Calcium-Binding Capacities of Different Brans under Simulated Gastrointestinal pH Conditions. In Vitro Study with ^{45}Ca

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The present study was performed to investigate calcium-binding characteristics of different brans under simulated gastrointestinal pH conditions and to explore the significance of dietary fiber, oxalate, and phytate for calcium binding. Different brans (rice, rye, soy, fine wheat, coarse wheat, and oat) and $CaCl_2$ solution containing ^{45}Ca were incubated at 37 °C at gastric pH (2.2) followed by buffering steps of 1 degree from pH 3.0 to pH 8.0. Total calcium binding and calcium-binding capacity of the pH 2.2 soluble bran fraction were determined. Additionally, oxalate and phytate contents of brans and solubility profiles of phytic acid were investigated. Calcium-binding capacities of brans showed a clear pH dependence. At gastric pH calcium binding was low in all brans, ranging from 0.022 to 0.040 mmol of calcium/g of bran. Soy bran, nearly phytate-free, showed higher binding values up to pH 4.0 and lower values between pH 5.0 and 8.0. In all other brans, binding values increased strongly with increasing pH in the quantitative order rice bran > coarse wheat bran > fine wheat bran > rye bran > oat bran. The solubility profiles indicate that in the cases of rye, wheat, and rice bran phytate accounts for 70−82% of their total calcium-binding capacities. The results suggest that dietary fiber makes no important contribution to calcium binding, except for soy and oat brans. Oxalate plays only a minor role in calcium binding by brans.

Keywords: *Calcium binding; phytate; dietary fiber; bran; ^{45}Ca*

INTRODUCTION

Hypercalciuria is one of the most important risk factors for calcium oxalate stone formation. A calcium excretion above the normal range (≥5 mmol/24 h) is observed in ∼60% of patients. In patients with absorptive hypercalciuria dietary calcium restriction is often not effective enough to reduce urinary calcium excretion below the critical value of 8 mmol/24 h (*1*). Due to the risk of severe side effects of thiazides such as hypotension, hypokalemia, hyperuricemia, and impairment of glucose tolerance, this medication is recommended only in cases of pronounced hypercalciuria.

The application of bran may be an alternative in these patients because bran can bind calcium during gastrointestinal transit (*2*) and thus reduce calcium absorption (*3*), urinary excretion (*4*, *5*), and stone recurrence (*6*). Weaver et al. found a linear calcium binding to wheat bran in vitro over a wide range of calcium levels (*3*).

Brans differ widely with regard to their physical and chemical properties, so different calcium-binding capacities of brans might be expected. Gastrointestinal pH conditions may also exert a great influence on the calcium-binding properties of brans; there is evidence for a strong pH dependence of calcium-binding capacity of brans. Phytate and dietary fiber are known to influence calcium absorption due to binding of calcium in the gastrointestinal tract. Therefore, the present study was performed to investigate calcium-binding characteristics of different brans under simulated gastrointestinal pH conditions and to explore the significance of dietary fiber and phytate for calcium binding.

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MATERIALS AND METHODS

Calcium Binding to Brans. Calcium-binding capacity was determined in rice, rye, soy, fine wheat, coarse wheat, and oat bran. Therefore, 1 g of each bran and 50 mL of an acidic $CaCl_2$ solution (pH 2.2) containing ^{45}Ca were mixed. The calcium concentration of the test solution amounted to 12.5 mmol/L, which is equivalent to 25 mg of calcium/g of bran (= 0.625 mmol of Ca/g of bran). The calcium/bran relationship chosen for investigation corresponded to the recommendations for calcium intake of the German Society of Nutrition (1000 mg/day) (*7*) and to the amount of bran (40 g/day) that has been found in human studies to be effective in decreasing calcium excretion (*4*, *5*). Each tube also contained 18500 Bq of ^{45}Ca. The test solution was incubated at 37 °C and adjusted to pH 2.2 with hydrochloric acid, simulating mean gastric pH conditions.

