



Antioxidant capacity of phytic acid purified from rice bran

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ABSTRACT. Rice bran is a by-product of rice processing industry, with high levels of phytic acid or phytate. Considering phytic acid antioxidant activity, its various applications and its high concentration in rice bran, this study had the objective of evaluating the antioxidant capacity of purified phytic acid from rice bran using three different methods. Using of 2,4,6-tripyridil-s-triazine or method of FRAP (Ferric Reducing Antioxidant Power), reducing Fe^{2+} activity was not detected for standard or purified phytic acid. With BPS (bathophenanthroline) method, the Fe^{2+} chelator activity of standard phytic acid and rice bran phytic acid were dependent on the concentration and contact time and were observed IC_{50} values of 4.39 mg mL^{-1} and IC_{50} of 7.54 mg mL^{-1} , respectively. By the deoxyribose method, the standard phytic acid inhibited the hydroxyl radical with an IC_{50} of 0.70 mg mL^{-1} while the rice bran phytic acid showed a maximum inhibitory activity of 40% associated to its chelating capacity and confirm this important antioxidant capacity.

Keywords: rice bran, phytic acid, iron ion, hydroxyl radical.

Capacidade antioxidante do ácido fítico purificado de farelo de arroz

RESUMO. O farelo de arroz é um subproduto da indústria de processamento de arroz, com níveis elevados de ácido fítico ou fitato. Considerando a atividade antioxidante do ácido fítico, suas diversas aplicações e sua alta concentração no farelo de arroz, este estudo teve como objetivo avaliar a capacidade antioxidante do ácido fítico purificado de farelo de arroz utilizando três diferentes métodos. Usando 2,4,6-tripiridil-s-triazina ou método de FRAP (Ferric Reducing Antioxidant Power), a atividade redutora de Fe^{+2} não foi detectada no padrão ou no ácido fítico purificado. Com o método de BPS (bathophenanthroline), a atividade quelante do Fe^{+2} de ácido fítico padrão e ácido fítico purificado de farelo de arroz foi dependente da concentração e tempo de contato e foram observados valores de IC_{50} de 4,39 mg mL^{-1} e IC_{50} de 7,54 mg mL^{-1} , respectivamente. Pelo método de deoxyribose, o ácido fítico padrão inibiu o radical hidroxila com um IC_{50} de 0,70 mg mL^{-1} enquanto que o ácido fítico do farelo de arroz apresentou uma atividade inibitória máxima de 40% associado a sua capacidade quelante e confirma a sua importante capacidade antioxidante.

Palavras-chave: farelo de arroz, ácido fítico, íon ferro, radical hidroxil.

Introduction

Phytic acid or 1, 2, 3, 4, 5, 6 inositol hexakisphosphate (IP6) is a negatively charged molecule in a wide pH range, with 12 replaceable protons (TSAO et al., 1997). These characteristics allow the formation of compounds based on its chelating capacity with polyvalent metals, especially divalent and trivalent cations, making them bio-unavailable (GRAF; EATON, 1990). Phytic acid or phytate is distributed in different rice components; 80% of the phytic phosphorus is concentrated in the pericarp and the aleurone, 7.6% is concentrated in the germen, and 1.2% is concentrated in the endosperm (O'DELL et al., 1972). Rice bran is composed of pericarp, aleurone, and germen, which shows a high phytic

acid content of $5.88 \pm 0.09\%$ (CANAN et al., 2011).

Phytic acid is an effective and economically feasible antioxidant for preserving biological material that are sensitive to oxygen due to its chelating capacity of multivalent metallic ions, especially iron, zinc, and calcium (GRAF et al., 1987). The iron chelating capacity makes it catalytically inactive, making phytic acid a strong inhibitor of iron-mediated hydroxyl radical ($\cdot\text{OH}$) production (GRAF; EATON, 1985). In addition, it alters the redox potential of iron, keeping it in its ferric form (Fe^{3+}). This effect offers protection against oxidative damage because Fe^{2+} causes production of lipid oxidicals, whereas Fe^{3+} is relatively inert (EMPSON et al., 1991; GRAF et al., 1987).

