Tempe Fermentation of Whole Grain Barley Increased Human Iron Absorption and *In Vitro* Iron Availability

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Abstract: In this study, iron absorption from a tempe fermented whole-grain barley meal was measured, and results were compared to *in vitro* estimation of available iron from an equivalent meal. The tempe meal (TM) was prepared from barley fermented with *Rhizopus oligosporus* to reduce the phytate content <0.5 μmol/g. Boiled barley with preserved phytate content was used as reference meal (BBM). Iron was added to obtain a total content of 3 mg meal in the human study, and to the *in vitro* meals 7 mg of iron was added. The iron absorption from TM and BBM was 5.5±1.5% and 3.0±0.7% respectively, and *in vitro* iron availability was 4.9±0.2% in TM and 1.7±0.1% in BBM (absorbed fraction of total iron/meal).

To conclude, iron absorption from a barley meal was improved by reducing the phytate content *via* tempe fermentation, and iron absorption was predicted by direction in the *in vitro* experiments.

Keywords: Iron absorption, *in vitro* iron availability, whole grain, vegetarian, tempe, tempeh fermentation.

INTRODUCTION

Vegetarianism has often been associated with possible nutritional inadequacy, especially with regard to iron. The absorption of iron for vegetarians is lower than for non-vegetarians [1-3]. In a study by Waldmann *et al.* [4], as many as 40% of young female vegans were found iron deficient and Alexander *et al.* [5] found that vegetarians had lower serum ferritin concentrations than age-sex matched omnivores. Since iron is a necessary micronutrient, there is a need for the development of novel vegetarian products with high iron bioavailability.

Whole grain cereals are often low in iron availability due to high contents of phytate (*myo*-inositolhexaphospate, $InsP_6$) and iron-binding polyphenols. Nevertheless, there are benefits from including cereals in the diet, like reduced risk of developing cardio vascular diseases, diabetes and obesity [6-9]. Barley is an underutilized cereal in Sweden, mainly used for animal feed [10]. Antioxidants and β-glucans are abundant in whole barley kernels [11] and compared to crops such as sorghum, millet, rice and beans, barley has a low polyphenol content [12-16]. However, the phytate content is more substantial [17] and hence, the main iron absorption inhibitor in barley. Therefore, to increase iron absorption from barley foods, degradation of the phytate content is required. Other important food factors affecting iron absorption are e.g. ascorbic acid and meat (enhancers), as well as polyphenols and calcium (inhibitors) [18, 19].

Phytate is hydrolysed via intermediate *myo*-inositol-phosphates ($InsP_5$ to $InsP_1$) into *myo*-inositol and inorganic phosphate. Degradation of phytate can be achieved during food processing through the addition of exogenous phytases [20], by micro-organisms producing phytase or by endogenous cereal phytases [21]. Most cereal phytases are reported to have their pH optima between 4.5 and 6.0 and temperature optima between 45 and 55°C [22]. In the study by Greiner *et al.* [23] barley was found to have two phytate-degrading enzymes – P1 and P2 – with optimal conditions reported to be 45°C, pH 5.0, and 55°C, pH 6.0, respectively. The micro fungus *Rhizopus oligosporus* used to ferment the vegetarian food tempe [24], has a phytase with optimal capacity at 65°C and pH 5.0 [25]. *R. oligosporus* has also previously been found to reduce phytate content of e.g. soybeans and oilcakes during fermentation, although not to very low levels [26-28]. However, effective enhancement of iron absorption requires almost complete degradation or removal of phytate. Hallberg *et al.* [29] and Brune *et al.* [30] found that, in order to markedly increase iron absorption, the total content of phytate-P from a meal should not exceed 10 mg. Additionally, the phytate to iron (InsP/Fe) molar ratio should be <1 and preferably <0.5 to achieve satisfactory iron absorption [31]. Nevertheless, we recently reported that it is possible to substantially reduce the phytate content and concomitantly preserving minerals in barley during tempe fermentation [32].

To assess the effect on iron bioavailability from reducing the phytate content in foods, the most reliable approach is considered conducting a human study. However, there is also a need for less time-consuming but valid models for screening of foods. Cost-effective *in vitro* models, capable of assessing the factors influencing iron availability, could therefore serve as important tools in predicting bioavailability [33].

To our knowledge, no studies in humans have been performed to determine the effect of low phytate tempe prod-

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1874-2882/08

ucts on iron absorption. The aim of the present investigation was to measure human iron absorption from tempe fermented whole grain barley and a reference boiled barley meal, and to compare the results with *in vitro* estimation of iron availability in equivalent meals using a computer-controlled gastrointestinal model.