One gram of each bran was suspended in 50 mL of the solution, mixed thoroughly, and incubated at 37 °C for 30 min at pH 2.2. After incubation, aliquots were centrifuged for 9 min at 13600*g*. An aliquot of the filtrate was sampled and the radioactivity of the unbound ^{45}Ca determined. The sampling was followed by buffering steps of 1 degree with Tris (1.25 mol/L) varying from pH 3.0 to 8.0. Calcium binding at each pH value was calculated on unbound ^{45}Ca from the clear supernatant of the centrifuged sample, analyzed by liquid scintillation counting with respect to a control (labeled $CaCl_2$ solution without bran) and the initial calcium concentration.

Calcium Binding to pH 2.2 Soluble Bran Fraction. After incubation of bran with $CaCl_2$ solution at 37 °C for 30 min at pH 2.2, the insoluble fraction of the bran suspension was vacuum-filtered and the amount of calcium bound to insoluble components was added to the clear filtrate. The clear filtrate was adjusted with Tris to pH 6.0 and 8.0, respectively. The determination of calcium binding by the soluble bran components was performed according to the method described above.

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Table 1. Constituents of Brans

	rice	rye	soy	wheat (fine)	wheat (coarse)	oat
protein[a] (%)	17.4	12	14	17	14−16	17.8
fat[a] (%)	0.7	5−7	3	4	4−7	8.9
starch/sugar[a] (%)	10.7	12−14		18	5−6	39.5
total dietary fiber (%)	33.2	40.7	58.4	47.9	48.8	20
insoluble dietary fiber (%)	28.3	36.1	50.2	44.4	45.8	10.8
soluble dietary fiber (%)	4.9	4.6	8.2	3.5	3.0	9.2
ash (%)	15.8	5.9	5.8	7.5	7.6	3.2
water[a] (%)	6	9	7	7	9	8
potassium (mg/g)	18.8	13.4	17.0	14.9	14.9	6.7
calcium (mg/g)	12.7	1.0	0.9	0.7	0.9	0.8
magnesium (mg/g)	16.2	4.2	2.4	5.4	6.1	2.1
oxalate (mg/g)	1.22	0.90	0.22	2.10	2.20	0.21
phytic acid (mg/g)	95.88	43.30	1.71	51.43	54.20	0.53
inorganic phosphate (μmol/g)	65.2	9.6	32.5	37.1	37.6	21.1

[a] Manufacturer-supplied data.

Calcium Binding to Brans at Different Calcium Concentrations. Calcium binding by rye, soy, fine and coarse wheat bran, was assessed by mixing 1 g of each bran with various quantities of calcium in 50 mL of $CaCl_2$ solution containing ^{45}Ca. Calcium concentrations varied from 10 mg (0.250 mmol) of calcium/g of bran to 25 mg (0.625 mmol), 50 mg (1.250 mmol), 100 mg (2.500 mmol, and 150 mg (3.750 mmol) of calcium/g of bran. After incubation of each bran at 37 °C for 8 h, aliquots were centrifuged for 9 min at 13600*g*. Determination of calcium binding by each bran at different calcium concentrations was performed according to the method described above.

Solubility Profiles of Phytic Acid. Additionally, solubility profiles of phytic acid were investigated in phytate-rich rice, rye, fine and coarse wheat bran corresponding to the design described above. Phytic acid contents of brans and of a control without bran dissolved in the $CaCl_2$ solution at different pH values were analyzed according to the method of Latta and Eskin (*8*).

Analysis of Bran Constituents. Mineral content of brans was determined after dry ashing and dissolving of ash in hydrochloric acid. Quantitative analysis was performed for potassium by flame photometry and for calcium and magnesium by atomic absorption spectrophotometry. Oxalic acid was determined by ion chromatography after acidic extraction. The content of soluble and insoluble fiber was measured with the Fibretec System (Tecator AB) in the homogenized bran. Phytic acid content of brans was analyzed according to the method of Latta and Eskin (*8*). The content of inorganic phosphate in brans was calculated from total phosphate content and organic-bound phosphate, both determined according to the method described by the AOAC (*9*).

