Production Process for High-Quality Pea-Protein Isolate with Low Content of Oligosaccharides and Phytate

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A process for pea-protein isolate production, resulting in low content of phytate and oligosaccharides, has been developed. Oligosaccharides were removed from the protein fraction through ultrafiltration. Ultrafiltration of 50- and 100-kD molecular-weight cutoffs (MWCOs) were tested, and both effectively separated the oligosaccharides from the protein. Phytate degradation was achieved by incubation of the pea-protein solution by addition of exogenous phytase enzyme. An almost complete degradation of inositol hexa-, penta-, tetra-, and triphosphates was reached using an incubation time of 1 h. The reduced content of oligosaccharides and inositol phosphates is likely to result in reduced flatulence and improved mineral bioavailability. These qualities of the pea-protein isolate make it a suitable protein source for infant formula production.

Keywords: *Pisum sativum; oligosaccharides; inositol phosphate; phytate; phytase*

INTRODUCTION

Pea is an important grain legume, as both human food and animal feed. A valuable part of the pea is the protein fraction, which can be extracted and further purified into a protein isolate. However, peas, as other legumes, contain a variety of antinutritional and antiphysiological factors which negatively influence the quality of their proteins. It is therefore of great importance to develop a production process resulting in a protein isolate devoid of the antinutritional and antiphysiological factors. High-quality pea-protein isolates with improved nutritional and physiological properties can beneficially replace other protein sources, for example soy protein, in several applications.

In this work the process was aimed at producing a protein isolate suitable for use in infant formula. Therefore, the attention was directed at reducing the content of oligosaccharides and phytate in the protein isolate. The oligosaccharides are considered to cause flatulence in man and animals (*1−3*), which obviously is a disadvantage when used as an ingredient in infant formula. Phytate is a recognized inhibitor of iron and zinc absorption in man (*4−7*), and phytate has also been shown to decrease the protein availability in peas (*8*). An infant formula with low content of oligosaccharides and phytate is therefore likely to result in reduced flatulence and improved mineral bioavailability.

Because phytate is associated with the protein in legumes and many other seeds (*9−11*), a purified protein isolate normally contains increased levels of phytate. To dephytinise the protein isolate, the possibility of enzymatic degradation of phytate was evaluated. Two approaches were compared: degradation by the endog-enous phytase present in the pea, and addition of a commercial exogenous phytase to the protein solution. Previously, processes for dephytinised protein isolate have been developed for soy protein (*12−14*), where the differences in solubility of phytate and soy protein at specific pHs were used as the dephytinisation method. The advantages of using an enzymatic dephytinisation is that it works also for proteins with similar solubility-versus-pH relationship as phytate, and that the pH can be customized during the other process steps such as ultrafiltration.

Another important aspect was to minimize the growth of microorganisms during the pea-protein production process. Control of the microbiological quality can be obtained by minimizing the processing time, thereby restricting the growth of microorganisms. Pasteurization treatments were also included. Ultrafiltration of pea-protein solution was evaluated for removal of the oligosaccharides.

The content of pea proteinase inhibitors, lectins, and saponins, and in vitro digestibility of the protein during the processing, were evaluated in a parallel study (*15*).
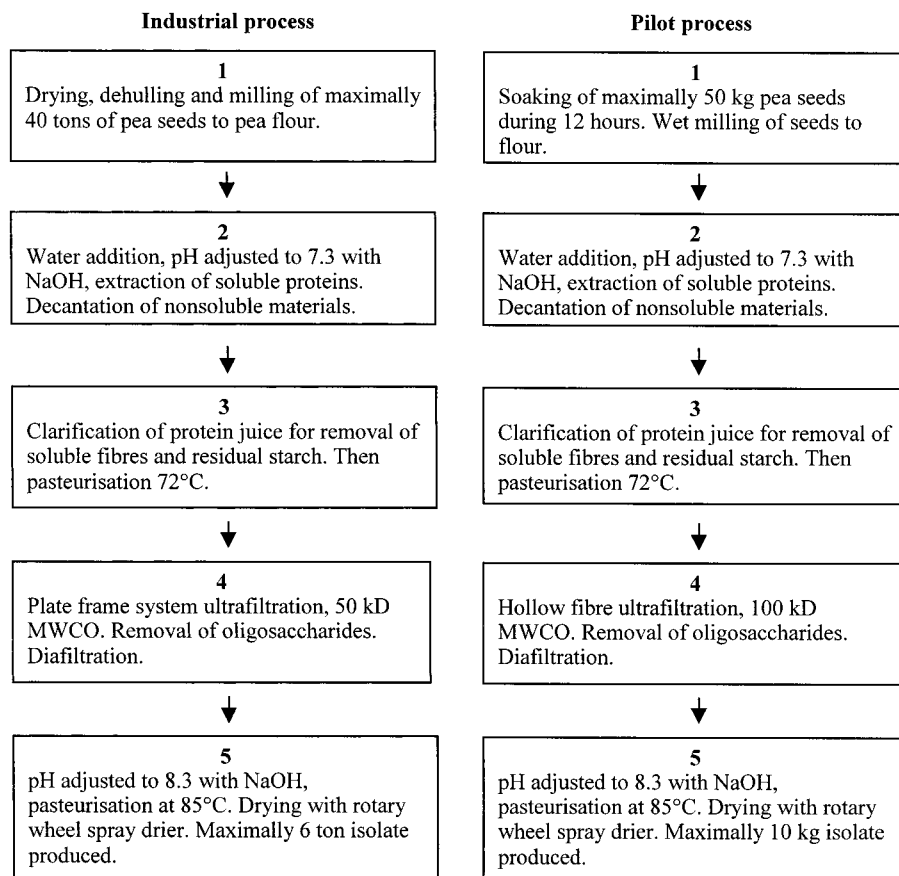
MATERIALS AND METHODS

