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Comparison of irradiated phytic acid and other antioxidants for antioxidant activity

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Abstract

A comparative study was undertaken to evaluate the antioxidant activities of irradiated phytic acid and commonly used antioxidants, including ascorbic acid, tocopherol and butylated hydroxyl anisole (BHA). Phytic acid was irradiated at 0, 10 and 20 kGy, and, after irradiation, radiolytic degradation and increments of antiradical and antioxidant activity were observed. Phytic acid irradiated at 20 kGy, showed significantly higher DPPH radical-scavenging capacity than ascorbic acid at the 800 μM level, while a scavenging effect was not seen in non-irradiated phytic acid ($P < 0.05$). Ferric reducing/antioxidant power (FRAP) of phytic acid was significantly increased by irradiation; however, ascorbic acid, tocopherol and BHA showed higher FRAP values than phytic acid. Antioxidant activity of phytic acid in the lipid models was higher than that of the other antioxidants during storage and, especially phytic acid (800 μM) irradiated at 20 kGy showed the highest antioxidative ability among the antioxidants tested at 3 weeks of storage.

Keywords: Irradiation; Phytic acid; Antioxidant; DPPH; FRAP

1. Introduction

Phytic acid (myo-inositol hexaphosphate, IP_6) is widely found in cereals, nuts, legumes, oil seeds, pollen, and spores, constituting 1–5% (Graf & Eaton, 1990). Phytic acid is historically considered to be an antinutrient. Structurally, phytic acid contains phosphorus, which binds minerals such as calcium, iron, and zinc, causing a decrease of their bioavailability in human and animal models (Reddy, Pierson, Sathe, & Salunkhe, 1989). However, recently, phytic acid has been reported to be an antioxidant (Graf & Eaton, 1990), anticarcinogenic (Shamsuddin, Vucenik, & Cole, 1997), and hypoglycemic or hypolipidemic (Rickard & Thompson, 1997). Phytic acid is considered to be an antioxidant, because it is a potent inhibitor of iron-catalyzed hydroxyl radical formation by chelating the free iron and then blocking its coordination site (Graf & Eaton, 1990). Epidemiological studies have shown lower incidence of colon cancer in populations consuming a vegetarian-type diet; however, the mechanism is still unclear (Shamsuddin et al., 1997). Furthermore, lower inositol phosphates, such as IP_4 and IP_3, may play roles in mediating cellular responses and have been noted as having a function in second messenger transduction systems (Berridge & Irvine, 1989). In phytic acid-rich foods, trials for reducing phytic acid, including physical or chemical processing, genetic manipulation or enzymatic hydrolysis have been performed (Harland & Harland, 1980; Siddhuraju, Makkar, & Becker, 2002). Actually, some cereals, including corn, barley, and rice mutants, have been developed that have significantly lower levels of phytic acid without reducing the total phosphorus, and these should prove valuable for swine and poultry feed ingredients (Larson, Rutger, Young, & Raboy, 2000).

Gamma irradiation is well known as the best method for eliminating pathogenic and spoilage microorganisms without compromising the nutritional properties and sensory quality of foods, and its use has gradually been

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increasing worldwide (WHO, 1999). Additionally, new trials for the purpose of reducing toxic or hazardous compounds such as volatile *N*-nitrosamines (Ahn et al., 2002; Ahn, Kim, Jo, Lee, & Byun, 2002), biogenic amines (Kim et al., 2003), allergenicity of foods (Lee et al., 2001), or for developing a new processing procedure, such as the production of low-salt fermented foods (Byun et al., 2000) have been reported. Reduction of antinutritional compounds by irradiation has also been reported (Duodu, Minnaar, & Taylor, 1999; Sattar, Neelofar, & Akhtar, 1990). Duodu et al. (1999) reported that irradiation reduced phytic acid levels in foods. However, phytic acid in cereals plays an important role in inhibiting oxidation during preservation or storage (Peterson, 2001). Therefore, both reduction of phytic acid contents and antioxidation properties are important starting points in developing methods for reducing the phytic acid levels in foods. A previous study demonstrated that irradiation induced the radiolysis of phytic acid, while antiradical and antioxidant activities were increased (Ahn, Kim, Yook, & Byun, 2003). Therefore, this study was designed to compare the antioxidative activity of irradiated phytic acid with those of other antioxidants, which are commonly used in the food industry.