Statistical Methods. The results are expressed as means ± standard deviation (SD). Nonparametric Mann−Whitney rank-sum test was used to compare calcium-binding capacity and phytate solubility profiles between different brans. Calcium binding and solubility of phytate of bran at different pH values were compared by Wilcoxon matched pairs signed rank test. The α level for significance was set at $P < 0.05$. All P values are two-tailed.

RESULTS

Components of Brans with Calcium-Binding Properties. Total dietary fiber content was highest in soy bran, whereas rice bran contained only about half and oat bran one-third of the amount of fiber present in soy bran (Table 1). Moreover, the highest proportions of soluble dietary fiber were determined in soy and oat brans. The phytic acid content varied considerably among brans. Phytic acid content was highest in rice, lowest in soy and oat brans, and similar in wheat and rye brans. Although oxalic acid content was low in all brans, the oxalic acid content of wheat bran was ∼10 times that of soy and oat brans, respectively. Rye and rice brans contained about half of the amount present in wheat bran. Content of inorganic phosphate was low in all brans.

Calcium Binding to Brans. Calcium-binding capacities of brans showed a clear pH dependence (Figure 1). At gastric pH 2.2 calcium binding was low in all brans. Calcium binding remained constantly low up to pH 4.0 in all brans, except soy bran. Starting at pH 4.0, binding values increased in rice, rye, oat, and wheat brans with increasing pH values, leading to very different calcium-binding capacities at duodenal/jejunal pH 6 and ileal pH 8, respectively. The calcium-binding capacity of soy bran, nearly phytate-free, increased significantly from pH 2.2 to 4.0. Calcium binding of soy bran was ∼4 times higher at pH 4.0 and significantly lower between pH 5.0 and 8.0 compared to all other tested brans. In the other brans, binding values increased strongly with increasing pH in the quantitative order rice bran > coarse wheat bran > fine wheat bran > rye bran > oat bran. The maximum calcium-binding capacity was achieved in all brans at pH 8.0 (Table 2).

Calcium Binding to pH 2.2 Soluble Bran Fraction. To estimate the significance of phytate in calcium-binding capacity, an isolation of pH 2.2 soluble bran components was performed. Bran phytate is highly soluble at pH 2.2, whereas dietary fiber components are insoluble under these conditions (*10*, *11*). The calcium-binding capacities of soluble soy bran fraction at pH 6.0 (7%) and pH 8.0 (9%) were lowest among all brans, whereas binding levels of rice, rye, and wheat brans were highest with values ranging from 74 to 86% of total binding capacity. The calcium-binding capacity of the soluble oat bran fraction amounted to 27% at pH 6.0 and 34% at pH 8.0. Calcium binding of the pH 2.2 soluble fraction increased with increasing pH (pH 6.0 and 8.0) in the quantitative order rice bran > coarse wheat bran > fine wheat bran > rye bran > oat bran > soy bran (Table 3).

Calcium Binding to Brans at Different Calcium Concentrations. The calcium-binding capacity varied among brans (Figure 2). Binding capacity was lowest in soy bran at each calcium concentration, whereas binding capacities of rye, fine and coarse wheat brans differed depending on the calcium concentration. Calcium-binding capacity was highest at the lowest calcium concentration (0.250 mmol of Ca/g of bran), ranging from 49% in soy bran to 78% in coarse wheat bran, and decreased with increasing concentration of calcium. At