One way of fighting oxidative stress and lipid oxidation in food is to use antioxidants to avoid, or decrease, the triggering of oxidative reactions (ARUOMA, 1998). The most commonly used synthetic antioxidants are butylhydroxyanisole (BHA), butylhydroxytoluene (BHT), propyl gallate (PG), and tert butylhydroquinone (TBHQ). The use of these antioxidants has been questioned due to safety concerns; therefore, natural antioxidants are being widely investigated (FERNÁNDEZ-LÓPEZ et al., 2005; FRANKEL et al., 1996). The main natural antioxidants that stand out are ascorbic acid (LEE; HENDRICKS, 1995; MANCINI et al., 2006), vitamin E (OLIVO et al., 2001), vitamin A and carotenoids (PALACE et al., 1999), phenolic compounds (FRANKEL et al., 1996), plant extracts (FERNÁNDEZ-LÓPEZ et al., 2005; SILVA et al., 2010), γ -oryzanol (BUTSAT; SIRIAMORNUN, 2010; LAI et al., 2009) and phytic acid (GRAF; EATON, 1990; EMPSON et al., 1991; FILGUEIRAS et al., 2009; GRAF et al., 1987; LEE; HENDRICKS, 1995; HARBACH et al., 2007; SOARES et al., 2004; STODOLAK et al., 2007).

Filgueiras et al. (2009) confirmed the antioxidant potential of purified phytic acid from corn germ through deoxyribose and bathophenanthroline tests; they concluded that the antioxidant capacity grows with the increase in purified phytic acid concentration. The antioxidant action of phytic acid of alcoholic extracts obtained from wild rice significantly reduced the thiobarbituric acid reactive substances (TBARS) when added to beef and pork fat stored under refrigeration (WU et al., 1994).

A number of methods have been developed to determine the antioxidant capacities of chemical compounds (PRIOR et al., 2005). However, there is no precision, simple and universal method that allows an appropriate comparison of results (HALLIWELL; CHIRICO, 1993). Moreover, various methods are used to evaluate different antioxidant mechanisms. The Ferric Reducing Antioxidant Power (FRAP) method measures the ferric reduction of 2,4,6-tripyridyl-s-triazine (TPTZ) to a colored product (SANCHÉZ-GONZÁLEZ et al., 2005). The bathophenanthroline (BPS) method uses the Fe^{+2} ion chelating system (BOLANN; ULVIK, 1987). The deoxyribose method observes the decrease in build-up of TBARS resulting from degradation of deoxyribose sugar by the hydroxyl radicals promoted by the Fenton reaction (HALLIWELL et al., 1987).

Rice bran is a by-product of rice processing industry, with high levels of phytic acid or phytate. Recently, it was established an appropriate analytical technique for extraction and purification of phytic

acid in rice bran IRGA 417 with high purity and yield (CANAN et al., 2011). Considering phytic acid antioxidant activity, its various applications and its high concentration in rice bran, this study had the objective of evaluating the antioxidant capacity of purified phytic acid from rice bran using the FRAP, BPS, and deoxyribose methods.

Material and methods

Materials

Phytate was purified from rice bran IRGA 417 with purity of $78.19 \pm 0.86\%$ as described by Canan et al. (2011). Dodeca-sodium phytate from rice was used as a standard (P0109, Sigma), with purity $\geq 90\%$. Reagents 2-deoxy-D-ribose and 6-hydroxi-2, 5, 7,8-tetramethylchroman-2-carboxylic acid (TROLOX) were obtained from Sigma (St. Louis, USA); bathophenanthroline disulfonic acidic (BPS) and 2,4,6-tri-pyridyl-s-triazine (TPTZ) were obtained from Acros Organics (Geel, Belgium). Other reagents were obtained from various commercial suppliers and were of a grade suitable for analysis.

Reducing capacity of iron ion by phytic acid using the Frap Method

The reducing power of phytic acid was evaluated according to Sánchez-González et al. (2005) with some changes. For the analysis, 3 mL of FRAP reagent and 100 μL of different standard phytic acid concentrations or of purified rice bran acid (0.028, 0.141, 0.353, 0.706, 1.412, 1.765, 2.824 and 7.060 mg mL^{-1}) were added; the reading of absorbance at 593 nm (HUANG et al., 2005) was conducted after 30 minutes at 37°C . The FRAP reagent was used as a blank, which consisted of sodium acetate buffer (0.3 mmol L^{-1} and pH 3.6) contained 10 mmol L^{-1} TPTZ, 40 mmol L^{-1} HCl, and 20 mmol L^{-1} ferric chloride at a volume proportion of 10:1:1, respectively. This solution was prepared at the moment of analysis. The solution was incubated at 37°C for 30 minutes. Reducing power was expressed as μmol equivalents of Trolox per gram of standard phytic acid or of rice bran. The tests were conducted in triplicate.