The purpose of this study was to evaluate the antiradical and antioxidant activity of phytic acid after irradiation, compared with ascorbic acid, tocopherol and BHA.

2. Materials and methods

2.1. Sample preparation

Extra pure phytic acid sodium salt, L-ascorbic acid, DL-α-tocopherol and BHA (butylated hydroxyl anisole) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Phytic acid sodium salt and ascorbic acid were dissolved in deionized distilled water, and tocopherol and BHA were prepared in ethanol at final concentrations of 800, 400, 200 and 100 μM, respectively. Phytic acid sodium salt is soluble in water and does not require conversion to the free phytic acid form. The phytic acid solution was transferred into a 50 ml glass tube and then irradiated in a cobalt-60 irradiator (Nordion International, Ottawa, Ontario, Canada). The source strength was ca. 100 kCi with a dose rate of 5 kGy h^{-1} at 11 ± 0.5 °C. Dosimetry was performed using 5 mm diameter alanine dosimeters (Bruker Instruments, Rheinstetten, Germany), and the free-radical signal was measured using a Bruker EMS 104 EPR Analyzer. The absorbed doses in this study were 0, 10 and 20 kGy, and the actual doses were within ±2% of the target dose.

2.2. Determination of phytic acid

After irradiation, the content of phytic acid was determined by the method of Latta and Eskin (1980). The sample (3 ml) was placed in 15 ml conical tubes and 1 ml of the modified Wade reagent (0.03% $FeCl_3 \cdot 6H_2O$ and 0.3% sulfosalicylic acid in distilled water) was added. The solution was mixed with a vortex mixer for 5 s, and the mixture was centrifuged at 3000 rpm for 10 min. The supernatant was read at 500 nm by using a spectrophotometer (Uvikon XL, Bio-Tek Instruments, Milano, Italy).

2.3. DPPH radical-scavenging capacity

The free radical-scavenging effect was estimated according to the method of Blois (1958) with some modification. The sample (1 ml) was added into the 0.2 mM 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical (Sigma Co., St. Louis, MO, USA, 1 ml). The mixture was shaken and left to stand for 30 min at room temperature and measured at 517 nm with a spectrophotometer (Bio-Tek Instruments). The DPPH radical-scavenging capacity was estimated from the difference between the absorbances with or without samples and expressed as a percentage of DPPH scavenging during storage for 2 weeks at 4 °C.

2.4. FRAP assay

The FRAP (ferric reducing/antioxidant power) assay was performed as previously described by Benzie and Strain (1996) using a spectrophotometer (Bio-Tek Instruments) with the function of an auto-rate assay. In the FRAP assay, reductones in the sample reduce the Fe (III)/tripyridyltriazine complex, present in stoichiometric excess, to the blue ferrous form, with an increase in the absorbance at 593 nm. Absorbance readings were taken after 0.5 s and every 30 s thereafter during the monitoring period for 5 min, and then the readings at 4 min were used as the FRAP value.

2.5. Antioxidant activity in soybean oil emulsion

The antioxidant activity of a sample in a 2% oil-in-water emulsion was measured during storage for 3 weeks at 37 °C. Oil emulsion was prepared with 2 ml of soybean oil plus 97 ml of deionized distilled water and 1 ml of the sample, and the mixture was homogenized with the addition of 1 ml of Tween 20 as an emulsifier. Soybean-oil emulsion with deionized distilled water was used as a control. Lipid oxidation was determined as a 2-thiobarbituric acid reactive substance (TBARS) value by using a spectrophotometer (Bio-Tek instruments) as described by Ahn, Olson, Jo, Love, and Jin (1999) with some modifications.

2.6. Statistical analysis

The experiment, from sample preparation to analysis, was repeated in triplicate, and the data were then analyzed by SAS software (SAS Institute, Cary, NC). The general linear model procedure was applied and Duncan's multiple range test was used to compare the mean values at $P < 0.05$. Mean values and pooled standard errors of the mean (SEM) were recorded.

3. Results and discussion

3.1. Radiolytic characteristics of phytic acid

Degradation of phytic acid, dissolved in deionized distilled water at various concentrations (800, 400, 200 and 100 μM), was caused by gamma irradiation (Fig. 1).