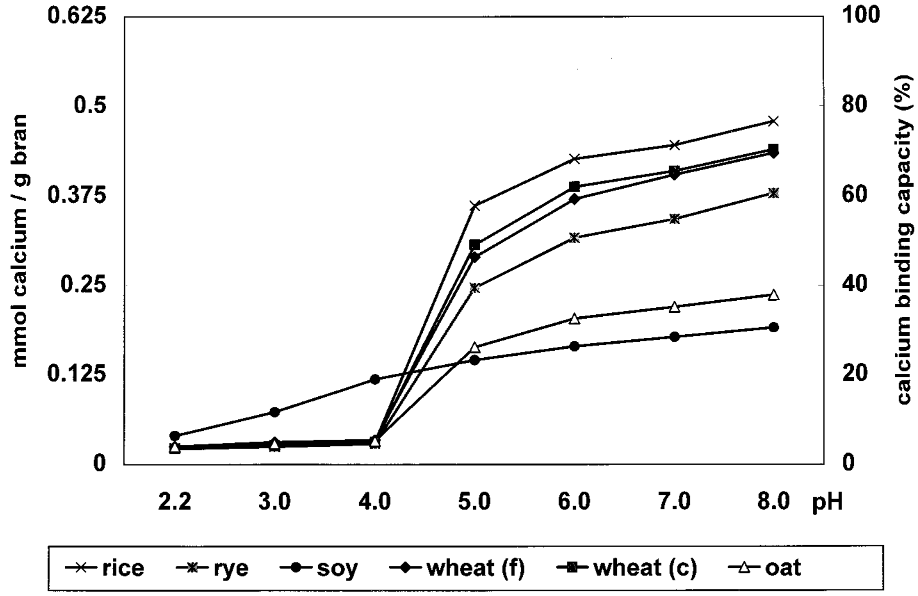

Figure 1. Calcium-binding capacity of brans under simulated gastrointestinal pH conditions ($n = 6$).

Table 2. Calcium Binding (Millimoles per Gram of Bran) by Brans at Different pH Values ($n = 6$; Mean ± SD)

	pH 2.2	pH 3.0	pH 4.0	pH 5.0	pH 6.0	pH 7.0	pH 8.0
rice	0.023 ± 0.004	0.027 ± 0.002	0.031 ± 0.004	0.362 ± 0.004	0.427 ± 0.002	0.446 ± 0.002	0.479 ± 0.003
rye	0.022 ± 0.002	0.025 ± 0.003	0.029 ± 0.003	0.247 ± 0.011	0.317 ± 0.007	0.343 ± 0.007	0.379 ± 0.008
soy	0.040 ± 0.004	0.073 ± 0.003	0.119 ± 0.005	0.146 ± 0.004	0.165 ± 0.003	0.178 ± 0.002	0.191 ± 0.003
wheat (fine)	0.025 ± 0.001	0.031 ± 0.003	0.034 ± 0.004	0.290 ± 0.004	0.371 ± 0.002	0.405 ± 0.002	0.435 ± 0.002
wheat (coarse)	0.022 ± 0.000	0.026 ± 0.004	0.031 ± 0.004	0.307 ± 0.005	0.388 ± 0.003	0.410 ± 0.002	0.440 ± 0.002
oat	0.025 ± 0.003	0.029 ± 0.002	0.033 ± 0.004	0.164 ± 0.003	0.204 ± 0.003	0.220 ± 0.002	0.237 ± 0.003
P[a]	*	*	*	#	#	+	+

[a] * = significant difference between soy and all other brans; # = significant difference between all brans; + = significant difference between all brans, exept fine and coarse wheat brans.

Table 3. Calcium Binding (Millimoles per Gram of Bran) by pH 2.2 Soluble Bran Fraction ($n = 6$; Mean + SD)[a]

	pH 6.0			pH 8.0		
	total binding	binding of pH 2.2 soluble fraction	% of total binding	total binding	binding of pH 2.2 soluble fraction	% of total binding
rice	0.427 ± 0.002	0.364 ± 0.004	85.2	0.479 ± 0.003#	0.414 ± 0.005*	86.4
rye	0.317 ± 0.007	0.245 ± 0.008	77.3	0.379 ± 0.008#	0.297 ± 0.005*	78.4
soy	0.165 ± 0.003	0.011 ± 0.004	6.7	0.191 ± 0.003#	0.017 ± 0.003	8.9
wheat (fine)	0.371 ± 0.002	0.278 ± 0.005	74.9	0.435 ± 0.002#	0.322 ± 0.003*	74.0
wheat (coarse)	0.388 ± 0.003	0.299 ± 0.003	77.1	0.440 ± 0.002#	0.338 ± 0.004*	76.8
oat	0.204 ± 0.003	0.055 ± 0.006	27.0	0.237 ± 0.003#	0.080 ± 0.004*	33.8

[a] # = significant difference in total binding of bran between pH 6.0 an 8.0; * = significant difference in binding of pH 2.2 soluble fraction between pH 6.0 and 8.0.

the highest calcium concentration (3.750 mmol of Ca/g of bran) only between 12 and 17% of calcium was bound by brans.

