination of free-ferulic acid

6.1 Ferulic acid extraction

Freshly ground sample (1 g) was added to a 20-mL capped tube with 10 mL *n*-hexane, shaken for 30 min (60°C), and filtered (Whatman paper #1) to remove the fats in the sample. The defatting process was repeated twice and the residue was dried under a hood. Defatted sample powder was dispersed in 10 mL methanol and sonicated for 2 hr with occasional shaking without light and air. The extract was filtered through filter paper (Whatman paper, #1). Methanol extract was filtered through a 0.45-μm nylon filter and analyzed by high-performance liquid chromatography (HPLC) [2,27].

6.2 HPLC analysis

Chromatography was performed on an HPLC workstation consisting of a Waters 600E multisolvent delivery system (Millipore, Billerica, MA, USA) and Waters 470 scanning fluorescence detector (Millipore), which was operated with an excitation wavelength of 312 nm and filter passing wavelength beyond 418 nm on the emission side of the flow cell. Separation was performed using a Phenomenex (Torrance, CA, USA) reverse phase C18 column (250 × 4.6 mm, packed with ODS-Hypersil) connected to a guard column (4 × 3 mm, packed with ODS-Hypersil, Phenomenex). The elution profile of the mobile phase was a 1 mL/min linear gradient from 5% A : 95% B to 30% A : 70% B, over 35 min. Solvent A consisted of 0.1% (v/v) trifluoracetic acid in acetonitrile; solvent B was 0.1% (v/v) trifluoracetic acid in HPLC-grade water. The flow rate was 1 mL/min at ambient temperature and samples were loaded every 1 hr. A ferulic acid standard was run with each sample. The chromatograms were automatically analyzed using Nelson Analytical software (version 4.1, Manchester, NH, USA).

7. Data analysis

The data were obtained by random samples of five measurements from each wheat flour product. All processing methods are obviously different by nature, and thus all data are just summarized by the mean and standard deviation.

Results and Discussion

1. FRSA during the baking process

The FRSAs determined at each step of the bread baking process, with and without the addition of ferulic acid, are shown in Table 2. The wheat flour extract showed an FRSA of 38%, while the mixed dough showed an activity of 34%. During the baking process, the most predominant change in activity occurred in the fermented dough. After fermentation, the FRSA was increased to 55.6%. The FRSA of baked bread crumb was higher than that of unfermented dough and lower than that of fermented dough. This increase in fermentation may be explained by the fermentation process during which bound antioxidant components are hydrolyzed and released as free forms, which are then active to scavenge free radicals [28] and increase the FRSA of fermented dough. It has been reported that bound antioxidants are also released by sprouting, and an increase in free antioxidants and FRSA was observed in sprouted cereal [29]. During the baking process, high temperatures may affect the activities of free radical-scavenging compounds, causing the FRSA of the bread crumb to decrease compared to that of fermented dough. However, the baked bread crumb showed some degree of FRSA. FRSA of the bread can be explained by the Maillard reaction products [19,30]; previously, Maillard reaction products were shown to lower lipid oxidation in a flour-lipid dough mixture [31].

Table 2. Free radical scavenging activities (%) of hard wheat flour, dough, and bread with and without ferulic acid

	Control	Ferulic acid added
Flour	38.1 ± 6.5	
Dough	34.2 ± 10.8	76.5 ± 11.8
Fermented dough	55.6 ± 1.4	92.6 ± 2.1
Baked bread	41.5 ± 3.4	78.2 ± 6.2

Free radical scavenge activity = [(abs blank − abs sample)/abs blank] × 100.
Equal amounts (250 μg/g) of ferulic acid were added.

The addition of 250 μg/g of ferulic acid increased the FRSA of the un-fermented and fermented doughs, as well as the baked bread crumb, compared to the FRSA of the control products (Table 2). The control showed a similar pattern of increasing FRSA as the products with added of ferulic acid, where the fermented dough showed the highest activity, and the activity of the baked bread crumb decreased compared to that of the dough.

2. FRSA of baked or extruded products

Changes in the FRSA for different cooking methods are shown in Table 3. All processing methods are obviously different by nature, and thus all data are just summarized by the mean and standard deviation. The control cookies showed the highest FRSA compared to any other product. This may be

Table 3. Free radical scavenging activities (%) of wheat flour products

	Control	Ferulic acid added
Hard wheat flour	38.1±6.5	
Soft wheat flour	33.9±2.1	
Bread	41.5±3.4	78.2±6.2
Cookie	87.6±8.2	87.2±7.0
Cracker	31.0±1.2	39.3±1.0
Extrudate	16.4±1.5	26.8±2.2

Free radical scavenge activity = [(abs blank − abs sample)/abs blank] × 100.
Equal amounts (250 μg/g) of ferulic acid were added.

