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Effect of soaking process on nutrient bio-accessibility and phytic acid content of brown rice cultivar

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ABSTRACT

Grain soaking treatment could reduce phytates improving mineral and protein bioavailability. To evaluate the effects of soaking process with lactic acid on phytates and nutrient content (Fe, Zn, and proteins) from whole rice grain, a surface response methodology with a 3^2 factorial experimental design was used. The independent variables were time (24, 36 and 48 h) and temperature (35°, 45° and 55 °C) of soaking.

The results showed that soaking decreased total phosphorus (TP) in the range of 35–92.4 mg/100 g compared to 255 mg/100 g in whole rice. The losses of phytate in the grains were between 87 and 91%, the remnant phytic acid phosphorus in the grains being between 15.1 and 20.9 mg/100 g, and the most effective treatment was soaking at 45 °C for 48 h. The losses of the different nutrients were high in all of the different soaking treatments, reaching 50% for Fe, more than 64% for Zn, and around 45% for proteins. Although protein digestibility and mineral dialyzability were improved, acid soaking of brown rice would not be suggested as a useful process to reduce phytates from whole rice grains, if the production of a staple food is the main objective.

1. Introduction

Cereals are members of the grass family (*Poaceae* or *Gramineae*) and produce dry one-seeded fruits (caryopsis) which are commonly called a kernel or grain. All cereals consist of a fruit coat (pericarp) surrounding the seed. The seed contains an embryo (germ) and an endosperm surrounded by a nucellar epidermis and a seed coat (testa). In addition, some cereals, such as rice, oats, and barley retain their husk during threshing, which must be removed to produce acceptable foods for humans. The bran consists of outer layers of the grain of cereals, removed during the process of milling and used as a source of dietary fiber. It generally comprises the fruit wall, seed wall, aleurone layer, and small amounts of the starchy endosperm and germ. Whole grain (WG) cereals and pseudocereals were defined by the American Association of Cereal Chemist International (AACCI) in 1999 as "the intact, ground, cracked, or flaked caryopsis (kernel or seed), whose principal anatomical components – the starchy endosperm, germ, and bran – are present in the same relative proportions as they exist in the intact caryopsis" (Frolich & Aman, 2010).

WG have always been recognized for their contribution of traditional nutrients, B vitamins, minerals, and dietary fiber to the diet. More recently, WG have been shown to be a good source of bioactive components (e.g. phytoestrogens and plant sterols) and antioxidants. In fact, the *in vitro* antioxidant activity of WG foods has been shown to be at parity with that of vegetables and fruits (Jones, Reicks, Adams, Fulcher, & Marquart, 2004). The effects of consumption of one type of WG do not necessarily reflect the magnitude of benefits for other WG due to the diversity of WG in terms of macronutrients, micronutrients, and bioactive components (Jones et al., 2002).

Food processing may produce either beneficial or deleterious effects on nutrient bioavailability. Regarding minerals, processing could increase the content of some minerals, destroy some inhibitors or form beneficial complexes between minerals and matrix components. The impact can be negative by deactivating enzymes that degrade inhibitors or by generating insoluble metal compounds (e.g. oxidation, precipitation) (Watzke, 1998).

Phytates are important inhibitors of mineral absorption, whose biosynthesis occurs only in the aleurone and embryo but not in the starchy endosperm. Regarding brown rice, the content of phytates is around 1300–2700 mg kg^{-1} (at 14 g moisture/100 g sample) (Juliano, 1985, p. 45). Hayakawa, Toma, and Igaue (1989) reported

Abbreviations: WG, whole grains; PA, phytic acid; WFP, water free phosphorus; WPPA, water phosphorus phytic acid; GPPA, grain phosphorus phytic acid; TP, total phosphorus; DML, dry matter losses; PL, protein losses; PD%, proteins digestibility; NPN, non protein digestion; DM%, dialyzable mineral.

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that inositol polyphosphate of the embryo and bran of mature brown rice is mainly hexaphosphate (IP6), with minor amounts of pentaphosphate (IP5), and tetraphosphate (IP4) reflecting some hydrolysis during storage, since only inorganic P and IP6 are present in the developing rice grain, while the endosperm has only the IP6.