Solubility Profiles of Phytic Acid. The phytic acid contents of brans differed widely (Table 1). Phytic acid content was highest in rice (95.9 mg/g) and lowest in soy bran (1.7 mg/g) and oat bran (0.53 mg/g). At pH 4 between 83 and 93% of total phytic acid content was released into the $CaCl_2$ solution. Buffering to pH 6.0 led to a strong precipitation of phytate among all brans. No further changes in phytic acid solubility occurred after adjustment to pH 8 (Figure 3). The formation of insoluble calcium phytate started on transition from pH 4.0 to 5.0 and was finished at pH 6.0 (Table 4).

DISCUSSION

Calcium binding by bran can be due to the phytic acid, dietary fiber, uronic acid, and oxalic acid content of brans (*12*). Phytic acid has been assumed to play the major role in calcium binding. Phytic acid is able to bind mineral elements strongly due to its phosphate groups (*13*). Degree and tightness of calcium binding to phytic acid depend on pH, temperature, and ionic strength (*14*). To distinguish between the effect of dietary fiber and phytic acid on calcium-binding capacity, fractionation of the bran suspension was performed at pH 2.2, because at pH 2.2 the highest proportion of bran phytates is released into solution. The formation of insoluble calcium phytate requires that phytate in brans is released into solution, so that calcium instead of potassium and magnesium is bound.

The in vitro study with ^{45}Ca confirmed the ability of cereal brans from rice, rye, and wheat and of legume bran from soybean to bind calcium (*15*). The different brans showed a clear pH dependence with regard to their calcium-binding capacities, especially in the pH range of the small intestine. Starting from very low binding values at gastric pH 2.2 calcium-binding capacity increased continuously with increasing pH in all brans. A reason for the low calcium binding at gastric pH might be that most of the acid groups, for example, the phosphate ester of phytic acid, the carboxyl ester of

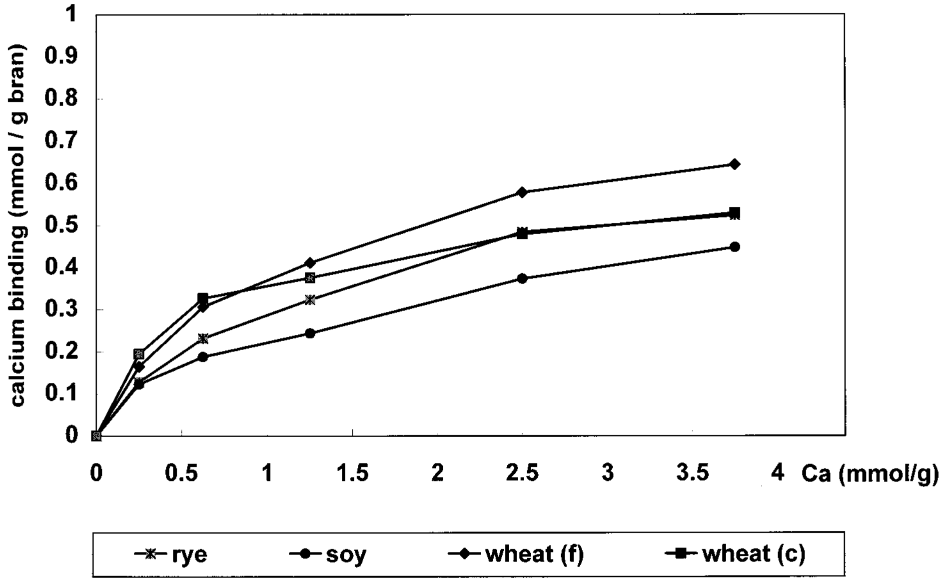

Figure 2. Calcium binding of brans at different calcium concentrations (n = 8).