Materials. All reagents used were of analytical grade. Exogenous phytase liquid solution, Phytase Novo L (5000 FYT/g) was obtained from Novo Nordisk A/S, Bagsværd, Denmark. (One FYT is the amount of enzyme that releases 1 μmol inorganic orthophosphate from 5 mM sodium phytate in one minute at pH 5.5 and 37 °C). In laboratory experiments pH was adjusted with citrate buffer, and a water bath with controlled incubation temperature was used. For industrial and pilot plant runs the pH adjustments were done with HCl and NaOH.

Methods. The enzymatic dephytinisation of pea-protein isolate was first studied in laboratory scale using 10−80-mL sample volume size, to estimate the necessary enzyme dosage and incubation time for a complete dephytinisation. Laboratory scale experiments were done at Chalmers University of Technology. Provital Industrie SA supplied standard processed

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Industrial process

1
Drying, dehulling and milling of maximally 40 tons of pea seeds to pea flour.

↓

2
Water addition, pH adjusted to 7.3 with NaOH, extraction of soluble proteins. Decantation of nonsoluble materials.

↓

3
Clarification of protein juice for removal of soluble fibres and residual starch. Then pasteurisation 72°C.

↓

4
Plate frame system ultrafiltration, 50 kD MWCO. Removal of oligosaccharides. Diafiltration.

↓

5
pH adjusted to 8.3 with NaOH, pasteurisation at 85°C. Drying with rotary wheel spray drier. Maximally 6 ton isolate produced.

Pilot process

1
Soaking of maximally 50 kg pea seeds during 12 hours. Wet milling of seeds to flour.

↓

2
Water addition, pH adjusted to 7.3 with NaOH, extraction of soluble proteins. Decantation of nonsoluble materials.

↓

3
Clarification of protein juice for removal of soluble fibres and residual starch. Then pasteurisation 72°C.

↓

4
Hollow fibre ultrafiltration, 100 kD MWCO. Removal of oligosaccharides. Diafiltration.

↓

5
pH adjusted to 8.3 with NaOH, pasteurisation at 85°C. Drying with rotary wheel spray drier. Maximally 10 kg isolate produced.

Figure 1. Flowchart of industrial and pilot standard processes for production of pea-protein isolates. For production of the dephytinised protein isolates the processes were modified between steps 4 and 5. The ultrafiltrated protein retentate was adjusted to pH 5.2 with HCl and the temperature was set to 55 °C. The phytase was added and the mixture was incubated while being stirred during 1 or 2 h. The dephytinised protein was then processed to the end product as described in step 5.

pea-protein isolates with high phytate content which were used as starting materials for the enzyme incubations. Depending on sample volume, 0.5–9.6 g of phytate-containing protein isolate was dissolved in 10 or 80 mL of citrate buffer, pH 5.5. The phytase enzyme was added and the samples were incubated in a 55 °C water bath while being shaken. The exogenous phytase incubations were performed at pH 5.5 and 55 °C as recommended by the phytase manufacturer. Incubation solutions with 5% to 12% dry matter (protein isolate) were studied, in accordance with the standard pea-protein production process. With consideration to mass transfer and growth of bacteria, the incubation times were restricted to 0.5–2 h. Laboratory experiment data are also presented later in Table 4. The laboratory incubations were accomplished by adding HCl to a final concentration of 0.5 M. Thereby the phytase was inactivated, and the inositol phosphates were simultaneously extracted for further analyses, as described below. The results obtained from the laboratory experiments were then used to include the phytase incubation in the standard pilot and industrial processes for the production of dephytinised protein isolate.