At pH values normally occurring in foods, as well as under physiological conditions, phytic acid is negatively charged and has the potential to bind cations or other positively charged functional groups of molecules. Phytic acid forms complexes with minerals and trace elements *in vitro* and can influence bioavailability *in vivo* (Egli, Davidsson, Juillerat, Barclay, & Hurrell, 2003). High bioavailability of trace elements from the diet is of special importance for complementary foods based on cereals for infants; however, cereals are usually high in phytic acid, resulting in low iron bioavailability. Removal or degradation of phytic acid has been reported to increase absorption of both iron (Davidsson et al., 1994; Hurrell et al., 1992) and zinc (Kivistö, Cederblad, Davidsson, Sandberg, & Sandström, 1989).

Several processing techniques applied to seeds allow phytates hydrolysis, such as enzyme activation, improving enzyme activity present in the seed or activated during process such as soaking, germination, cooking and fermentation (Egli et al., 2003). Few researches have studied the effect of soaking process on phytase activity and phytic acid hydrolysis from cereals (Lestienne, Icard-Vernière, Mouquet, Picq, & Trèche, 2005; Liang, Han, Nout, & Hamer, 2009). However there are no studies about the effect of acid soaking on phytic acid content and nutrient losses of brown rice, in order to improve protein digestibility and mineral bio-accessibility.

The objective of this work was to evaluate the effect of soaking process (time and temperature) with lactic acid on phytic acid and nutrient content, mineral bio-accessibility and protein digestibility from brown rice (whole grain).

2. Materials and methods

2.1. Rice sample

Whole grains of brown rice (*Oryza sativa*) Fortuna variety were supplied by Molino Los Cerillos, Trimacer (Santa Fe, Argentina).

2.2. Soaking

To evaluate the effect of time (t) and temperature (T) during soaking on phytic acid and nutrient content, a surface response methodology was used. A 3^2 factorial experimental design with three central points was selected, resulting in 11 experiments. Factor levels were temperature (35, 45 and 55 °C) and soaking time (24, 36 and 48 h).

Approximately 25 g of brown rice was soaked with 6.6 g/L lactic acid solution (1:2 g:mL) in the different experimental conditions.

The moistened seeds were dried in an oven at 80 °C and kept at 4 °C until the analysis. The soaking liquid was analyzed for free phosphorus and phytic acid phosphorus.

2.3. Free phosphorus, phytic acid phosphorus and total phosphorus (TP)

Free phosphorus and phytic acid phosphorus from soaking water (WFP and WPPA, respectively), and phytic acid phosphorus and total phosphorus from brown rice (GPPA and TP, respectively) were determined according to AOAC Anion-Exchange Method and AOAC Phosphorus Method (AOAC, 1995; AOAC, 1993, chap. 26, pp. 334–339).

2.4. Chemical composition

Moisture and ash were determined by gravimetric measure according to AOAC 935.29 and AOAC 923.03 (AOAC, 1995), respectively. The protein content was determined by Kjeldahl method (AOAC 920.53) using 5.95 as nitrogen-to-protein conversion factor (AOAC, 1995). Assessment of minerals (Fe and Zn) was made by Flame Atomic Absorption Spectroscopy after dry ashing, using an Atomic Absorption spectrophotometer Analyst 300 (Perkin Elmer).

2.5. Protein digestibility

Protein digestibility (PD%) was determined as described by Rudloff and Lönnerdal (1992) with modifications. Approximately 2.3 g of sample was dispersed in 10 mL of distilled water, adjusted to pH 2 with 6 mol/L HCl and pepsin was added in order to have 1/15 and 1/20 enzyme/protein ratio. The samples were kept in dark, in a shaking water bath at 37 °C for 30 min. Then, the pH was gradually increased to 7.0 with 1 mol/L $NaHCO_3$ and 2.5 mL of pancreatin solution (0.4 g/100 mL 0.1 mol/L $NaHCO_3$) was added and incubated for 1 h at 37 °C. Digested samples were immediately placed in boiling water for 10 min to inactivate the enzymes. On 5 mL aliquot of the digested samples, 5 mL of 200 g/L TCA was added, stayed 30 min at 4 °C and centrifuged 30 min at 5000 × *g*. PD % was defined as non protein nitrogen (NPN) after digestion, in relation to the total nitrogen content (TN).