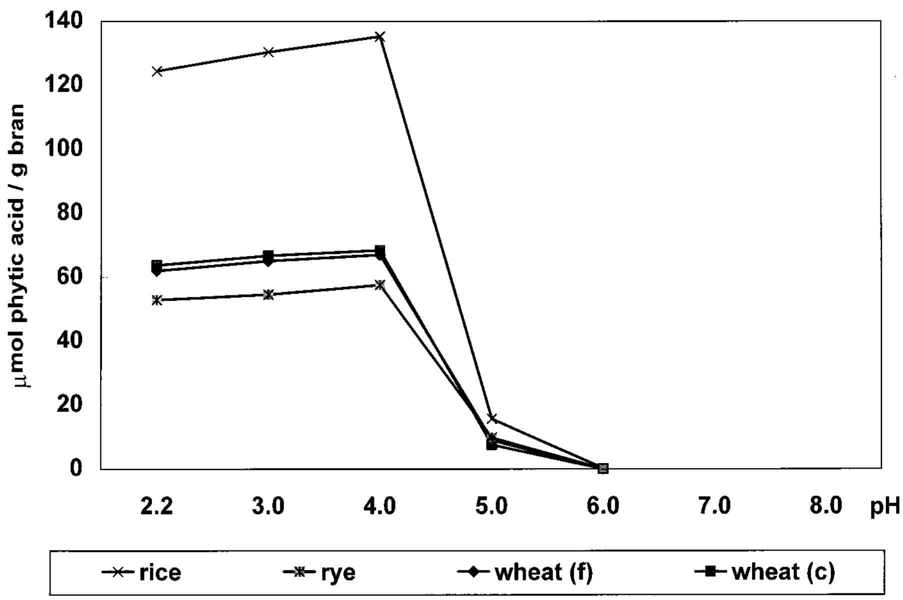

Figure 3. Solubility profiles of phytic acid of brans under simulated gastrointestinal pH conditions (n = 6).

Table 4. Solubility Profiles of Phytic Acid (Micromoles per Gram of Bran) of Brans at Different pH Values (n = 6; Mean + SD)[a]

	pH 2.2	pH 3.0	pH 4.0	pH 5.0	pH 6.0	pH 8.0
rice	124.46 ± 2.12	130.4 ± 1.35*	135.3 ± 2.21*#	15.7 ± 0.73+	0.26 ± 0.60°	0.22 ± 0.91
rye	52.95 ± 1.47	54.6 ± 1.56	57.6 ± 2.14*	9.8 ± 0.42+	0.05 ± 0.77°	0.01 ± 0.47
wheat (fine)	62.06 ± 1.71	65.1 ± 1.26*	67.1 ± 1.39*	9.0 ± 0.32+	0.02 ± 0.35°	0.01 ± 0.52
wheat (coarse)	63.79 ± 1.57	66.8 ± 0.93*	68.5 ± 1.15*	7.6 ± 0.33+	0.03 ± 0.21°	0.02 ± 0.39

[a] * = significant difference versus pH 2.2; # = significant difference versus pH 3.0; + = significant difference versus pH 4.0; ° = significant difference versus pH 5.0.

uronic acids, and oxalic acid, are undissociated under these conditions, thus having only a few free binding sites for calcium. Adjustment to pH 5.0 resulted in a varying increase in calcium binding.

With regard to the course of calcium binding, three different types of brans can be distinguished. The nearly phytate-free soy bran bound more calcium at gastric pH compared with all other brans. Binding capacity was highest at pH 4.0 but lowest in the pH range of 5.0–8.0. The proportions of the pH 2.2 soluble bran fraction of total calcium binding were 7% at pH 6.0 and 9% at pH 8.0, which indicates that the highest quantity of total calcium binding in soy bran is due to the fiber-containing insoluble bran fraction.