MATERIALS AND METHODS

Raw Material and Preparation of Meals

The high ß-glucan (6.3%) and high amylose (40.7%) variety barley, cv. Karmosé [34], was obtained from Svalöf Weibull AB, (Svalöv, Sweden). This variety was chosen since the barley tempe was simultaneously optimised to induce low glycemic response, examined in a separate study [35]. Production processes were performed at Cerealia R&D (Järna, Sweden).

Table 1. Nutrient Content (Dry Weight) in a Portion of a TM (Tempe Meal) and a BBM (Boiled Barley Meal) of the Human and the *in vitro* Studies

		Human study (n = 8)		*In vitro* study (n = 4)		
		TM	**BBM**	**TM**	**BBM**	
Meal size	w.w.	115	115	57	57	g
	d.m.	56	56	28	28	g
Protein		2.4	3.2	1.2	1.6	g
Fat		0.9	1.0	0.45	0.5	g
Carbohydrates		14.8	17.2	7.4	8.6	g
Dietary fibre		3.0	4.3	1.5	2.2	g
Starch		12.0	17.7	6	8.9	g
Water		64.4	70.6	32.2	35.3	g
Iron (in kernels)		1.9	1.1	0.95	0.55	mg
Iron (total)		3.0	3.0	7.8	7.5	mg
		54	54	139	134	µmol
Phytate-P		6.5	72.3	3.3	36.2	mg
$InsP_{3-6}$		41	452	20	226	µmol
Molar ratio (InsP/Fe)		0.8	8.4	0.4	1.7	
Energy		328	384	164	192	kJ
		78	92	39	46	kcal

To produce the tempe meal (TM), fermentations were performed according to Eklund-Jonsson *et al.* [30]. Briefly, barley kernels were pearled for 5 min in a laboratory scale grinder (Strechel und Schrader, Germany), and the pearling residue was collected. The husk was removed from the pearling residue and discarded. Pearled kernels were soaked in lactic acid (0.3% lactic acid; 6+4 h of soaking at 48°C), boiled (10 min) and drained before the pearling residue was returned. The fungal strain, *Rhizopus oligosporus* ATCC 64063, was produced according to Feng *et al.* [36]. The drained kernels were mixed with the pearling residue (kernel/residue mixture; pH 4.5, water content 54%), inoculated with *R. oligosporus* and allowed to ferment for 27 h at 32°C. Tempe cakes were stored at minus 20°C until the day of serving. The boiled barley meal (BBM) was produced by pearling kernels for 1 min to remove husk, and simmering kernels in water (70 min) with NaCl (human study, 0.75 g; *in vitro* study, 0.38 g per portion). The nutrient contents per portion of the TM and BBM are shown in Table **1**.

Human Study – Experimental Design and Subjects

Iron absorption from the test meals was measured using two different radio iron tracers ^{55}Fe (BBM) and ^{59}Fe (TM) and each subject served as its own control. The meals were all served in a fasting state in an ABBA order, where A was the TM and B was the BBM. The test meals were given on four consecutive days and no food or drink was allowed for 3h after the meals. Two weeks after the last serving, the total retention of ^{59}Fe was measured by whole-body counting and blood samples were taken to determine the content of ^{55}Fe and ^{59}Fe in blood. The principal design of the human study has been described in detail by Hallberg [37].

The endogenous iron content of the meals was 1.9 mg (TM) and 1.1 mg (BBM). Iron was added (as $FeSO_4$* $7H_2O$) up to 3 mg per portion, in order to reach equal iron content in the meals, and to increase the accuracy in iron absorption measurement. This resulted in molar ratios (InsP/Fe) of 0.8 (TM) and 8.4 (BBM). The size of the test meals were ~115 g (w.w.) and according to the energy content, meals were suitable as a snack or part of a bigger meal. The TM and BBM had slightly different nutrient compositions, with higher macronutrient levels in BBM and higher endogenous iron content in TM (Table **1**). Water (200 ml) was served with each meal and before serving the TM, 0.75 g NaCl was added per portion (~115 g w.w.).