$$\mathrm{PD\%} = 100 \times \mathrm{NPN/TN}$$

2.6. Mineral dialyzability

A modification of the widespread *in vitro* Miller, Schricker, Rasmussen, and Van Campen (1981) method, according to Drago, Binaghi, and Valencia (2005) was followed. The samples were ground previously and then prepared to 10 g solid/100 g dispersion using deionized water. Aliquots (25 g) of homogenized samples were adjusted to pH 2.0 with 4 mol/L HCl and after addition of 0.8 mL pepsin digestion mixture (160 g/L pepsin (Sigma P-7000) solution in 0.1 mol/L HCl), were incubated at 37 °C for 2 h in a shaking water bath. At the end of pepsin digestion, dialysis bags containing 20 mL 0.15 mol/L PIPES (piperazine-N,N′-bis [2-ethane-sulfonic acid] disodium salt) buffer (Sigma P-3768) were placed in each flask and were incubated for 50 min in a shaking water bath at 37 °C. Pancreatin-bile mixture (6.25 mL of 25 g/L bile (Sigma B-8631), 4 g/L pancreatin (Sigma P-1750) solution in 0.1 mol/L $NaHCO_3$) was then added to each flask and the incubation continued for another 2 h. Then, bag contents were weighed and analyzed for its mineral content by flame atomic absorption spectroscopy. Mineral dialyzability was calculated from the amount of each dialyzed mineral expressed as a percentage of the total amount present in each sample.

$$\text{Dialyzable Mineral (DM\%)} = [D/(W \times A)] \times 100$$

Where: D is the total amount of dialyzed mineral (μg); W is the weight of sample (g) and A is the concentration of each mineral in the sample (μg/g).

2.7. Statistical analysis

Each experiment was performed at least in duplicate and each assay was performed by triplicate. Response surface and analysis of Variance was carried out using the software Statgraphics Plus 5.1. The statistical differences among samples were determined using the LSD (*least significant difference*) test. The accepted level of significance was $p < 0.05$.

3. Results and discussion

The effects of soaking treatments of brown rice on soaking WFP, WPPA, GPPA, TP, DML, PL, Fe and Zn retention in the grain are presented in Table 1. The degree of significance (p values) of the polynomial regression model coefficients, corresponding to each response are shown in Table 2.

The ANOVA results shows that lack of fit was not significant ($p > 0.05$) in all cases, which means that regression models obtained are adequate to explain the effect of factors (T and t) on each response.

3.1. Effect of soaking treatment on soaking WFP

It is observed (Table 2) that only the linear terms (t and T) and T^2 were significant. The maximum release of WFP was 71.5 mg free phosphorus/100 g of soaked grain (DM) and corresponded to 35 °C 48 h.

Fig. 1A shows the response surface for WFP, which increased with soaking time and presented a maximum at 45 °C.

Free phosphorus leaching can occur because it could be released by diffusion in soaking water and/or endogenous phytase could hydrolyze phytic acid during soaking releasing free phosphorous. This is in agreement with the fact that the acid conditions of the treatments (presence of lactic acid in soaking water), promotes the phytates enzymatic hydrolysis (liberating P as free P and mono, di, tri, P-inositol), because the endogenous phytases have the optimum activity at pH 4–5 and 40–50 °C (Yoshida, Tanaka, & Kasai, 1975).

3.2. Effect of soaking treatment on WPPA

For this response, the effect of all terms corresponding to the regression model was significant, except for t. Fig. 1B shows the response surface for WPPA, which increased with soaking temperature and the higher values were obtained at 55 °C. The content of WPPA depends on the diffusion of phytic acid to the water and its hydrolysis for the action of phytase activity.

3.3. Effect of soaking treatment on GPPA

In this case, none of the terms of the polynomial were significant ($p > 0.05$). However, the significance of t (p: 0.0541) and T^2 (0.0512) are not negligible. Moreover, the differences among experimental values are not too high, so the experimental error could overlap the effect of each experimental factor.