Industrial and pilot plant processing were made at Provital SA's production plant in Warcoing, Belgium. As the details of the industrial and pilot processes are proprietary, only a general description of the continuously linked process steps can be given as summarized in Figure 1. The method is basically a standard wet process, widely used for grain legume protein isolation. The pea protein was first extracted in water from pea flour, thus obtaining a protein juice. The protein juice was made from 11 to 13% pea flour dissolved in water, resulting in totally 10–12% dry matter and 2–3% protein in the start solution. The nonsoluble carbohydrates and fibers were then removed by continuous centrifuge decantation (Alfa-Laval, Sweden). To also remove the soluble fiber and residual starch, a clarification treatment followed by a continuous

centrifuge clarifier (Alfa-Laval, Sweden) was used. After decantation and clarification of the protein juice, its volume was decreased by 20% and the dry matter content was decreased to 4%. The protein juice was further purified by ultrafiltration (UF), using 50- or 100-kD molecular-weight-cutoff (Romicon, Koch Membrane Systems Inc. Wilmington, MA). During UF the solubilized protein juice is fed, under pump pressure, through a membrane filter that separates the components on the basis of their molecular size. Molecules smaller than the membrane filter, such as oligosaccharides, are removed. Protein juice volume decreased by an additional $^2/_3$ (UF concentration factor = 3), resulting in approximately 6% protein content and 7% dry matter content. The protein-enriched retentate was then continuously diafiltered with three parts water per one part protein juice (diafiltration factor = 3). After diafiltration the solution was dried to produce protein isolate flour, obtaining about 65% protein yield in the final product.

The process treatments were performed at room temperature except where other temperatures are stated (Figure 1). To understand the influence of the process on the quality of the protein isolate, representative samples were collected from the processes after the different process stages, 1–5 in Figure 1. The samples were analyzed, and the chemical composition during processing and in the final protein isolates was recorded.

Sample preparations and analyses of inositol triphosphate–inositol hexaphosphate (IP_3–IP_6) were done according to Sandberg and Ahderinne (*16*) with some minor modifications (*17*). Analyses and sample preparations for quantification of oligosaccharides were done according to Carlsson et al. (*18*). Protein content determinations were supplied by Provital SA, using the Kjeldahl method for analyses. The microbiological quality controls were supplied by VTT Biotechnology and Food Research in Finland. Species-specific and total microbial

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Table 1. Content of Oligosaccharides (OS) during Standard Industrial Pea-Protein Processing

process sample designation[a]	saccharose (mg/g)	raffinose (mg/g)	stachyose (mg/g)	verbascose (mg/g)	ajugose (mg/g)	Σ OS (mg/g)	protein (%)
1 pea seeds: starting material	24.0 ± 1.3[b]	8.0 ± 0.1	20.5 ± 0.1	24.5 ± 1.0	0.3 ± 0.3	77.3 ± 3.7	25 ± 1
2 extracted protein juice	57.4 ± 2.4	19.2 ± 0.1	47.2 ± 2.0	51.7 ± 2.9	0.9 ± 0.1	176 ± 8	63 ± 2
3 decanted, clarified, and pasteurized protein juice	63.3 ± 2.2	20.5 ± 0.3	47.3 ± 0.2	50.5 ± 0.3	0.8 ± 0.1	182 ± 3.5	64 ± 2
4 protein retentate after ultrafiltration (50 kD MWCO)	3.5 ± 0.4	1.5 ± 0.1	3.1 ± 0.2	3.9 ± 0.1	0.1 ± 0.1	12.1 ± 0.9	90 ± 1
5 end product after second pasteurization and drying	2.8 ± 1.2	1.5 ± 0.1	3.0 ± 0.2	3.9 ± 0.1	0.5 ± 0.3	11.7 ± 1.9	92 ± 2

[a] The samples are numbered according to the process steps shown in Figure 1. [b] All results represent mean values ± SD from two process runs.