Table 2. Age and Weight Distribution, and Haematological Measurements Among Subjects (n=8) of the Human Study

	Mean ± SEM	**Range**
Age	33±4	21-53
Weight	73±5	60-106
Haemoglobin (g/l)	139±5	127-170
Serum ferritin (µg/l)	19±3	6-29
Total Iron-Binding Capacity TIBC, (µmol/l)	80±5	57-100
Blood sedimentation (mm)	7±3	1-11
Transferrin saturation (%)	22±3	10-31

Ten subjects were recruited by advertising on the campus of Göteborg University. The project was approved by the Ethics Research Committee of the Göteborg University and by the Radioisotope Committee at the Sahlgrenska University Hospital, Göteborg. The volunteers were given oral and written information about the aims and procedures prior to the study. The subjects height and weight were measured and blood samples were taken to establish; haemoglobin (g/l), serum ferritin (µg/l), transferrin saturation (%), blood sedi-

mentation (mm) and total iron-binding capacity (TIBC, µmol/l) (Table **2**). Blood samples were analysed at the Central Laboratory Sahlgrenska University Hospital, Göteborg. At the time of recruitment, non of those recruited were consuming nutrient supplements, they were all non-pregnant and without known gastrointestinal disorders. However, during the study, two subjects were excluded due to infection on the basis of high blood sedimentation values (>15 mm). The eight accepted subjects, two men and six women were aged 21-53, and thereof five were regular blood donors.

In Vitro Study Method

A computerised dynamic gastrointestinal model – TIM (TNO gastroIntestinal Model) described in detail by Minekus *et al*. [38], was used for the *in vitro* experiments (Fig. **1**). The model has previously been used for simulated digestion of cereal products to estimate iron dialyzability [39-41]. The model simulates gastric emptying and small intestinal passage of foods in the human intestinal tract with good accuracy and reproducibility [38, 42]. Physiological amounts of digestive juices are secreted [38] into the four compartments of the model - stomach, duodenum, jejunum and ileum. The composition of the different digestive juices and the experimental conditions are according to Haraldsson *et al*. [41].

During the experiment, the intestinal fluids were dialyzed through the semi-permeable hollow fibre filters (Hospal hemodialyzer HG-400 (cut-off 3-5 kD), Gambro, Renal Products, Lund, Sweden), allowing for dissolved nutrient and small complexes to diffuse across the membrane. The dialysed fluids were collected in the jejunal and ileal containers where samples were taken for determination of iron. Each experiment was repeated four times using a protocol suitable for semi-solid foods.

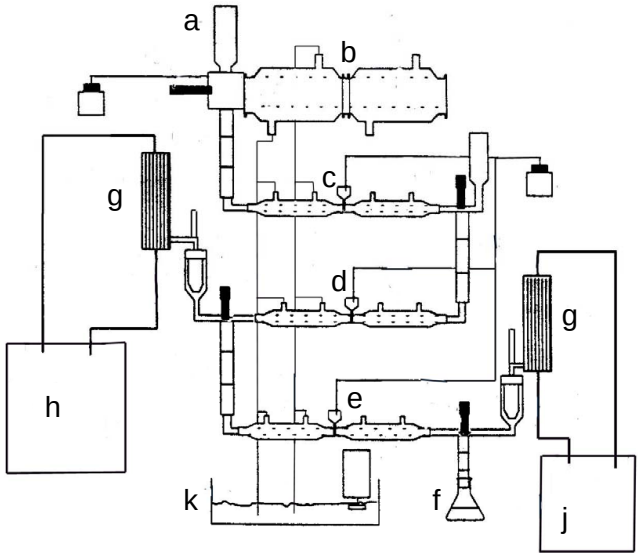

Fig. (1). Schematic picture of the TNO gastrointestinal *in vitro* model (TIM).

[a]Inlet, [b]Stomach, [c,d,e]Small intestine – duodenum, jejunum and ileum, [f]Ileo-cecal valve (outlet), [g]Semi-permeable hollow fibre filters (dialysis filters), [h,j]Jejunal and ileal containers, [k]Water bath.

Compared to the human study meals, the *in vitro* meals were halved due to model limitations. The meals included 55 g (w.w.) and were homogenised for 30 or 60 s (TM and BBM, respectively), using a household mixer. The homogenisation method was designed after authentic chewing and the average particle size was approximately 1x1x2 mm. Due to large dilution of intestinal fluids occurring in the TIM experiments, the amount of added iron was increased to enable accurate determination. To the meals, 7 mg of iron (1250 µl, 0.1M $FeSO_4$ in 0.1 M HCl) was added up to a total of 7.8 mg in a TM and 7.5 mg in a BBM, including the endogenous iron. This resulted in molar ratios (InsP/Fe) of 0.4 for the TM meal and 1.7 for the BBM meal. Water was added up to a total volume of 300 ml before introducing the meal into the gastric compartment.