Fig. 2A shows the response surface for GPPA. Soaking reduced phytate levels in the grains from 87 to 91%, considering that brown rice used in the experiments have a GPPA content of 174.9 mg/100 g. The lowest GPPA value was obtained at 45 °C – 48 h soaking, this value representing about 10% of that corresponding to brown rice without soaking. As was mentioned before, phytase activity is higher at this temperature, promoting phytic acid hydrolysis (Yoshida et al., 1975). These results indicate that soaking treatment in the presence of lactic acid is an effective treatment to reduce phytic acid from whole grain rice.

3.4. Effect of soaking treatment on TP

ANOVA results indicate that the only significant term was t. Fig. 2B shows the response surface for TP. The lower TP values were obtained at 48 h soaking. Considering that TP content in brown rice grains was 255 mg P/100 g DM sample, Phosphorus losses varied between 64% and 86%.

3.5. Effect of soaking treatment on DML

DML during soaking process depended on t and T, being the effects of both experimental factors significant for all terms, except for that of T^2 (Table 2). Experimental values of DML showed that lixiviation increases with t. These losses could be explained taking into account that acid conditions would increase cell wall permeability for soluble solids (Liang et al., 2009).

3.6. Effect of soaking treatment on PL

PL followed similar tendency as that of DML. ANOVA results indicate that the terms t, t^2, and T^2 were significant. PL values varied between 33.2% and 45.4%, the maximum values were obtained at 45 °C. These results could be explained taking into account that, on one hand, acid conditions favor rice globulin dispersibility (Ohishi, Kasai, Shimada, & Hatae, 2003), which would increases with t and T and, on the other hand, some protein denaturalization would occur at higher temperature (55 °C) reducing protein diffusion (Bourdillon, 1951; O'dell & De Boland, 1976; Smith & Rockis, 1957).

3.7. Retention of minerals (Fe and Zn)

As it is expected, Fe retention decreased with t and the minimum Fe retention (maximum losses) were obtained at 45 °C (Fig. 3A). The effect of both experimental factors was significant (t and T^2) and the losses due to the soaking were between 51 and 69%.

According to Lestienne et al. (2005) Fe content of seeds soaked for 24 h at 30 °C was significantly lower than unsoaked seeds in all species except in maize and the biggest reduction of Fe content occurred in rice grains (60%).

Table 1
Effect of soaking treatments on water free phosphorous (WFP), water phosphorus phytic acid (WPPA), grain phosphorus phytic acid (GPPA), total phosphorus (TP), dry matter losses (DML), protein losses (PL), Fe and Zn retention (%).

Soaking treatments	WFP mg/100 g	WPPA mg/100 g	GPPA mg/100 g	TP mg/100 g	DML mg/100 g	PL mg/100 g	Fe Retention %	Zn Retention %
35 °C 24 h	47.8	41.5	20.9	92.4	11.8	33.2	49.2	36.0
35 °C 36 h	51.9	18.4	18.5	65.0	14.9	37.4	39.2	26.6
35 °C 48 h	71.5	14.5	17.9	75.0	15.5	37.0	36.0	26.0
45 °C 24 h	45.8	21.0	19.1	85.0	12.3	36.9	41.2	32.0
45 °C 36 h	66.2	20.5	17.0	66.0	14.9	45.2	33.8	23.0
45 °C 36 h	63.5	26.0	18.0	71.0	14.7	45.4	31.2	21.5
45 °C 36 h	68.4	22.0	19.0	57.0	14.4	45.2	33.4	25.0
45 °C 48 h	66.0	19.9	15.1	35.0	15.3	44.9	34.0	28.0
55 °C 24 h	27.4	57.7	24.0	81.3	12.5	36.9	40.7	30.8
55 °C 36 h	32.6	35.2	20.2	65.0	13.8	36.8	39.0	22.0
55 °C 48 h	47.5	67.7	20.9	65.0	13.1	38.9	35.0	26.6

Table 2
Degree of significance (p values) of the polynomial regression model coefficients, corresponding to each response.