Chelating capacity of iron ion by phytic acid using the BPS Method

The chelating capacity of Fe^{2+} ion by phytic acid was determined according to Bolann and Ulvik (1987) using BPS. First, the contact time between Fe^{2+} ion and standard phytic acid was evaluated for different times in the medium of the reaction before adding BPS. In this test, 50 $\mu\text{mol L}^{-1}$ of $(\text{NH}_4)_2\text{Fe}(\text{SO}_4)_2$ and 50 μL of standard phytic acid

in 4.41, 17.65, and 141.20 $\mu\text{g mL}^{-1}$ concentrations were kept in contact for 0, 15, 30, 60, or 120 minutes in 2 mL of a reaction medium containing 125 mmol L^{-1} sucrose, 65 mmol L^{-1} KCl, and 10 mmol L^{-1} Tris-HCl at 7.4 pH (medium I). Afterwards, 0.2 mmol L^{-1} of BPS was added to the reaction mix, which was kept at room temperature for 15 minutes. The chelating capacity of iron was determined by the formation of the $\text{Fe}_2(\text{BPS})_3$ colored compound, and readings on the spectrophotometer were performed at 530 and 700 nm according to Casagrande et al. (2006).

After defining a best contact time of 60 minutes for the reaction between phytic acid and the Fe^{2+} ion before the addition of BPS, standard phytic acid solutions or purified phytic acid from rice bran with 3.530 mg mL^{-1} in 0.02 mol L^{-1} HCl were prepared. Afterwards, they were diluted with deionized water to obtain concentrations of 2.2, 4.4, 8.8, 35.3, 88.3, 176.5, 353.0, and 1765.0 $\mu\text{g mL}^{-1}$. To determine the concentration response curve, 50 $\mu\text{mol L}^{-1}$ of $(\text{NH}_4)_2\text{Fe}(\text{SO}_4)_2$ and 50 μL of the different concentrations of standard phytic acid or purified phytic acid from rice bran were kept at room temperature for 60 minutes in 2 mL of medium I. BPS at 0.2 mmol L^{-1} was then added, and the procedure was followed as previously described. At the same time, the blank (reaction medium) and the positive control (reaction medium + Fe^{2+} + BPS) were assessed. Tests were performed in triplicate.

The antioxidant capacity (%) was expressed as the Fe^{2+} ion chelating capacity by phytic acid and estimated as $100 - (100A_s/A_0)$, where, A_s was the absorbance of the sample (530 nm – 700 nm) and A_0 was the absorbance of the positive control (530 – 700 nm).

Inhibiting capacity of hydroxyl radicals by phytic acid using the Deoxyribose Method

The reduction in the formation of substances reactive to thiobarbituric acid (TBA) from deoxyribose sugar degradation by the hydroxyl radical generated by the Fenton reaction was determined according to Halliwell et al. (1987). A standard phytic acid solution at 14.12 mg mL^{-1} was prepared with deionized water; it was later diluted to obtain 0.028, 0.141, 0.353, 0.706, 1.412, 1.765, 2.824, and 7.060 mg mL^{-1} concentrations. For purified phytic acid from rice bran, a saturated solution in deionized water was prepared and filtered through qualitative filter paper. The phytic acid content was quantified (2.280 mg mL^{-1}) according to the procedure described by Latta and Eskin (1980) with a change from resin to Dowex-Agx-4 according to Ellis and Morris (1986).

Afterwards, dilutions of 0.023, 0.045, 0.091, 0.280, 0.285, 0.456, and 1.140 mg mL^{-1} were obtained. Test tubes containing 1 mL of KH_2PO_4 -KOH buffer (20 mmol L^{-1} and pH 7.4), 100 $\mu\text{mol L}^{-1}$ of ascorbate, 500 μL of different concentrations of standard phytic acid or purified phytic acid from rice bran, 2.8 mmol L^{-1} of deoxyribose, 1 mmol L^{-1} of H_2O_2 , 50 $\mu\text{mol L}^{-1}$ of $\text{Fe}_3\text{Cl}_6\text{H}_2\text{O}$, and 52 $\mu\text{mol L}^{-1}$ of EDTA were then prepared. Tubes containing the reaction mixtures were incubated at 37°C for 30 minutes. After this period, 1 mL of 1% TBA, prepared in 50 mmol L^{-1} of NaOH, 100 μL of NaOH 10 mol L^{-1} , and 500 μL of H_3PO_4 20% was added to the tubes, and they were incubated at 85°C for 20 minutes. Readings were conducted on a spectrophotometer at 535 nm at room temperature. The blank (absence of deoxyribose), positive control (absence of phytic acid), and negative control (absence of iron) were tested simultaneously. Antioxidant capacity was expressed as hydroxyl radical inhibition % by phytic acid and estimated as $100 - (100A_s/A_0)$, where, A_s was the absorbance of the sample (535 nm) and A_0 was the absorbance of the positive control (535 nm).