Chemicals

For the human study, isotopes ^{59}Fe and ^{55}Fe were purchased from Perkin Elmer Life Science (USA). The isotope solutions ^{55}Fe and ^{59}Fe were prepared in 0.1 mol/l HCl and 55.5 kBq was given of ^{59}Fe and 148 kBq of ^{55}Fe. ^{59}Fe was checked for activity in a whole body counter at the Radiation Physics Department (Göteborg) before use. In the *in vitro* experiments, porcine bile extracts (B-8631), porcine stomach mucosal pepsin A (2260 units/mg, P-7012), bovine pancreatic trypsin (7500 N-α-benzoyl-L-arginine ethyl ester (BAEE) units/mg T-4665), porcine pancreatin (4*U.S.P., P-1750) (Sigma-Aldrich, Stockholm, Sweden) and lipase (150,000 units/mg, F-AP 15) (Amano Enzyme Inc. Nagoya, Japan) were used.

Analytical Methods

Preparatory Analyses

Tempe and reference material were analysed for inositol phosphates ($InsP_{3-6}$) and iron content. Samples were freeze dried (Hetosicc, Heto Birkerød, Denmark) and finely ground in a coffee grinder (Krups 75, type 203E). Determination of dry matter was performed on a moisture balance (Precisa Ha 300, Zürich, Switzerland). InsP analyses were performed according to Carlsson *et al*. [43] (method CV = ±2.7%, for barley samples) by extraction with HCl (0.5 M) and quantification with High Performance Ion Exchange Liquid Chromatography (HPIC). Iron content was determined in samples completely dissolved by heating in HCl and HNO_3 and deionised water in a high-pressure microwave oven (Ethos Plus, Milestone Microwave Laboratory Systems). Iron analysis was performed by HPIC according to Fredrikson *et al*. [44] (method CV = ±1.2% for Fe contents >1 ppm).

Human Iron Absorption

The analysis of ^{55}Fe and ^{59}Fe concentrations in blood was undertaken with minor modifications of the method by Eakins and Brown [45], using a liquid-scintillation spectrometer (Tri-Carb, Packard Instruments, San Antonio). Relative absorption of the isotopes ^{55}Fe and ^{59}Fe was determined in the blood, while the absolute absorption of ^{59}Fe was measured by whole-body counting. The absolute absorption of ^{55}Fe was calculated from the absolute absorption of ^{59}Fe and the relative absorption of ^{55}Fe and ^{59}Fe in the blood. Procedures and methods have previously been described by Björn-Rassmussen *et al*. [46]. The results were statistically evaluated by t-Test: Paired Two Sample for Means.

In Vitro Iron Availability

The samples from the collected dialysates were analysed for iron content with minor modifications by HPIC using the

Table 3. Iron Absorption from a TM and a BBM Meal (~115 g, w.w.) of the Human Study

	TM		BBM	
Subjects	[a]µg	[b]%	[a]µg	[b]%
F [BD]	111	3.70	93	3.10
F	48	1.60	45	1.50
F	102	3.40	39	1.30
F[BD]	156	5.20	132	4.40
M[BD]	399	13.30	171	5.70
F[BD]	66	2.20	18	0.60
F	93	3.10	69	2.30
M[BD]	336	11.20	156	5.20
Mean	164*	5.5*	90	3.0
SEM	46	1.5	20	0.7

F = Female, M = Male, [BD]Blood donor, [a]The amount of iron absorbed from the meals, [b]Iron absorption expressed as a fraction of total iron, *The value is significantly different from the value of the reference meal ($p < 0.05$).

method by Fredrikson *et al*. [44]. To 0.8 ml of the dialysate sample, 0.1 ml of ascorbic acid (20 mg/ml) was added and samples were spiked with 0.1 µg Fe/ml sample (0.1 ml, 1 µg/ml). The results were statistically evaluated using t-Test: Paired Two Sample for Means.

RESULTS

The human iron absorption and the *in vitro* available iron from tempe fermented barley meals (TM) and boiled barley meals (BBM) are presented in Tables 3 and 4. In the human study, the iron absorption was significantly ($p<0.05$) increased by 94±3% from 3.0±0.7% in BBM to 5.5±1.5% in TM. The combination of high serum-ferritin and high serum-Fe values in a subject, resulted in low absorption values, and vice versa. *In vitro* available iron was significantly ($p<0.001$) increased from 1.7±0.1% in BBM to 4.9±0.2% in TM.