The phytate-rich cereal brans rice, rye, fine and coarse wheat showed a strong increase in binding values only during transition from pH 4.0 to 5.0. The quantitative order of binding was rice > coarse wheat > fine wheat > rye bran. The proportion of pH 2.2 soluble fractions of total binding in these brans was between 74 and 86%. Therefore, the contribution of the fiber-containing insoluble bran fraction to total calcium binding in these brans is relatively small.

The course of calcium binding in the nearly phytate-free oat bran is comparable to that of the other cereal brans, but binding capacities at different pH values ranged between those of rye and soy brans. The proportion of the pH 2.2 soluble oat bran fraction of total binding amounted to only 27% at pH 6.0 and 34% at pH 8.0, which indicates the important role of the fiber-containing insoluble fraction of oat bran in total calcium-binding capacity compared with all other cereal brans.

The solubility profiles of phytate indicate that in the cases of rye and coarse and fine wheat bran phytate accounts for ~70% and in rice bran for 82% of their total calcium-binding capacities. Calcium binding and decrease in solubility of phytate started on transition from pH 4.0 to 5.0 and was finished at pH 6. At pH 4.0 between 83 and 93% of phytate in rice, rye, and wheat brans was released into solution, which corresponds to results reported in the literature (*11*). Formation of insoluble, nonabsorbable calcium phytate was finished to an extent of 90% already at pH 5.0.

The calcium-binding capacity of phytic acid was found to be between 4.25 and 4.68 mol of calcium/mol of phytic acid. Each mole of phytate maximally binds 6 mol of calcium, but according to the results of Evans and Pierce (*16*) and Nolan et al. (*17*) only 5 mol of calcium is bound per mole of phytate on average. The difference from the maximal calcium binding by phytate may be due to the magnesium and calcium content of the brans.

Hydrolysis of phytate by endogenous phytases in bran can diminish calcium binding in the gastrointestinal tract of humans (*18*). The amount of endogenous phytase activity varies with the origin and the method of processing of the foodstuff. Of the cereal brans, only rye and wheat brans are rich in phytase (*19*). An inactivation of the endogenous phytase is caused by the usual heat treatment of brans. Due to the high content of unsaturated fatty acids, thermal treatment of brans is performed to reduce lipase and peroxidase activities.

Supposing that oxalic acid in brans is completely released into solution under simulated gastrointestinal pH conditions, it is suggested that maximally 0.010–0.024 mmol of calcium is bound per gram of bran.

An involvement of dietary fiber in calcium binding is expected in all brans, but it plays a prominent role in binding capacity only in soy and oat brans. The high content of pectine in soy bran, with a high proportion of carboxylic groups, implicates a substantial contribution of these fiber components to the calcium-binding capacity of soy bran. Protein, especially of soy bran, can also contribute to calcium binding. Kroll (*20*) found an in vitro binding capacity of soy protein of 0.26 mmol of calcium/g of protein at pH 7. A protein content of 14% in soy bran may therefore result in a potential binding capacity of ~0.04 mmol of calcium/g of bran. In cereal brans, calcium binding to protein is less probable without the involvement of phytic acid (*21*). In all other brans lignin, hemicellulose, and tannin, in the case of wheat bran, are suggested to play a more important role. The results suggest that dietary fiber makes no important contribution to calcium binding, except in soy and oat brans.

LITERATURE CITED

(1) Hesse, A.; Tiselius, H. G.; Jahnen, A. *Urinary Stones. Diagnosis, Treatment and Prevention of Recurrence*; Karger: Basel, Switzerland, 1997.

(2) James, W. P.; Branch, W. J.; Southgate, D. A. Calcium binding by dietary fibre. *Lancet* **1978**, *1* (8065), 638–639.

(3) Weaver, C. M.; Heaney, R. P.; Teegarden, D.; Hinders, S. M. Wheat bran abolishes the inverse relationship between calcium load size and absorption fraction in women. *J. Nutr.* **1996**, *126*, 303–307.