Method precision

The precision of the iron ion chelating capacity analysis by the standard phytic acid using the BPS method was determined by repeatability (intra-test) and intermediate (inter-test) precision. Repeatability for the test was evaluated on the same day, with samples of the same concentrations (40 and 180 $\mu\text{g mL}^{-1}$ of standard phytic acid). The intermediate precision was determined using the same concentrations and compared to the analysis on three different days. Repeatability (intra-test) and intermediate (inter-test) precisions of the deoxyribose test were evaluated by Filgueiras et al. (2009). Similar assessments of the FRAP method were not determined because it was negative for phytic acid.

Statistic analysis

To evaluate the influence of time on the BPS method, the data were statistically analyzed using a one-way ANOVA followed by Bonferroni's multiple comparisons *t*-test (Program GraphPad Prisma, Version 4.00, 2003). The concentrations of standard phytic acid or purified phytic acid from rice bran required to inhibit 50% of the oxidative process (IC_{50}) were estimated with the same software, using a hyperbolic curve. The results were expressed as mean \pm standard error of the mean (SEM) and considered significantly different when $p < 0.05$ was obtained.

Results and discussion

Reducing capacity of iron ion by phytic acid using the FRAP Method

The FRAP method determines the ferric reduction of TPTZ to a colored product. The reaction detects compounds with redox potential < 0.7 V (the redox potential of Fe^{+3} -TPTZ). Under acidic conditions, the Fe^{2+} ion forms a compound of yellowish color with 2,4,6-tri(2-pyridyl)-s-triazine (Fe^{2+} -TPTZ), which can be measured in a spectrophotometer at 593 nm (SÁNCHEZ-GONZÁLEZ et al., 2005).

The Fe^{3+} ion or iron chelate may start lipid peroxidation reactions in the presence of a Fe^{+3} to Fe^{+2} reducing agent (BRAUGHLER et al., 1986) because Fe^{2+} is a source of oxyradicals and lipid peroxidation, whereas Fe^{3+} is relatively inert (EMPSON et al., 1991; GRAF et al., 1987). In this test, no reducing capacity of iron ion by standard phytic acid or by purified phytic acid from rice bran using TPTZ was detected, confirming the results of Lee and Hendricks (1995) they observed that adding phytic acid provoked the oxidation of Fe^{+2} to Fe^{+3} . According to the same authors, this factor also characterizes phytic acid as an inhibitor of the formation of hydroxyl radicals by the Fenton reaction; consequently, there is a related ability to inhibit the formation of TBARS by the degradation of deoxyribose. That is, phytic acid alters the redox potential of iron, keeping it in its ferric form (Fe^{3+}).

Chelating capacity of iron ion by phytic acid using the BPS Method

BPS is a strong chelator of iron ion, and it forms with this ion a colored compound with strong absorptions at 530 nm. Transition metals such as iron may catalyze the generation of reactive oxygen species. For example, the hydroxyl radical ($\cdot\text{OH}$) and the superoxide anion ($\text{O}_2^{\cdot-}$) may propagate lipid peroxidation through a chain reaction (HALLIWELL; CHIRICO, 1993).

Figure 1 shows the effect of different concentrations of standard phytic acid and the contact times with iron required for the formation of complex Fe^{2+} and phytic acid before the addition of BPS. The chelating capacity of standard phytic acid was significantly dependent on the phytic acid concentration, i.e., it increased with the rise in concentration of phytic acid from 0.110 to 3.530 $\mu\text{g mL}^{-1}$. It also increased with the time of contact with iron for the formation of the Fe^{2+} -phytic acid complex before the addition of the BPS, thus confirming the potential of phytic acid in chelating

Fe^{2+} ions. However, a greater affinity between BPS and Fe^{2+} than between phytic acid and Fe^{2+} was observed since when phytic acid and BPS were simultaneously added in the reaction medium, i.e., at time 0, a low chelating capacity of phytic acid by Fe^{2+} ions occurred. However, with a previous contact of 15, 30, 60, or 120 minutes between standard phytic acid and Fe^{2+} before the addition of BPS, the chelating capacity of phytic acid by Fe^{2+} ions significantly increased ($p < 0.05$).

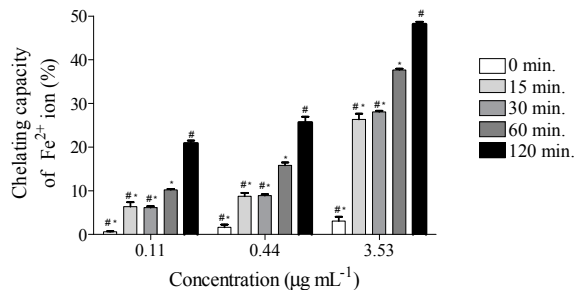


Figure 1. Chelating capacity of the Fe^{2+} ion by the standard phytic acid. The phytic acid concentrations used were 0.11, 0.44, and 3.53 $\mu\text{g mL}^{-1}$. BPS was added immediately or 15, 30, 60, or 120 minutes after iron addition. Statistical analysis was conducted using a one-way ANOVA, followed by the Bonferroni's multiple comparisons *t*-test. *Significant statistical differences relative to BPS added after 120 minutes and # Significant statistical difference relative to BPS added after 60 minutes ($p < 0.05$) ($n = 3$).