Table 4. Iron Available from a TM and a BBM Meal (~57 g, w.w.) of the *in vitro* Study

	TM		BBM	
	[a] µg	[b] %	[a] µg	[b] %
I	368.2	4.7	95.5	1.3
II	371.4	4.8	138.1	1.8
III	348.1	4.5	140.0	1.9
IV	428.7	5.5	124.6	1.7
Mean	379*	4.9*	125	1.7
SEM	17	0.2	10	0.1
[c]CV (%)	9.1		16.5	

[a]The amount of iron available after simulated digestion, [b]Iron availability expressed as a fraction of total iron, [c]coefficient of variation, *The value is significantly different from the value of the reference meal ($p < 0.001$).

DISCUSSION

The influence of phytate content reduction in whole grain barley on iron absorption and iron availability was investigated. In both human and *in vitro* studies, the phytate-P content of the TM was below 10 mg (6.5 mg / 3.3 mg), and in the BBM above 10 mg (72.3 mg / 36.2 mg). The InsP/Fe molar ratio of the TM was below 1 in both the human and the *in vitro* studies (ratios 0.8 and 0.4), while the InsP/Fe ratios of the BBM of the human and *in vitro* studies were above 1 (ratios 8.4 and 1.7). In the study by Brune *et al.* [28], $InsP_6$ was found to inhibit iron absorption, and $InsP_5 - InsP_3$ were also suggested to influence absorption, which was later confirmed by Sandberg *et al.* [47]. In the present study, the $InsP_{3-6}$ content of the TM was reduced to 0.36 µmol/g (w.w.) by an optimised tempe fermentation process [30], therefore an increase in absorption and availability of iron was expected.

Human absorption of iron was increased by 94±0.3% from the low phytate (41 µmol $InsP_{3-6}$ or 6.5 mg phytate-P) barley meal compared to the high phytate (452 µmol $InsP_{3-6}$ or 72.3 mg phytate-P) barley meal. Despite the low phytate-P content the iron absorption was relatively low from TM (5.5±1.5%) as well as from BBM (3.0±0.7%). According to the study by Brune *et al.* [28], phytate-P content corresponding to those of the TM and BBM meals were expected to result in ~13% and ~7.5% iron absorption respectively. The low iron absorption may be explained by the fact that barley kernels in both TM and BBM were largely intact, and of a high amylose/ß-glucan variety [34], factors which have been suggested to induce a slow digestion and a low glycemic response [35, 48, 49]. Consequently, the release of iron from the food matrix might be restrained. This theory is supported by the study of Björn-Rasmussen *et al.* [50] where iron absorption was lower from a meal with polished whole rice kernels compared to a rice flour meal prepared from the polished rice. On the other hand, the isotope method assumes labelling iron to be fully exchanged in food during preparations, which might not be the case with intact kernels [51]. In the study on unpolished rice by Björn-Rasmussen *et al.* [50], the mean iron absorption of an extrinsic tracer was significantly higher (4.60±1.90%) than of an intrinsic tracer (2.72±1.07%) from the same meal, suggesting that structures such as the aleurone layer impair the rate of diffusion of extrinsic tracers into the kernels, which then would result in a false to high absorption of the extrinsic tracer. However, our study shows similar results to the study by Larsson *et al.* [52] where the iron absorption from malted, milled oat porridge with low phytate content (231 µmol $InsP_{3-6}$ or 37.0 mg phytate-P) was increased to 6.0% from 4.4% compared to a high phytate (452 µmol $InsP_{3-6}$ or 83.7 mg phytate-P) milled oat porridge meal.

To accurately predict iron absorption using *in vitro* methods presents a challenge, since a number of the factors involved in human iron absorption are difficult to mimic [33]. Nevertheless, attempts can be made to simulate the different stages of bioavailability *in vitro* [33]. Dialyzability is designed to predict the first step – availability. Using the TIM model, the digestion processes are simulated, allowing for measurements of the dialyzability. The *in vitro* and the human study were both performed on identically prepared test meals, however, the *in vitro* meals were halved and the

amount of added iron was increased due to the nature of the model. The *in vitro* obtained data were validated against the human absorption data.

The *in vitro* availability was found to increase nearly three times from 1.7±0.1% of total iron available in BBM (226 μmol $InsP_{3-6}$ or 36.2 mg phytate-P) to 4.9±0.2% in TM (20 μmol $InsP_{3-6}$ or 3.3 mg phytate-P). The *in vitro* results for the TM correspond well to the human study, but the results from the BBM shows a larger discrepancy. Since iron becomes chelated by phytate and a large dilution of sample fluids occurs during the *in vitro* experiment, dialyzable iron concentration can become very low in a high-phytate meal and may therefore be underestimated. Nevertheless, the direction of the results are consistent with the results from the human study, i.e. iron availability is increased from TM as compared to the reference meal. The gastrointestinal model has also previously been used to evaluate iron availability from low phytate meals. The results of the present study concur with those of Haraldsson *et al.* [41], where iron availability from barley porridge was increased from 0.4% in unprocessed high phytate barley (511 μmol $InsP_{3-6}$ or 85.7 mg phytate-P) up to 7.9% in low phytate malted barley (13 μmol $InsP_{3-6}$ or 2.2 mg phytate-P). Furthermore, *in vitro* results on dephytinized pea and soy protein meals, described by Sandberg [53], also compare well with the human study by Davidsson *et al.* [54] using identical meals.