Table 2. Content of Oligosaccharides (OS) and Protein in Pea-Protein Isolates from Pilot and Industrial Processes, with Two Different Ultrafiltrations

UF membrane cutoff size	ΣOS in pea seeds (mg/g)	ΣOS in isolate (mg/g)	protein in pea seeds (%)	protein in isolate (%)
industrial 50 kD MWCO[a]	77 ± 2	11.7 ± 1.9	25 ± 1	92 ± 2
pilot 100 kD MWCO[b]	75 ± 2	4.4 ± 1.3	25 ± 1	89 ± 2

[a] Mean values ±SD from two process runs, using the same pea variety. [b] Mean values ±SD from five process runs, using five different pea varieties.

analyses were done. Plate count was used as the microbiological analysis method, giving the number of colony-forming units/g sample after incubation on Petri plates. Duplicate samples were analyzedexcept as otherwise indicated. All results correspond to the dry weight of the sample.

RESULTS AND DISCUSSION

Removal of Oligosaccharides. Table 1 shows the mean content of oligosaccharides during two runs of the industrial standard process, which is described in Figure 1. The oligosaccharides are water soluble and thus concentrated with the protein during the water extraction. Consequently, the content of oligosaccharides in the pea seeds influences the content of oligosaccharides in the protein juice. The decantation and clarification treatments separate the less soluble compounds, such as fibers and starch, from the protein solution and do not influence the oligosaccharides. The ultrafiltration (50-kD MWCO) dramatically reduces the content of oligosaccharides in the protein juice. In the pilot plant, ultrafiltration of 100-kD MWCO was tested for comparison with the industrial 50-kD filter. The two ultrafiltrations are reported in Table 2. The results indicate that 100-kD MWCO ultrafiltration removes the oligosaccharides more effectively than 50 kD, without significantly reducing the protein yield or the protein content in the isolate. However, ultrafiltration with the 50-kD MWCO filter should be sufficient for the separation of the oligosaccharides from the protein, because the oligosaccharides are smaller than 50 kD. The improvement in removal of oligosaccharides with the pilot 100-kD UF compared to that of the industrial 50-kD UF is therefore suggested to be due to the differences in washing time and water quantity during pilot and industrial UF processing. The two systems also differ in their UF mechanisms, as the 50-kD industrial UF is a plate frame system and the 100-kD pilot UF is a hollow fiber system. Furthermore, different pea varieties were used in the pilot and industrial processes but the contents of protein and oligosaccharides in these pea varieties are similar. Finally, the content of oligosac-

charides in both the pilot and industrially processed isolates are acceptable for use in infant formula production.

Phytate Content during Standard Process. Table 3 shows the mean content of inositol phosphates during two runs of the industrial standard process. It is well-known that the phytate is complexed to protein in the pea (*9, 11*), which explains why the phytate content increases as the protein content increases. As a consequence, the phytate content in the final protein isolate is influenced by the phytate content in the pea seeds used for starting material. As seen in Table 3, the phytate content does not increase to the same extent as the protein content during the ultrafiltration at neutral pH, indicating a partial separation of inositol phosphates and protein. Table 3 also shows that the relative reductions of IP_4 and IP_5 are higher than the relative reduction of IP_6 after ultrafiltration. This UF effect may be explained by the fact that some of the inositol phosphates are not complexed to the protein at neutral pH, as described by Rham and Jost (*14*), and Reddy et al. (*11*). The noncomplexed inositol phosphates are thus separated from the protein juice during ultrafiltration because the molecular size of the inositol phosphates is smaller than the 50-kD UF membrane. The complexing between protein and inositol phosphates is dependent on the phosphate groups on the inositol ring (*11*). The complex strength between protein and inositol phosphates is proportional to the number of phosphate groups, and IP_6 binds more strongly to the protein than do the lower inositol phosphates (IP_3-IP_5).

Degradation of Phytate by Endogenous Phytase. It has previously been shown that the phytate in pea flour can be dramatically reduced in approximately 10 h by incubation at 45 °C and pH 7.5 (*19*). However, large-scale incubation of pea flour during this length of time is impossible because of the growth of microorganisms. Considering that growth of microorganisms is slower in whole pea seeds than in pea flour, soaking of whole dehulled pea seeds at 45 °C was tested. However, up to 20 h of soaking of pea seeds did not result in any significant phytate degradation. The degradation of phytate by phytase is a water-mediated reaction. According to an internal study by Provital Industrie SA the water migration into the seeds requires approximately 3 h. However, it is suggested that the main reason for the slow phytate degradation in whole pea seeds is the limited accessibility between the phytase and the phytate, or by possible inhibitors. It was concluded that the endogenous pea phytase is not sufficiently effective for degrading phytate at the conditions considered possible for an industrial process.