Therefore, 60 minutes was established as the contact time between the phytic acid and Fe^{2+} for a study on the chelating capacity of phytic acid, though it was significantly different from the 120-minute time. Furthermore, the formed complex at 120 minutes of contact was instable as observed by a low absorbance of the positive control, which is probably due to the oxidation of iron when exposed for a prolonged time in solution before the addition of BPS (Figure 2).

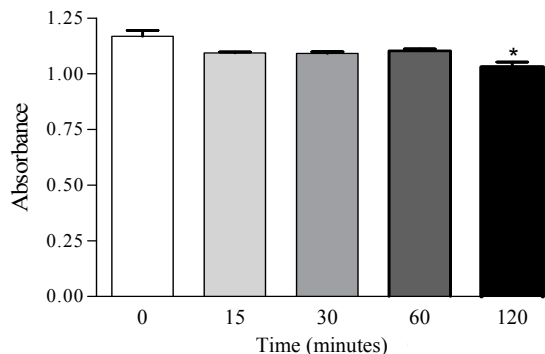


Figure 2. Absorbance of positive controls at time points of 0, 15, 30, 60, and 120 minutes. The statistical analysis was conducted using a one-way ANOVA, followed by the Bonferroni's multiple comparisons *t*-test. *Significant statistical differences relative to BPS added immediately (zero time) ($p < 0.05$) ($n = 3$).

When comparing the chelating capacity of Fe^{2+} from standard phytic acid and purified phytic acid from rice bran after 60 minutes of BPS addition, IC_{50} values of $4.39 \mu\text{g mL}^{-1}$ and $7.54 \mu\text{g mL}^{-1}$ were observed, respectively (Figure 3A and B). These results demonstrate that standard phytic acid has a greater chelating capacity of Fe^{2+} ions, as compared to purified phytic acid from rice bran. Similar results were also described by Filgueiras et al. (2009) who evaluated the chelating capacity of purified phytic acid from corn germ using the BPS test and observed a higher chelating capacity of Fe^{2+} ions by standard phytic acid.

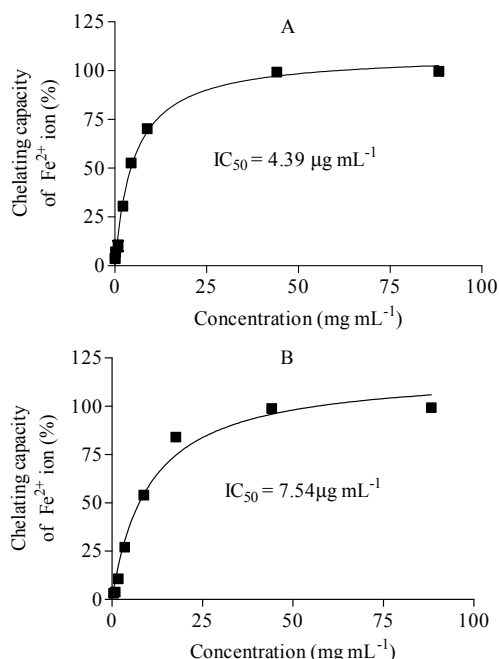


Figure 3. Chelating capacity of standard phytic acid (A) or of phytic acid obtained from rice bran (B) by Fe^{2+} ion through a bathophenanthroline test. Results expressed as mean \pm SEM ($n = 3$).

Although there is evidence that the antioxidant capacity of phytic acid depends on the chelating of Fe^{2+} ion, the interaction mechanism between phytic acid and minerals is not yet totally clear (FEBLES et al., 2001). The results of the bathophenanthroline test confirm that phytic acid forms a chelate with iron, making it catalytically inactive (GRAF; EATON, 1985). This effect offers protection against oxidative damage, as Fe^{2+} causes production of oxyradicals and lipid peroxidation (EMPSON et al., 1991; GRAF et al., 1987). This in turn leads to the development of objectionable flavors; fading and loss of the nutritional value of food due to the destruction of vitamins and essential fatty acids; and the production of potentially toxic substances such as cholesterol oxides and malonaldehyde. Malonaldehyde is the greatest

secondary product of lipid oxidation (GARDNER, 1979); it shows a cytotoxic, carcinogenic, mutagenic effect (DIANZANI, 1993). It is detected with 2-thiobarbituric acid, which is the basis for many tests used to investigate rancidification (HALLIWELL, 2009).