Table 4. Free form ferulic acid content (μg/g) of cereal products

Flour	Products	Control	Ferulic acid added
Hard wheat	Flour	2.8±0.3	
	Bread	4.9±0.8	72.9±9.1
	Extrudate	3.8±0.0	45.83±5.5
Soft wheat	Flour	0.9±0.0	
	Cookie	2.8±0.4	65.1±10.7
	Cracker	1.8±0.0	12.9±0.5

Equal amounts (250 μg/g) of ferulic acid were added.

because of the Maillard reaction products in the cookies [19,29]. The bread crumb, crackers, and extrudate showed little or no browning (data not shown). Particularly, the extrudate had no Maillard color development, and its FRSA was rather low compared to the hard wheat flour.

The increase in FRSA by adding ferulic acid was large in the bread. The differences in FRSA with and without the addition of ferulic acid were small among the cracker or cookie products, and these differences were lower than in the other products. The thin and small shapes of the cookies and crackers may have allowed for more direct heat contact, causing a decrease in FRSA although they contained ferulic acid.

3. Residual free ferulic acid content

Ferulic acid contents of flour and products are shown in Table 4. Before processing, the concentration of ferulic acid in hard wheat flour was 2.8 μg/g. Hard wheat flour contained more endogenous ferulic acid than soft wheat flour. Bread contained more free ferulic acid than hard wheat flour, which may have been released from the bound ferulic acid during fermentation. The baking process affected the residual free phenolic acid content of the flour. Residual ferulic acid content was slightly increased to 4.9 μg/g in bread supplemented with ferulic acid. In general, phenolic acids are known to be susceptible to heat, which causes their oxidation and degradation [32]. However, our data showed that the residual ferulic acid content after baking at high temperature was increased rather than decreased. A previous study [33] showed a recovery rate of 73.8∼80.9% of added phenolic acids after baking. This result suggests that bread retains some of its antioxidant activity despite the baking process. Cracker contained a lower amount of free ferulic acid compared to cookie and bread. Cracker showed higher FRSA than the extrudate, as shown in Table 3, but the free ferulic acid content of cracker was lower than that in the extrudate.

Conclusions

Antioxidants are added to food because of their potential health benefits in reducing free radicals. However, some active compounds of antioxidants are sensitive to heat and oxygen, and determining their activity after cooking is difficult. When ferulic acid was added to baked and extruded wheat flour products in this study, the baked bread crumb was most effective in retaining the FRSA of the compound. In contrast, the cookies and crackers were not effective in retaining the FRSA of added ferulic acid. During the bread-baking process, fermentation increased the FRSA of the dough. Residual ferulic acid content after baking at high temperature increased rather than decreased and some of the added ferulic acid remained after baking.

References

1. Temple NJ. Antioxidant and disease: more questions than answers. Nutr Res 2000;20:449-459.
2. Hatcher DW, Kruger JE. Simple phenolic acids in flours prepared from Canadian wheat: relationship to ash content, color, and polyphenol oxidase activity. Cereal Chem 1997;74:337-343.
3. Adom KK, Liu RH. Antioxidant activity of grains. J Agric Food Chem 2002;50:6182-6187.
4. Beta T, Nam S, Dexter JE, Sapirsteini HD. Phenolic content and antioxidant activity of pearled wheat and roller-milled fractions. Cereal Chem 2005;82:390-393.
5. Li W, Shan F, Sun S, Corke H, Beta T. Free radical scavenging properties and phenolic content of Chineses black-grained wheat. J Agric Food Chem 2005;53:8533-8536.
6. Moore J, Hao Z, Zhou K, Luther M, Costa J, Yu L. Carotenoid, tocopherol, phenolic acid, and antioxidant properties of Maryland-grown soft wheat. J Agric Food Chem 2005;53:6649-6657.
7. Perez-Jimenez J, Saura-Calixto F. Literature data may underestimate the actual antioxidant capacity of cereals. J Agric Food Chem 2005;53:5036-5040.