This is a model study on the effect of phytate removal on the iron availability and absorption. Very simple meals were chosen in order not to have any interfering effects from other food items. Iron was added to increase the accuracy in measuring the inhibitory effect of phytate, and to have equal content of iron in the test meals. The added iron could be considered as coming from a second food item in a composite meal.

Hence, this investigation demonstrates that fractional iron absorption can be improved by phytate content reduction *via* tempe fermentation of whole grain barley. Barley tempe could therefore contribute to novel vegetarian products with high nutritional value and high iron bioavailability. The experiments also show that it is technically feasible to reproducibly determine relative availability of iron from cereal products with the *in vitro* model, and that it is a potential tool for prediction of *in vivo* bioavailability.

ACKNOWLEDGEMENTS

We would like to acknowledge Rickard Jonsson and Therese Christerson at Svalöf Weibull AB for providing the cultivars and sharing important knowledge, and Ingmar Börjesson, Per Berthelson and Lena Rimsten at Cerealia R&D for producing the tempe and contributing their expertise. The investigation was financed by VINNOVA Swedish Agency for Innovation Systems (Dnr 2004-02301).

REFERENCES

[1] Hunt JR, Roughead ZK. Nonheme-iron absorption, fecal ferritin excretion, and blood indexes of iron status in women consuming controlled lactoovovegetarian diets for 8 wk. Am J Clin Nutr 1999; 69(5): 944-52.

[2] Hurrell RF. Influence of vegetable protein sources on trace element and mineral bioavailability. J Nutr 2003; 133(9): 2973S-7S.

[3] Wilson AK, Ball MJ. Nutrient intake and iron status of Australian male vegetarians. Eur J Clin Nutr 1999; 53(3): 189-94.

[4] Waldmann A, Koschizke JW, Leitzmann C, Hahn A. Dietary iron intake and iron status of German female vegans: results of the German vegan study. Ann Nutr Metab 2004; 48(2): 103-8.

[5] Alexander D, Ball MJ, Mann J. Nutrient intake and haematological status of vegetarians and age-sex matched omnivores. Eur J Clin Nutr 1994; 48(8): 538-46.

[6] Anderson JW. Whole grains and coronary heart disease: the whole kernel of truth. Am J Clin Nutr 2004; 80(6): 1459-60.

[7] Kris-Etherton PM, Hecker KD, Bonanome A, *et al.* Bioactive compounds in foods: their role in the prevention of cardiovascular disease and cancer. Am J Med 2002; 113(9 Suppl 2): 71-88.

[8] Liu S, Stampfer MJ, Hu FB, *et al.* Whole-grain consumption and risk of coronary heart disease: results from the Nurses' Health Study. Am J Clin Nutr 1999; 70(3): 412-9.

[9] Montonen J, Knekt P, Jarvinen R, Aromaa A, Reunanen A. Whole-grain and fiber intake and the incidence of type 2 diabetes. Am J Clin Nutr 2003; 77(3): 622-9.

[10] Jordbrukverket. Swedish Board of Agriculture; Usage of barley - Extract from The Eurostat report 2005-2006.

[11] Duffus CM, Cochrane MP. Formation of the barley grain - morphology, physiology, and biochemistry. In: MacGregor AW, Bhatty RS, editors. Barley: Chemistry and Technology. Edinburgh, Scotland, UK: American association of cereal chemists, Inc. St. Paul, Minnesota, USA; 1993; pp. 31-67.

[12] Lolas GM, Markakis P. Phytic acid and other phosphorous compounds of beans. J Agric Food Chem 1975; 23(1): 13-5.

[13] Quinde-Axtell Z, Baik BK. Phenolic compounds of barley grain and their implication in food product discoloration. J Agric Food Chem 2006; 54(26): 9978-84.