The precision of the BPS test was also evaluated using standard phytic acid. The intra-test coefficients of variance (CV) ranged from 6.34 to 9.39% for a concentration of $40 \mu\text{g mL}^{-1}$ and 2.89 to 4.22% for a concentration of $180 \mu\text{g mL}^{-1}$. The inter-test CVs for concentrations of 40 and $180 \mu\text{g mL}^{-1}$ were 9.84 and 5.99%, respectively. According to Agência Nacional de Vigilância Sanitária (ANVISA), a CV lower than 15% indicates that the method shows a good precision.

Inhibiting capacity of hydroxyl radicals by phytic acid using the Deoxyribose Method

The deoxyribose method was used to test whether phytic acid is efficient in inhibiting hydroxyl radicals, which act in the degradation of deoxyribose sugar, forming substances reactive to TBA. In Figure 4A, it can be observed that standard phytic acid showed an IC_{50} of 0.70 mg mL^{-1} , whereas purified phytic acid from rice bran showed a maximum inhibiting capacity of hydroxyl radicals, close to 40% (Figure 4B). This effect is probably due to the low purity (60%) of the rice bran sample as compared to the standard ($\geq 90\%$). Also, at the maximum concentration in solution, it was not soluble.

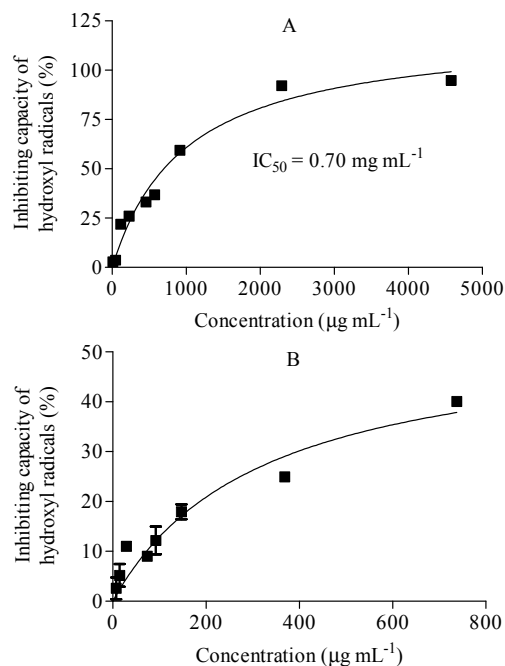


Figure 4. Inhibiting capacity of hydroxyl radicals by standard phytic acid (A) or phytic acid obtained from rice bran (B) through a deoxyribose test. Results expressed as mean \pm SEM ($n = 3$).

The formation of hydroxyl radicals catalyzed by iron requires an availability of at least one reactive coordination site, and many chelators increase the catalytic function of this ion. However, there are few chelators that preserve the solubility of iron and make it totally inert, as does phytic acid (GUTTERIDGE, 1984). In aqueous solutions, the six coordination sites of trivalent iron are occupied by water or hydroxide ions. Many chelating agents dislocate five of those binders and form a pentatoothed chelate with water, occupying the sixth coordination site. In the case of EDTA, which was used in the deoxyribose test, the formation of a hexatoothed chelate occurred. However, due to its small size, the chelate was distorted and produced a seventh site that is available for water, increasing the solubility of the iron. Phytic acid uniquely occupies the sixth coordination site and dislocates all of the coordination water of the Fe^{+3} -phytate complex (GRAF et al., 1984). It is one of the rare chelators that, when in molar proportions of 0.25 or more of phytate to iron, blocks the generation of free radicals (GUTTERIDGE, 1984).

Conclusion

In the method that uses BPS, the purified phytic acid from rice bran showed an iron ion chelating capacity, which was dependent on the concentration and contact time with the iron for the formation of the Fe^{2+} -phytic acid complex before the addition of the BPS. Moreover, the hydroxyl radical inhibition by phytic acid further confirmed the important antioxidant capacity observed.

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References

ARUOMA, O. I. Free radicals, oxidative stress, and antioxidants in human health and disease. **Journal of the American Oil Chemists' Society**, v. 75, n. 2, p. 199-212, 1998.

BOLANN, B. J.; ULVIK, R. J. Release of iron from ferritin by xanthine oxidase. Role of the superoxide radical. **Biochemistry Journal**, v. 243, n. 1, p. 55-59, 1987.