(4) Jahnen, A.; Heynck, H.; Gertz, B.; Classen, A.; Hesse, A. Dietary fibre: the effectiveness of a high bran intake in reducing renal calcium excretion. *Urol. Res.* **1992**, *20*, 3–6.

(5) Gleeson, M. J.; Thompson, A. S.; Mehta, S.; Griffith, D. P. Effect of unprocessed wheat bran on calciuria and oxaluria in patients with urolithiasis. *Urology* **1990**, *35*, 231–234.

(6) Ebisuno, S.; Morimoto, S.; Yasukawa, S.; Ohkawa, T. Results of long-term rice bran treatment on stone recurrence in hypercalciuric patients. *Br. J. Urol.* **1991**, *67*, 237–240.

(7) Deutsche Gesellschaft für Ernährung ÖGE, SGE, SVE (German Society of Nutrition). *Referenzwerte für die Nährstoffzufuhr (Recommendations for Nutrient Intake)*; Umschau/Braus: Frankfurt, Germany, 2000.

(8) Latta, M.; Eskin, M. A simple and rapid colorimetric method for phytate determination. *J. Agric. Food Chem.* **1980**, *28*, 1313–1315.

(9) Association of Official Analytical Chemists. Phytate in foods. Anion-exchange methods. *J. Assoc. Off. Anal. Chem.* **1986**, *69*, 356–357.

(10) Asp, N. G. Definition and analysis of dietary fibre. *Scand. J. Gastroenterol., Suppl.* **1987**, *129*, 16–20.

(11) Champagne, E. T.; Rao, R. M.; Liuzzo, J. A.; Robinson, J. W.; Gale, R. J. Miller, F. Solubility behaviors of the minerals, proteins and phytic acid in rice bran with time, temperature and pH. *Cereal Chem.* **1985**, *62*, 218–222.

(12) Kratzer, F. H.; Vohra, P. *Chelates in Nutrition*; CRC Press: Boca Raton, FL, 1986.

(13) Persson, H.; Baboo, M. N.; Frølich, W.; Nyman, M.; Asp, N. G. Binding of mineral elements by some dietary fibre components—in vitro (II). *Food Chem.* **1987**, *26*, 139–148.

(14) Graf, E. Calcium binding to phytic acid. *J. Agric. Food Chem.* **1983**, *31*, 851–855.

(15) Heynck, H.; Krampitz, G.; Hesse, A. Binding of calcium by brans under simulated gastrointestinal pH conditions. *In vitro* study with ^{45}Ca. *Eur. J. Clin. Nutr., Suppl. 3* **1995**, *49*, S250–S252.

(16) Evans, W. J.; Pierce, A. G. Calcium-phytate complex formation studies. *J. Am. Oil Chem. Soc.* **1981**, *58*, 850–851.

(17) Nolan, K. B.; Duffin, P. A.; McWeeny, D. J. Effects of phytate on mineral bioavailability. In vitro studies on Mg^{2+}, Ca^{2+}, Fe^{3+}, Cu^{2+} and Zn^{2+} (also Cd^{2+}) solubilities in the presence of phytate. *J. Sci. Food Agric.* **1987**, *40*, 79–85.

(18) Sandberg, A. S.; Andersson, H. Effect of dietary phytase on the digestion of phytate in the stomach and small intestine of humans. *J. Nutr.* **1988**, *118*, 469–473.

(19) Viveros, A.; Centeno, C.; Brenes, A.; Canales, R.; Lozano, A. Phytase and acid phosphatase activities in plant feedstuffs. *J. Agric. Food Chem.* **2000**, *48*, 4009–4013.

(20) Kroll, K. D. Effect of pH on the binding of calcium ions by soybean proteins. *Cereal Chem.* **1984**, *61*, 490–495.

(21) Rendleman, J. A. Cereal complexes: Binding of calcium by bran and components of bran. *Cereal Chem.* **1982**, *59*, 302–309.

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