[14] Rocha-Guzmán NE, González-Laredo RF, Ibarra-Pérez FJ, Nava-Berumen CA, Gallegos-Infante JA. Effect of pressure cooking on the antioxidant activity of extracts from three common bean (Phaseolus vulgaris L.) cultivars. Food Chem 2007; 100(1): 31-5.

[15] Towo E, Matuschek E, Svanberg U. Fermentation and enzyme treatment of tannin sorghum gruels: effects on phenolic compounds, phytate and *in vitro* accessible iron. Food Chem 2006; 94(3): 369-76.

[16] Maillard MN, Soum MH, Boivin P, Berset C. Antioxidant Activity of Barley and Malt: Relationship with Phenolic Content. LWT 1996; 29(3): 238-44.

[17] Steiner T, Mosenthin R, Zimmermann B, Greiner R, Roth S. Distribution of phytase activity, total phosphorus and phytate phosphorus in legume seeds, cereals and cereal by-products as influenced by harvest year and cultivar. Anim Feed Sci Technol 2007; 133(3-4): 320-34.

[18] Sandberg A-S, Rossander-Hulthén L, Türk M. Dietary *Aspergillus niger* phytase increases iron absorption in humans. J Nutr 1996; 126(2): 476-80.

[19] Sandberg A-S, Svanberg U. Phytate hydrolysis by phytase in cereals: effects on *in vitro* estimation of iron availability. J Food Sci 1991; 56(5): 1330-3.

[20] Konietzny U, Greiner R. Molecular and catalytic properties of phytate-degrading enzymes (phytases). Int J Food Sci Technol 2002; 37(7): 791-812.

[21] Greiner R, Jany KD, Larsson Alminger M. Identification and Properties of myo -Inositol Hexakisphosphate Phosphohydrolases (Phytases) from Barley (Hordeum vulgare). J Cer Sci 2000; 31(2): 127.

[22] Sutardi, Buckle KA. Characterization of extra- and intracellular phytases from *Rhizopus oligosporus* used in tempeh production. Int J Food Microbiol 1988; 6(1): 67-79.

[23] Casey A, Walsh G. Identification and characterization of a phytase of potential commercial interest. J Biotechnol 2004; 110: 313-22.

[24] Ramachandran S, Roopesh K, Nampoothiri KM, Szakacs G, Pandey A. Mixed substrate fermentation for the production of phytase by Rhizopus spp. using oilcakes as substrates. Proc Biochem 2005; 40(5): 1749-54.

[25] Sutardi, Buckle KA. Phytic acid changes in soybeans fermented by traditional inoculum and six strains of *Rhizopus oligosporus*. J Appl Bact 1984; 58: 539-43.

[26] Sutardi, Buckle KA. Reduction in phytic acid levels in soybeans during tempeh production, storage and frying. J Food Sci 1985; 50: 260-3.

[27] Hallberg L, Brune M, Rossander L. Iron absorption in man: Ascorbic acid and dose-dependent inhibition by phytate. Am J Clin Nutr 1989; 49(1): 140-4.

[28] Brune M, Rossander-Hultén L, Hallberg L, Gleerup A, Sandberg A-S. Iron absorption from bread in humans: Inhibiting effects of cereal fiber, phytate and inositol phosphates with different numbers of phosphate groups. J Nutr 1992; 122(3): 442-9.

[29] Hurrell RF. Phytic acid degradation as a means of improving iron absorption. Int J Vit Nutr Res 2004; 74(6): 445-52.

[30] Eklund-Jonsson C, Sandberg A-S, Larsson Alminger M. Reduction of phytate content while preserving minerals during whole grain cereal tempe fermentation. J Cer Sci 2006; 44(2): 154-60.

[31] Reddy MB, Cook JD. Assessment of dietary determinants of non-heme-iron absorption in humans and rats. Am J Clin Nutr 1991; 54(4): 723-8.

[32] Fairweather-Tait SJ. Iron nutrition in the UK: getting the balance right. Proc Nutr Soc 2004; 63(4): 519-28.

[33] Fairweather-Tait S, Lynch S, Hotz C, *et al*. The usefulness of *in vitro* models to predict the bioavailability of iron and zinc: a consensus statement from the HarvestPlus expert consultation. Int J Vit Nutr Res 2005; 75(6): 371-4.

[34] Ajithkumar A, Andersson R, Christerson T, Åman P. Amylose and ß-Glucan Content of New Waxy Barleys. Starch 2005; 57(6): 235-9.

[35] Alminger M, Eklund-Jonsson C. Whole-grain cereal products based on a high fibre barley or oat genotype lower post-prandial glucose and insulin responses in healthy humans. 2008 (Submitted).