The phytic acid solution at (100 μM) was degraded to over 90% by irradiation at 10 kGy; however, the degradation because more difficult as the concentration increased. The degree of degradation induced by irradiation differed according to the concentration of phytic acid, and these results indicated that the concentration had a great effect on the degree of radiolysis. Generally, from the radiolysis of water, hydroxyl radical, aqueous electrons and hydrogen atoms are produced. The hydroxyl radical is a powerful oxidizing agent, while the aqueous electrons and hydrogen atom are reducing agents; therefore, all foods containing water are likely to undergo both oxidation and reduction reactions during irradiation (Stewart, 2001). These radicals play an important role in the radiolysis of materials.

In this analytical method for the determination of phytic acid, the pink colour of the Wade reagent is due to the reaction between the ferric ion and sulfosalicylic acid (Latta & Eskin, 1980). In the presence of phytic acid, the ferric ion binds to the phosphate ester and is unavailable to react with the sulfosalicylic acid, resulting in a decrease of the pink colour's intensity. Results in this study imply that the radicals produced during irradiation may remove phosphorus for the phytic acid structure and, consequently, a reduction of the phytic acid content was observed in the reaction with the Wade reagent. However, further identification is needed to support these results.

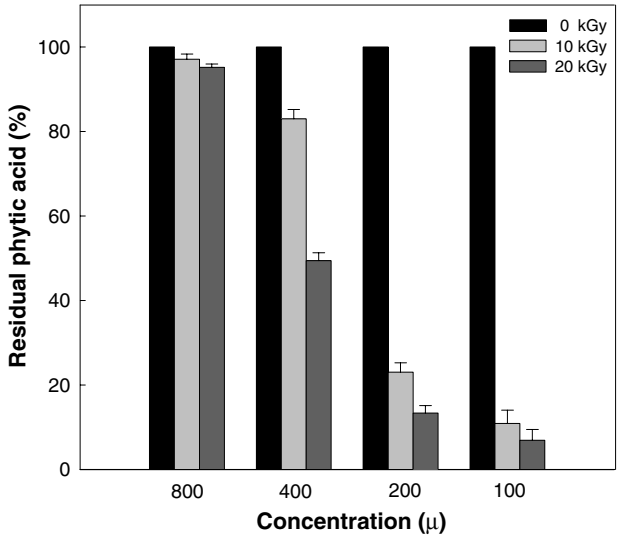

Fig. 1. Residual phytic acid (%) dissolved in deionized distilled water with various concentrations immediately after irradiation.

Recently, phytase has been widely used in animal feeds for the purpose of a hydrolyzing phytate to prevent the potential pollution of surface waters as well as for increasing the availability of phosphorus (Phillippy & Wyatt, 2001). So far, the best way to reduce phytic acid in the feed industry is enzymatic hydrolysis by phytase treatments. Duodu et al. (1999) reported that cooking did not decrease the phytic acid level in sorghum porridge, but the combination of cooking and irradiation at 1 kGy caused a significant decrease (40%). Similarly, treatment of soybean seeds with irradiation, alone or in combination with soaking, reduces the level of phytate compared to the non-irradiated control (Sattar et al., 1990). Accordingly, gamma irradiation offers a potential technique for reducing antinutrients by inducing radiolysis, accompanied with the microbiological safety advantage.

3.2. DPPH radical-scavenging effect

The ability of irradiated phytic acid and other antioxidants at various concentrations to scavenge the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical is shown in Table 1.

Non-irradiated phytic acid solution did not show DPPH radical-scavenging activity regardless of its concentration. However, after irradiation, a radical-scavenging ability of phytic acid was found. The maximum scavenging capacity of phytic acid was observed in samples irradiated at 20 kGy at the same concentration. Especially, when phytic acid at (800 μM) was irradiated at 20 kGy, the DPPH radical-scavenging capacity was significantly higher than that of ascorbic acid ($P < 0.05$). Ahn et al. (2003) reported that the irradiation dose was positively correlated with the DPPH radical-scavenging effects. Phytic acid has antioxidant functions by virtue of forming a unique iron chelate and it suppresses the iron-catalyzed oxidative reactions, serving as a potent antioxidant function in the preservation of seeds (Graf & Eaton, 1990). Also, by the same mechanism, dietary phytic acid may lower the incidence of colonic cancer and protect against other inflammatory bowel diseases. However, the free radical-scavenging activity of phytic acid has not yet been reported. A concentration effect of phytic acid was also observed, and radical-scavenging