8. Yu L, Haley S, Perret J, Harris M, Wilson J, Qian M. Free radical scavenging properties of wheat extracts. J Agric Food Chem 2002;50:1619-1624.
9. Zhou K, Laux JJ, Yu L. Comparison of swiss red wheat grain and fractions for their antioxidant properties. J Agric Food Chem 2004;52:1118-1123.
10. Zhou K, Yin JJ, Yu L. Phenolic acid, tocopherol and carotenoid compositions, and antioxidant functions of hard red winter wheat bran. J Agric Food Chem 2005;53:3916-3922.
11. Hung TT, Seib PA, Kramer KJ. Determination of L-ascorbyl 6-palmitate in bread making using reverse-phase high performance liquid chromatography with electrochemical detection. J Food Sci 1987;52:948-953.
12. Rogers DE, Malouf RB, Langemeier J, Gelroth JA, Ranhotra GS. Stability and nutrient contribution of beta-carotene added to selected bakery products. Cereal Chem 1993;70:558-561.
13. Hix DK, Klopfenstein CK, Walker CE. Physical and chemical attributes and consumer acceptance of sugar-snap cookies containing naturally occurring antioxidants. Cereal Chem 1997;74: 281-283.
14. Park H, Seib P, Chung OK, Seitz LM. Fortifying bread with each of three antioxidants. Cereal Chem 1997;74:202-206.
15. Ranhotra GS, Gelroth JA, Okot-Kotber BM. Stability and dietary contribution of vitamin E added to bread. Cereal Chem 2000;77:159-162.
16. Hsu CL, Hurang S, Chen W, Weng Y, Tseng CY. Qualities and antioxidant properties of bread as affected by the incorporation of yam flour in the formulation. Int J Food Sci Tech 2004;39: 231-238.
17. Yun JM, Lee HL, Hwang JK. Activation of ferulic acid in rice bran by extrusion and polysaccharidase treatment. Food Sci Biotechnol 2004;13:523-524.
18. Jeong YJ, Woo SM. Effect of germinated brown rice concentrate on free amino acid levels and antioxidant and nitrite scavenging activity in *Kimchi*. Food Sci Biotechnol 2006;15:351-356.
19. Jing H, Kitts DD. Comparison of the antioxidative and cytotoxic properties of glucose-lysine and fructose-lysine Maillard reaction products. Food Res Int 2000;33:509-516.
20. Dewanto V, Wu X, Liu RH. Processed sweet corn has higher antioxidant activity. J Agric Food Chem 2002;50:4959-4964.
21. Zhang D, Hamauzu Y. Phenolics, ascorbic acid, carotenoids and antioxidant activity of broccoli and their changes during conventional and microwave cooking. Food Chem 2004;88: 503-509.
22. Gorinstein S, Drzewiecki J, Leontowicz H, Leontowicz M, Najman K, Jastrzebski Z, et al. Comparison of the bioactive compounds and antioxidant potentials of fresh and cooked polish, Ukrainian, and Israeli garlic. J Agric Food Chem 2005;53: 2726-2732.
23. Lin CH, Chang CY. Textural change and antioxidant properties of broccoli under different cooking treatments. Food Chem 2005;90:9-15.
24. Turkmen N, Sari F, Velioglu S. The effect of cooking methods on total phenolics and antioxidant activity of selected green vegetables. Food Chem 2005;93:713-718.
25. American Association of Cereal Chemists. Approved methods of the AACC, Methods 10-10B, 10-54, and 54-40A. 10th ed. St. Paul (MN): The Association; 2000.
26. Lee L, Ng PKW, Steffe JF. A modified procedure (one-stage fermentation) for evaluating flour cracker-making potential. Food Eng Prog 2002;6:201-207.
27. Pusssayanawin, V, Wetzel DL, Fulcher RG. Fluorescence detection and measurement of ferulic acid in wheat milling fractions by microscopy and HPLC. J Aagric Food Chem 1988;36:515-520.
28. Saulnier L, Marot C, Elgorriaga M, Bonnin E, Thibault JF. Thermal and enzymatic treatments for the release of free ferulic acid from maize bran. Carbohydr Polym 2001;45:269-275.
29. Purnama M, Ser WY, Kyaw NS, Arntfield SD, Beta T. Antioxidant activities of cereal sprouts. AACC Internal annual meeting. 2005.
30. Borrellia RC, Mennellaa C, Barba F, Russo M, Russo GL, Krome K, et al. Characterization of coloured compounds obtained by enzymatic extraction of bakery products. Food Chem Toxicol 2003;41:1367-1374.
31. Wijewickreme AN, Kitts DD. Oxidative reactions of model Maillard reaction products and α-tocopherol in a flour-lipid mixture. J Food Sci 1998;63:466-471.
32. Leenhardt F, Lyan B, Rock E, Boussard A, Potus J, Chanliaud E. Wheat lipoxygenase activity induces greater loss of carotenoids than vitamin E during breadmaking. J Agric Food Chem 2006;54:1710-1715.
33. Han HM, Koh BK. Antioxidant activity of hard wheat flour, dough and bread prepared using various processes with the addition of different phenolic acids. J Sci Food Agric 2011;91: 604-608.