[36] Feng XM, Eriksson ARB, Schnurer J. Growth of lactic acid bacteria and *Rhizopus oligosporus* during barley tempeh fermentation. Int J Food Microbiol 2005; 104(3): 249-56.

[37] Hallberg L. Food iron absorption. In: Cook JD, editor. Methods in Haemathology. London; 1980.

[38] Minekus M, Marteau P, Havenaar R, Huis in't Veld JHJ. A multicompartimental dynamic computer-controlled model simulating the stomach and small intestine. ATLA 1995; 23: 197-209.

[39] Larsson M, Minekus M, Havenaar R. Estimation of the bioavailability of iron and phosphorus in cereals using a dynamic *in vitro* gastrointestinal model. J Sci Food Agric 1997; 74(1): 99-106.

[40] Salovaara S, Larsson Alminger M, Eklund-Jonsson C, Andlid T, Sandberg A-S. Prolonged transit time through the stomach and small intestine improves iron dialyzability and uptake *in vitro*. J Agric Food Chem 2003; 51(17): 5131-6.

[41] Haraldsson A-K, Rimsten L, Alminger M, Andersson R, Aman P, Sandberg A-S. Digestion of barley malt porridges in a gastrointestinal model: Iron dialysability, iron uptake by Caco-2 cells and degradation of [beta]-glucan. J Cer Sci 2005; 42: 243-54.

[42] Blanquet S, Antonelli R, Laforet L, Denis S, Marol-Bonnin S, Alric M. Living recombinant *Saccharomyces cerevisiae* secreting proteins or peptides as a new drug delivery system in the gut. J Biotechnol 2004; 110(1): 37-49.

[43] Carlsson N-G, Bergman E-L, Skoglund E, Hasselblad K, Sandberg A-S. Rapid analysis of inositol phosphates. J Agric Food Chem 2001; 49(4): 1695-701.

[44] Fredrikson M, Carlsson N-G, Almgren A, Sandberg A-S. Simultaneous and sensitive analysis of Cu, Ni, Zn, Co, Mn, and Fe in food and biological samples by ion chromatography. J Agric Food Chem 2002; 50(1): 59-65.

[45] Eakins J, Brown DA. An improved method for the simultaneous determination of iron-55 and iron-59 in blood by liquid scintillation counting. Int J Appl Rad Isotop 1966; 17(7): 391-7.

[46] Bjorn-Rasmussen E, Hallberg L, Isaksson B, Arvidsson B. Food iron absorption in man. Applications of the two-pool extrinsic tag method to measure heme and nonheme iron absorption from the whole diet. J Clin Invest 1974; 53: 247-55.

[47] Sandberg A-S, Brune M, Carlsson N-G, Hallberg L, Skoglund E, Rossander-Hulthén L. Inositol phosphates with different numbers of phosphate groups influence iron absorption in humans. Am J Clin Nutr 1999; 70: 240-6.

[48] Stevnebo A, Sahlstrom S, Svihus B. Starch structure and degree of starch hydrolysis of small and large starch granules from barley varieties with varying amylose content. Anim Feed Sci Technol 2006; 130(1-2): 23-38.

[49] Granfeldt Y, Eliasson A-C, Björck I. An examination of the possibility of lowering the glycemic index of oats and barley flakes by minimal processing. J Nutr 2000; 130: 2207-14.

[50] Bjorn-Rasmussen E, Hallberg L, Walker RB. Food iron absorption in man. II. Isotopic exchange of iron between labeled foods and between a food and an iron salt. Am J Clin Nutr 1973; 26(12): 1311-9.

[51] Sayers MH, Lynch SR, Charlton RW, Bothwell TH, Walker RB, Mayet F. Iron absorption from rice meals cooked with fortified salt containing ferrous sulphate and ascorbic acid. Am J Clin Nutr 1974; 31(3): 367-75.

[52] Larsson M, Rossander-Hulthen L, Sandstrom B, Sandberg AS. Improved zinc and iron absorption from breakfast meals containing malted oats with reduced phytate content. Am J Clin Nutr 1996; 76(5): 677-88.

[53] Sandberg AS. Methods and options *in vitro* dialyzability; benefits and limitations. Int J Vit Nutr Res 2005; 75(6): 395-404.

[54] Davidsson L, Dimitriou T, Walczyk T, Hurrell RF. Iron absorption from experimental infant formulas based on pea (*Pisum sativum*)-protein isolate: The effect of phytic acid and ascorbic acid. Br J Nutr 2001; 85(1): 59-63.

Received: December 23, 2007 Revised: March 27, 2008 Accepted: April 08, 2008