BRAUGHLER, J. M.; DUNCAN, L. A.; CHASE, R. L. The involvement of iron in lipid peroxidation. **The Journal of Biological Chemistry**, v. 261, n. 22, p. 10282-10289, 1986.

BUTSAT, S.; SIRIAMORNPUN, S. Antioxidant capacities and phenolic compounds of the husk, bran and endosperm of Thai rice. **Food Chemistry**, v. 119, n. 2, p. 606-613, 2010.

CANAN, C.; CRUZ, F. T. L.; DELAROZA, F.; CASAGRANDE, R.; SARMENTO, C. P. M.; SHIMOKOMAKI, M.; IDA, E. I. Studies on the extraction and purification of phytic acid from rice bran. **Journal of Food Composition and Analysis**, v. 24, n. 7, p. 1057-1063, 2011.

CASAGRANDE, R.; GEORGETTI, S. R.; VERRI, W. A. J. R.; JABOR, J. R.; SANTOS, A. C.; FONSECA, M. J. V. Evaluation of functional stability of quercetin as a raw material and in different topical formulations by its antilipoperoxidative activity. **American Association of Pharmaceutical Scientists**, v. 7, n. 1, p. 64-71, 2006.

DIANZANI, M. U. Lipid peroxidation and cancer. **Critical Reviews in Oncology/Hematology**, v. 15, n. 2, p. 125-147, 1993.

EMPSON, K. L.; LABUZA, T. P.; GRAF, E. Phytic acid as a food antioxidant. **Journal of Food Science**, v. 56, n. 2, p. 560-563, 1991.

ELLIS, R.; MORRIS, E. R. Appropriate resin selection for rapid phytate analysis by ion-exchange chromatography. **Cereal Chemistry**, v. 63, n. 1, p. 58-59, 1986.

FEBLES, C. I.; ARIAS, A.; HARDISSON, A.; RODRIGUEZ-ALVAREZ, C.; SIERRA, A. Phytic acid level in wheat flours. **Food Chemistry**, v. 74, n. 4, p. 437-441, 2001.

FERNÁNDEZ-LÓPEZ, J.; ZHI, N.; ALEZON-CARBONELL, L.; PÉREZ-ALVAREZ, J. A.; KURI, V. Antioxidant and antibacterial activities of natural extracts: application in beef meatballs. **Meat Science**, v. 69, n. 3, p. 371-380, 2005.

FILGUEIRAS, C. T.; CASAGRANDE, R.; SOARES, A. L.; SHIMOKOMAKI, M.; IDA, E. I. Avaliação da atividade antioxidante do ácido fítico de germe de milho. **Química Nova**, v. 32, n. 7, p. 1787-1791, 2009.

FRANKEL, E. N.; HUANG, S. W.; PRIOR, E.; AESCHBACH, R. Evaluation of antioxidant activity of rosemary extracts, carnosol and carnosic acid in bulk vegetable oils and fish oil and their emulsions. **Journal of the Science of Food and Agriculture**, v. 72, n. 2, p. 201-208, 1996.

GARDNER, H. W. Lipid hydroperoxide reactivity with proteins and amino acids: a review. **Journal of Agricultural and Food Chemistry**, v. 27, n. 2, p. 220-229, 1979.

GRAF, E.; EATON, J. W. Antioxidant functions of phytic acid. **Free Radical Biology and Medicine**, v. 8, n. 1, p. 61-69, 1990.

GRAF, E.; EATON, J. W. Dietary suppression of colonic cancer: fiber or phytate? **Cancer**, v. 56, n. 15, p. 717-718, 1985.