Source of variation	Response							
	WFP	WPPA	GPPA	TP	DML	PL	Fe Retention	Zn Retention
Time (t)	0.0087	0.1209	0.0541	0.0405	0.0056	0.0182	0.0168	0.0516
Temperature (T)	0.0088	0.0064	0.0861	0.3486	0.0324	0.1176	0.1046	0.1658
t^2	0.4364	0.0403	0.5873	0.3898	0.0190	0.0447	0.0825	0.0306
$t \times T$	0.5462	0.0227	0.9647	0.9453	0.0204	0.3787	0.1151	0.2404
T^2	0.0102	0.0138	0.0512	0.1576	0.0832	0.0065	0.0382	0.7184
Lack-of-fit	**0.0949**	**0.0571**	**0.4082**	**0.2047**	**0.3655**	**0.0859**	**0.2651**	**0.6534**

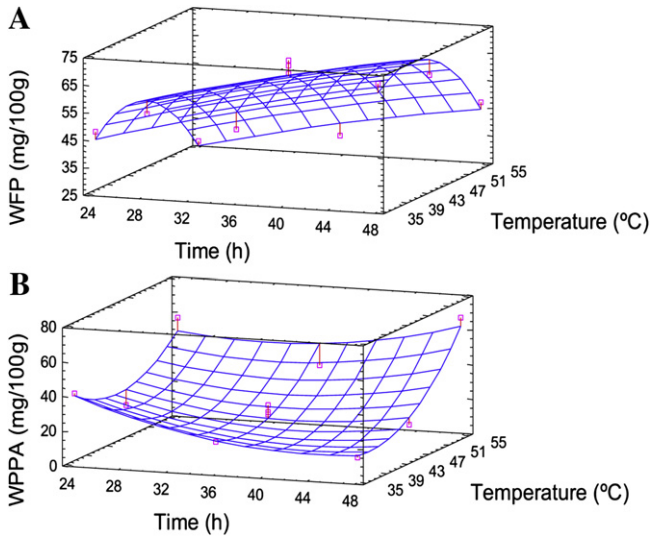

Fig. 1. Response surface for: A. Soaking water free phosphorus: WFP (mg P/100 g sample). B. Soaking water phosphorus phytic acid WPPA (mg P/100 g sample).

Retention of Zn showed similar tendency as Fe retention (Fig. 3B). However, Zn losses were higher than those of Fe (64–78.5%). This could be due to the fact that both minerals are linked to different molecules and located in different places in the seed. Zinc is found in a large number of enzymes and structural proteins (Lestienne et al., 2005).

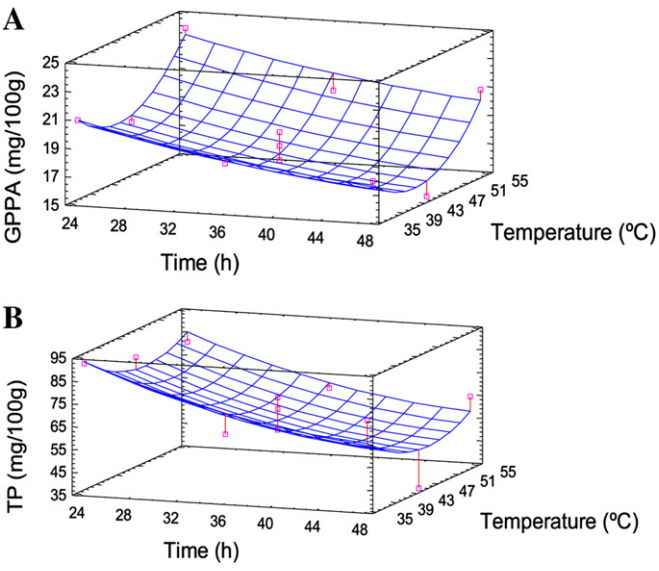

Fig. 2. Response surface for: A. Grain phosphorus phytic acid: GPPA (mg/100 g sample). B. Total phosphorus in grain: TP.