Table 1
DPPH radical-scavenging capacity (%) of irradiated phytic acid compared with other antioxidants with various concentrations

Concentration (μM)	Sample[a]						SEM[c]
	PA0	PA10	PA20	Ascorbate	Tocopherol	BHA	
800	-c[b]	69.2bw	77.8aw	73.7bx	79.1ax	77.4ax	2.97
400	-d	44.0cx	51.3cx	70.2bx	79.0ax	76.7abx	2.33
200	-d	22.6cy	36.7cy	62.7bx	78.8ax	64.2by	2.14
100	-d	10.7cz	11.1cz	16.2cy	67.0ay	48.4bz	4.99
SEM[d]	–	1.68	1.95	7.45	1.45	1.18	

[a] PA0, PA10, PA20: phytic acid irradiated at 0, 10, or 20 kGy, respectively.
[b] Different letters within same row (a–d) or column (w–z) differ significantly ($P < 0.05$).
[c] SEM: standard error of the mean ($n = 18$).
[d] SEM: standard error of the mean ($n = 12$).

capacity increased with increasing concentration. Ascorbic acid, tocopherol and BHA at 200 μM or above, showed about 60–70% of the DPPH scavenging capacity, while a similar ability of the phytic acid was observed at 800 μM. Nenadis, Zafiropoulou, and Tsimidou (2003) reported that artificial antioxidants (BHA and BHT) have effective antioxidant activity even at low levels (50 μM); however, the toxicity of BHA or BHT has to be considered in the use of these artificial antioxidants.

DPPH radical assays are based on the transfer of electrons from a donor molecule to the corresponding radical (Fogliano, Verde, Randazzo, & Ritieni, 1999). This indicates that irradiated phytic acid might be structurally changed to have an electron donating effect by irradiation. However, before proposing a mechanism for antiradical activity acquired by irradiation, structural identification of radiolytic products from phytic acid is needed.

3.3. FRAP value and antioxidant activity in oil emulsion

The FRAP (ferric reducing/antioxidant power) assay is a method for measuring the reducing power of antioxidants (Benzie & Strain, 1996), and comparative data are shown in Table 2. The FRAP value showed a dose-response pattern in all the samples, and BHA had the most powerful reducing effect. Reducing power in phytic acid was significantly increased by irradiation ($P < 0.05$). However, the FRAP value of phytic acid was relatively low compared with ascorbic acid, tocopherol and BHA.

The activities of phytic acid, ascorbic acid, tocopherol and BHA in preventing the lipid oxidation of emulsified soybean oil during storage are shown in Table 3. After 1 week of storage at 37 °C, TBARS value of the control (oil emulsion without antioxidants) was higher than those of the samples prepared with phytic acid or other antioxidants. A statistically significant difference was observed among the samples, and phytic acid is effective in inhibiting lipid oxidation. The concentration effect was shown in the sample treated with phytic acid, while ascorbic acid, tocopherol, and BHA did not show a significant concentration effect. However, irradiation had no effect on the antioxidant activity of phytic acid at 1 and 2 weeks. At 3 weeks, phytic acid had effectively inhibited lipid oxidation compared to the other antioxidants. Especially, irradiation effects show by 800 μM phytic acid, and phytic acid irradiated at 20 kGy significantly reduced the TBARS values in the lipids.

Part of the antioxidative effects of phytic acid in lipid-containing model systems can be explained by their radical-scavenging activity but, as the present results demonstrate, the hydrogen-donating ability of samples does not necessarily indicate activity in lipid model

Table 2
FRAP value (μM FRAP/each concentration of antioxidant) of irradiated phytic acid compared with other antioxidants at various concentrations