Degradation of Phytate by Exogenous Phytase. Incubation of the protein juice with added commercial

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Table 3. Content of Inositol Phosphates during Standard Industrial Pea-Protein Processing

process sample designation[a]	IP_6 (μmol/g)	IP_5 (μmol/g)	IP_4 (μmol/g)	IP_3 (μmol/g)	ΣIP_3-IP_6 (μmol/g)	protein (%)
1 pea seeds: starting material	12.6 ± 1.0	1.7 ± 0.5	0.3 ± 0.2	0	14.6 ± 1.7	25 ± 1
2 extracted protein juice	23.4 ± 0.6	7.0 ± 0.5	2.1 ± 0.6	0	32.5 ± 1.7	63 ± 2
3 decanted, clarified, and pasteurized protein juice	25.8 ± 1.3	5.0 ± 0.2	1.0 ± 0.1	0	31.8 ± 1.6	64 ± 2
4 protein retentate after ultrafiltration (50 kD MWCO)	24.3 ± 1.4	3.0 ± 0.1	0.6 ± 0.1	0	27.9 ± 1.6	90 ± 1
5 end product after second pasteurization and drying.	27.2 ± 5.5	2.9 ± 0.6	0.5 ± 0.1	0	30.6 ± 6.2	92 ± 2

[a] Samples are numbered according to the process steps shown in Figure 1. [b] Mean values $\pm$SD from two process run.

Table 4. Laboratory Scale Incubations of Protein Isolates with Phytase Additions, at pH 5.5 and 55 °C

incubation time and phytase dosage (FYT phytase/g protein isolate)	sample size (ml)	dry matter (%)	IP_6 (μmol/g)	ΣIP_3-IP_6 (μmol/g)
control 1[a]			29.6 ± 0.4	33.3 ± 0.5
1h, 1.2 FYT/g control 1	10	5	0.24 ± 0.05	9.8 ± 0.8
1h, 2.4 FYT/g control 1	10	5	0.12 ± 0.07	1.4 ± 0.4
2h, 1.2 FYT/g control 1	10	5	0	0.65 ± 0.04
2 h, 2.5 FYT/g control 1	10	5	0	0.73 ± 0.2
2 h, 5 FYT/g control 1	10	5	0	0.37 ± 0.05
control 2[a]			36.9 ± 1.6	40.8 ± 1.8
0.5 h, 2.5 FYT/g control 2	10	10	0	5.4 ± 0.2
1 h, 2.5 FYT/g control 2	10	10	0	1.0 ± 0
0.5 h, 5 FYT/g control 2	10	10	0	0.74 ± 0.09
control 3[a]			22.1 ± 2.0	25.8 ± 2.9
2 h, 2.5 FYT/g control 3	10	12	0	0.95 ± 0.44
2 h, 5 FYT/g control 3	10	12	0	0.42 ± 0.05
control 4[a]			18.3 ± 0.7	21.8 ± 0.9
1h, 10 FYT/g control 4	80	12	0	0.4 ± 0.1
2h, 10 FYT/g control 4	80	12	0	0

[a] The pea-protein isolates show varying IP_6-content, depending on the different pea varieties used as starting material. From a production of twelve different standard isolates, four isolates were chosen as phytase incubation substrates. The isolate with highest content of IP_3-IP_6 was included (control 2).

Table 5. Pea-Protein Isolates with Standard Processing, or with Added Phytase Processing

process (standard, or amount of phytase addition and incubation time)	protein in pea seeds (%)	ΣIP_3-IP_6 In pea seeds (μmol/g)	protein in isolate (%)	IP_6 in isolate (μmol/g)	ΣIP_3-IP_6 in isolate (μmol/g)
standard pilot[a]	25.3 ± 1.3	13.6 ± 1.7	89.0 ± 2.1	32.1 ± 3.4	36.8 ± 3.6
standard industrial	26.5 ± 1	14.9 ± 0.3	88	19.6 ± 0.2	22.4 ± 0.3
phytase 10 FYT[b]/g, 2h	26.5 ± 1	14.9 ± 0.3	86	0	1.2 ± 0.3
phytase 10 FYT/g, 2h	26.5 ± 1	14.9 ± 0.3	85	0.08 ± 0.03	0.08 ± 0.03
phytase 10 FYT/g, 1h	26.5 ± 1	14.9 ± 0.3	87	0.17 ± 0.02	0.17 ± 0.02