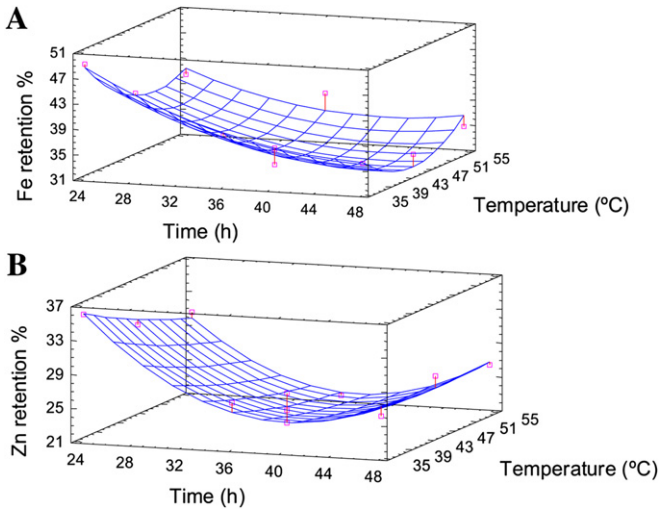

Fig. 3. Response surface for: A. Percentage of retention of Fe in the grain. B. Percentage of retention of retention of Zn in the grain.

Our results suggest that minerals would dissolve in acid soaking water together with some soluble components of rice grains, as was seen by Ohishi et al. (2003) in the soaking rice in 0.2 M acetic acid for 1 h at 20 or 50 °C.

3.8. Protein digestibility

As shown in Table 3, PD% increased after the softest soaking treatment (35 °C – 24 h) compared to brown rice without soaking, and higher time and temperature did not imply a further increase. This means that a reduction about the 88% of the content of PA in the above mentioned condition of soaking was enough to increase protein digestibility.

3.9. Mineral dialyzability

Table 3 shows that the softest soaking treatment (35 °C – 24 h) improved FeD% respect to brown rice without treatment, but increasing time and temperature did not imply a further increasing.

Table 3
Protein digestibility and mineral dialyzability of selected soaking treatments.

Sample	Protein digestibility (%)*	Mineral dialyzability (%)*	
		Zn	Fe
Brown rice	**74.9 ± 0.19**[a]	**9.89 ± 1.42**	**ND**
35 °C – 24 h	83.6 ± 2.23[b]	26.16 ± 1.36[a]	27.45 ± 0.6[a]
45 °C – 48 h	86.3 ± 0.18[b]	35.97 ± 3.25[b]	30.36 ± 2.05[a]

*Mean ± standard deviation; different letters mean significant differences between samples ($p < 0.05$); ND: Fe non-detected in dialyzate.

However, in the case of ZnD% greater time and higher temperature soaking, the better the dialyzability. This means that the 88% reduction of the content of phytic acid was enough to increase the bio-accessibility of Fe but not for Zn, where a reduction of phytic acid increased ZnD by 164.5% and 263.7% for 35 °C – 24 h and 45 °C – 48 h respectively, compared to brown rice.

4. Conclusion

Approximately 91% of phytic acid was removed from whole rice by soaking the grains in acidic conditions (45 °C – 48 h soaking). The reduction was caused by the activation of endogenous phytase and leaching of phytic acid in the soaking water. However, while protein digestibility and mineral dialyzability were improved, soaking process produced significant losses of minerals and proteins. Thus, acid soaking of brown rice would not be suggested as a useful process to reduce phytic acid from whole rice grains, if the production of a staple food that will not be fortified is the main objective. However, acid soaking rice could be useful to produce a low phytate starchy ingredient to be used in rice base formulated foods.

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Supporting Information Available: this information is available free of charge via the internet at http://pubs.acs.org.