Concentration (μM)	Sample[a]						SEM[c]
	PA0	PA10	PA20	Ascorbate	Tocopherol	BHA	
800	90.8ey[b]	136.1dex	181.0cd	325.8cx	698.4bw	1239.6aw	36.47
400	31.3ey	81.6dy	82.2dy	172.3cxy	377.9bx	875.9ax	9.50
200	21.3cx	34.1cz	41.8cz	47.0cy	197.9by	481.0ay	10.18
100	20.6cx	43.2cz	54.5cz	37.8cy	109.2bz	256.6az	11.95
SEM[d]	4.42	7.70	7.24	24.80	10.78	22.12	

[a] PA0, PA10, PA20: phytic acid irradiated at 0, 10, or 20 kGy, respectively.
[b] Different letters within same row (a–e) or column (w–z) differ significantly ($P < 0.05$).
[c] SEM: standard error of the mean ($n = 18$).
[d] SEM: standard error of the mean ($n = 12$).

Table 3
TBARS value of soybean-oil emulsion prepared with irradiated phytic acid compared with other antioxidants at various concentrations during storage for 3 weeks at 37 °C

Concentration (μM)	Sample[a]							SEM[c]
	Control	PA0	PA10	PA20	Ascorbate	Tocopherol	BHA	
0 week	0.01							
800		0.02	0.02	0.03	0.02	0.03	0.02	0.011
400		0.01	0.02	0.01	0.02	0.04	0.03	0.009
200		0.01	0.03	0.02	0.01	0.02	0.02	0.003
100		0.02	0.02	0.01	0.02	0.02	0.01	0.005
SEM[d]		0.012	0.008	0.010	0.007	0.005	0.002	
1 week	0.79							
800		0.15bcy[b]	0.16bcy	0.14cy	0.19a	0.17ab	0.13c	0.009
400		0.14by	0.15by	0.13by	0.21a	0.15b	0.16ab	0.015
200		0.16by	0.14by	0.14by	0.28a	0.23a	0.26a	0.042
100		0.26x	0.23x	0.21x	0.26	0.30	0.21	0.063
SEM[d]		0.026	0.029	0.006	0.024	0.073	0.069	
2 week	0.97							
800		0.13aby	0.12b	0.13by	0.20a	0.20a	0.15aby	0.017
400		0.13y	0.12	0.12y	0.21	0.22	0.13y	0.033
200		0.14by	0.13b	0.11by	0.24a	0.25a	0.29ax	0.024
100		0.19x	0.21	0.20x	0.29	0.27	0.23y	0.057
SEM[d]		0.014	0.041	0.017	0.063	0.038	0.020	
3 week	1.27							
800		0.22bxy	0.21b	0.18cy	0.30ab	0.33a	0.32a	0.039
400		0.24abx	0.19b	0.17by	0.33a	0.32a	0.32a	0.032
200		0.25abx	0.22b	0.21bx	0.23ab	0.33a	0.30a	0.030
100		0.25aby	0.23b	0.21by	0.25ab	0.33a	0.27ab	0.028
SEM[d]		0.013	0.18	0.008	0.039	0.030	0.074	

[a] PA0, PA10, PA20: phytic acid irradiated at 0, 10, or 20 kGy, respectively.
[b] Different letters within a same row (a–d) or column (w–z) differ significantly ($P < 0.05$).
[c] SEM: standard error of the mean ($n = 18$).
[d] SEM: standard error of the mean ($n = 12$).

systems. It can be assumed that several factors, such as metal chelating properties, interactions with an emulsifier or proteins, and the distribution between the oil and water phases are more complex in lipid systems and may be important for the antioxidant action (Schwarz et al., 2000). Graf and Eaton (1990) reported that phytic acid is remarkably unreactive and an extraordinarily stable antioxidant, which contrasted with other antioxidants, because many antioxidants function by reacting with activated oxygen species, thereby being consumed in the process. The antioxidant function of phytic acid is differentiated from antioxidants; however, irradiation can induce the antiradical activity through structural changes, as well as maintaining or increasing the antioxidant activity in lipids. This may suggest that irradiation might be used in developing or modifying a new function in materials.

In conclusion, gamma irradiation induces the radiolysis of phytic acid in an aqueous system, and the free radical-scavenging activity of degraded phytic acid is greatly increased. Phytic acid is present in many botanical foodstuffs; therefore, the present study provides important information on use of irradiation for reducing the levels of phytic acid and in increasing the antioxidant activity. More research is needed to provide adequate information for the food and feed industries for practical applications.

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