- GRAF, E.; EMPSON, K. L.; EATON, J. W. Phytic acid. A natural antioxidant. **The Journal of Biological Chemistry**, v. 262, n. 24, p. 11647-11650, 1987.
- GRAF, E.; MAHONEY, J. R.; BRYANT, R. G.; EATON, J. W. Iron-catalyzed hydroxyl radical formation. **The Journal of Biological Chemistry**, v. 259, n. 6, p. 3620-3624, 1984.
- GUTTERIDGE, J. M. C. Ferrous ion-EDTA-stimulated phospholipid peroxidation. **Biochemistry Journal**, v. 224, n. 3, p. 697-701, 1984.
- HALLIWELL, B. The wanderings of a free radical. **Free Radical Biology and Medicine**, v. 46, n. 5, p. 531-542, 2009.
- HALLIWELL, B.; CHIRICO, S. Lipid peroxidation: its mechanism, measurement, and significance. **American Society for Clinical Nutrition**, v. 57, n. 5, p. 715S-725S, 1993.
- HALLIWELL, B.; GUTTERIDGE, J. M.; ARUOMA, O. I. The deoxyribose method: a simple "test-tube" assay for determination of rate constants for reactions of hydroxyl radicals. **Analytical Biochemistry**, v. 165, n. 1, p. 215-219, 1987.
- HARBACH, A. P. R.; COSTA, M. C. R.; SOARES, A. L.; BRIDI, A. M.; SHIMOKOMAKI, M.; SILVA, C. A.; IDA, E. I. Dietary corn germ containing phytic acid prevents pork meat lipid oxidation while maintaining normal animal growth performance. **Food Chemistry**, v. 100, n. 4, p. 1630-1633, 2007.
- HUANG, D.; OU, B.; PRIOR, R. L. The chemistry behind antioxidant capacity assays. **Journal of Agricultural and Food Chemistry**, v. 53, n. 6, p. 1841-1856, 2005.
- LATTA, M.; ESKIN, M. A simple and rapid colorimetric method for phytate determination. **Journal of Agricultural and Food Chemistry**, v. 28, n. 6, p. 1313-1315, 1980.
- LAI, P.; LI, K. Y.; LU, S.; CHEN, H. H. Phytochemicals and antioxidant properties of solvent extracts from Japonica rice bran. **Food Chemistry**, v. 117, n. 3, p. 538-544, 2009.
- LEE, B. J.; HENDRICKS, D. G. Phytic acid protective effect beef round muscle lipid peroxidation. **Journal of Food Science**, v. 60, n. 2, p. 1313-1315, 1995.
- MANCINI, R. A.; HUNT, M. C.; SEYFERT, M.; KROPE, D. H.; HACHMEISTER, K. A.; HERALD, T. J.; JOHNSON, D. E. Effect of antioxidant solubility and concentration on discoloration of beef vertebrae marrow during display. **Journal of Food Science**, v. 71, n. 8, p. 484-494, 2006.
- O'DELL, B. L.; DE BOLAND, A.; KOIRTYOHANN, S. R. Distribution of phytate and nutritionally important elements among the morphological components of cereal grains. **Journal of Agricultural and Food Chemistry**, v. 20, n. 3, p. 718-723, 1972.
- OLIVO, R.; SOARES, A. L.; IDA, E. I.; SHIMOKOMAKI, M. Dietary vitamin E inhibits poultry PSE and improves meat functional properties. **Journal of Food Biochemistry**, v. 25, n. 4, p. 271-283, 2001.
- PALACE, V. P.; KHAPER, N.; QIN, Q.; SINGAL, P. K. Antioxidant potentials of vitamin A and carotenoids and their relevance to heart disease - protection of LDL by vitamin E but not by β -carotene. **Free Radical Biology and Medicine**, v. 26, n. 5-6, p. 746-761, 1999.
- PRIOR, R. L.; WU, X.; SCHAICH, K. Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. **Journal of Agricultural and Food Chemistry**, v. 53, n. 10, p. 4290-4302, 2005.
- SÁNCHEZ-GONZÁLEZ, I.; JIMÉNEZ-ESCRIG, A.; SAURA-CALIXTO, F. In vitro antioxidant activity of brewed using different procedures (Italian, espresso and filter). **Food Chemistry**, v. 90, n. 1-2, p. 133-139, 2005.
- SILVA, C. B.; CÂNDIDO, A. C. S.; SIMIONATTO, E.; FACCENDA, O.; SCALON, S. P. Q.; PERES, M. T. L. P. Atividade alelopática, antioxidante e teor de fenóis totais de *Hydrocotyle bonariensis* Lam. (Araliaceae). **Acta Scientiarum. Technology**, v. 32, n. 4, p. 413-420, 2010.
- SOARES, A. L.; OLIVO, R.; SHIMOKOMAKI, M.; IDA, E. I. Synergism between dietary vitamin E and exogenous phytic acid in prevention of warmed-over flavour development in chicken breast meat, Pectoralis major M. **Brazilian Archives of Biology and Technology**, v. 47, n. 1, p. 57-62, 2004.
- STODOLAK, B.; STARZYŃSKA, A.; CZYSZCZOŃ, M.; ŻYLA, K. The effect of phytic acid on antioxidant stability of raw and cooked meat. **Food Chemistry**, v. 101, n. 1, p. 1041-1045, 2007.
- TSAO, G. T.; ZHENG, Y.; LU, J. Adsorption of heavy metal ions by immobilized phytic acid. **Applied Biochemistry and Biotechnology**, v. 63-65, n. 1, p. 731-741, 1997.
- WU, K.; ZHANG, W.; ADDIS, P. B.; EPLEY, R. J.; SALIH, A. M.; LEHRFELD, J. Antioxidant properties of wild rice. **Journal of Agricultural and Food Chemistry**, v. 42, n. 1, p. 34-37, 1994.

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