References

AOAC international. (1995). In W. Horwitz (Ed.), *Official methods of analysis* (17th ed.)..
AOAC Phosphorus 964.06. (1993). *Methods of analysis*. Washington D.C.: Association of Official Analytical Chemistry.
Bourdillon, J. (1951). A crystalline bean seed protein in combination with phytic acid. *Journal of Biological Science, 189*, 65.
Davidsson, L., Galan, P., Kastenmeyer, P., Cherouvrier, F., Juillerat, M. A., Hercberg, S., et al. (1994). Iron bioavailability studied in infants: the influence of phytic acid and ascorbic acid in infant formulas based on soy isolate. *Pediatric Research, 36*(6), 816–822.
Drago, S. R., Binaghi, M. J., & Valencia, M. E. (2005). Effect of gastric digestion pH on iron, zinc and calcium availability from preterm and term starting infant formulas. *Journal of Food Science, 70*, S107–S112.
Egli, I., Davidsson, L., Juillerat, M. A., Barclay, D., & Hurrell, R. (2003). Phytic acid degradation in complementary foods using phytases naturally occurring in whole grain cereals. *Journal of Food Science, 68*(5), 1855–1859.
Frolich, W., & Aman, P. (2010). Whole grain for whom and why? *Food & Nutrition Research, 54*, 5056.
Hayakawa, T., Toma, Y., & Igaue, I. (1989). Purification and characterization of acid phosphatases with or without phytase activity from rice bran. *Agricultural and Biological Chemistry, 53*(6), 1475–1483.
Hurrell, R. F., Juillerat, M. A., Reddy, M. B., Lynch, S. R., Dassenko, S. A., & Cook, J. D. (1992). Soy protein, phytate, and iron absorption in humans. *American Journal of Clinical Nutrition, 56*(3), 573–578.
Jones, J. M., Reicks, M., Adams, J., Fulcher, G., & Marquart, L. (2004). Becoming proactive with the whole-grains message. *Nutrition Today, 39*(1), 10–17.
Jones, J. M., Reicks, M., Adams, J., Fulcher, G., Weaver, G., Kanter, M., et al. (2002). The importance of promoting a whole grain foods message. *Journal of the American College of Nutrition, 21*(4), 293–297.
Juliano, B. O. (1985). *Rice: Chemistry and technology* (2nd ed.). St. Paul, Minnesota (USA): American Association of Cereal Chemists, Inc, Gross Composition.
Kivistö, B., Cederblad, A., Davidsson, L., Sandberg, A. S., & Sandström, B. (1989). Effect of meal composition and phytate content on zinc absorption in humans from an extruded bran product. *Journal of Cereal Science, 10*(3), 189–197.
Lestienne, I., Icard-Vernière, C., Mouquet, C., Picq, C., & Trèche, S. (2005). Effects of soaking whole cereal and legume seeds on iron, zinc and phytate contents. *Food Chemistry, 89*, 421–425.
Liang, J., Han, B., Nout, M. J., & Hamer, R. J. (2009). Effect of soaking and phytase treatment on phytic acid, calcium, iron and zinc in rice fractions. *Food Chemistry, 115*, 789–794.
Miller, D. D., Schricker, B. R., Rasmussen, R. R., & Van Campen, D. (1981). An in vitro method for estimation of iron availability from meals. *American Journal of Clinical Nutrition, 34*, 2248–2256.
O'dell, B. L., & De Boland, A. (1976). Complexation of phytate with proteins and cations in corn grains and oilseed meals. *Journal of Agriculture and Food Chemistry, 24*, 804–808.
Ohishi, K., Kasai, M., Shimada, A., & Hatae, K. (2003). Effect of acetic acid added to cooking water on the dissolution of proteins and activation of protease in rice. *Journal of Agricultural Food Chemistry, 51*, 4054–4059.
Rudloff, S., & Lönnerdal, B. (1992). Solubility and digestibility of milk protein in infant formulas exposed at different heat treatments. *Journal of Pediatric Gastroenterology and Nutrition, 15*, 25–33.
Smith, A. K., & Rockis, J. J. (1957). Phytin elimination in soybean protein isolation. *Journal of the American Chemical Society, 79*, 633.
Watzke, H. J. (1998). Impact of processing on bioavailability examples of minerals in foods. *Trends in Food Science & Technology, 9*, 320–327.
Yoshida, T., Tanaka, K., & Kasai, Z. (1975). Phytase activity associated with isolated aleurone particles of rice grains. *Agricultural and Biological Chemistry, 39*(1), 289–290.