[a] Average from six runs. [b] 5% dry matter used during processing instead of the normal 12% dry matter.

phytase was evaluated in order to reach a rapid degradation of the inositol phosphates. The experiments with laboratory-scale incubations, 10- or 80-mL sample volume, are presented in Table 4. When using the protein isolate with highest phytate content (control 2) it appears that 30-min incubation with 5 FYT enzyme/g isolate is sufficient for a substantial IP_6-IP_3 degradation. It is evident that the IP_6 is degraded rapidly by the added phytase, and the following IP_5-IP_3 degradation requires more time. Because of the mass transfer in the forthcoming large-scale incubations, 30 min was estimated as the minimal incubation time. The results in Table 4 also show that the phytase works well at 5, 10, or 12% dry matter content, which consisted of 85–94% protein.

The total content of IP_3-IP_6 is of nutritional interest since a recent study by Sandberg et al. (*7*) showed that in breads containing IP_3-IP_6, also IP_3-IP_4 in the presence of IP_6, contributed a negative effect to the absorption of iron in humans. Therefore, it is of impor-

tance to determine the total content of IP_3-IP_6 during phytate degradation.

The enzymatic approach was then applied to larger scale production by introducing the phytase incubation step at the protein production process. To make a suitable amount of dephytinised protein isolate, the industrial and pilot processes were combined. Protein juice from the industrial process was tapped just after the ultrafiltration, then lead into the pilot process. Temperature and pH were adjusted, the phytase was added, and the protein juice was incubated for 1 or 2 h. The dephytinised pea protein treatment was completed according to pilot process step 5 in Figure 1. As seen in Table 5, the phytase-processed protein isolates were almost completely devoid of inositol phosphates, in contrast to the standard protein isolates. To maximize the yield of the pea protein, all but one of the process runs were done with 12% dry matter. Compared with the study by Rham and Jost (*14*), the present enzymatic approach facilitates more complete phytate removal

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with comparable protein yield. From the dephytinisation point of view, the phytase incubation can be done using either pilot or industrial process, depending on the desired quantity of dephytinised protein isolate to be produced.

Microbiological Quality. The microorganisms quantified in the final pea-protein isolates were enterobacteria; yeasts and molds; *Bacillus cereus*; aerobic spore-formers; clostridia; mesophilic lactic acid bacteria (LAB); and thermophilic LAB. The amount of each species was less than 50 cfu/g isolate.

CONCLUSIONS

Dephytinised pea-protein isolates with low content of oligosaccharides were produced. The dephytinisation was accomplished by introducing a phytase incubation step to a standard protein production process. Control of the microbiological quality was obtained by minimizing the processing time, thereby restricting the growth of microorganisms. The reduced content of oligosaccharides and inositol phosphates is likely to result in reduced flatulence and improved mineral bioavailability. The pea-protein isolates were used for test production of infant formulas. The availability of minerals from the pea-protein formulas will be compared with that of commercial infant formula based on soy protein.

ABBREVIATIONS USED

cfu, Colony forming unit; HPLC, high-performance liquid chromatography; IP$_3$, inositol triphosphate; IP$_4$, inositol tetraphosphate; IP$_5$, inositol pentaphosphate; IP$_6$, inositol hexaphosphate; ΣIP$_3$–IP$_6$, total amount IP$_3$ + IP$_4$ + IP$_5$ + IP$_6$; kD, 1000 atomic weights; LAB, lactic acid bacteria; MWCO, molecular-weight cutoff; OS, oligosaccharides.

ACKNOWLEDGMENT

LITERATURE CITED

(1) Rackis, J. J. Oligosaccharides of Food Legumes; Alpha-Galactosidase Activity and the Flatus Problem. In *Physiological Effects of Food Carbohydrates*; Jeanes, A., Hodge, J., Eds.; American Chemical Society: Washington, DC, 1975; pp 207–222.

(2) Reddy, N. R.; Salunkhe, D. K.; Sharma, R. P. Flatulence in rats following ingestion of cooked and germinated black gram and a fermented product of black gram and rice blend. *J. Food Sci.* **1980**, *45*, 1161–1164.

(3) Olson, A. C.; Gray, G. M.; Gumbmann, M. R.; Wagner, J. R. Nutrient Composition of and Digestive Response to Whole and Extracted Dry Beans. *J. Agric. Food Chem.* **1982**, *30*, 26–32.

(4) Cheryan, M. Phytic acid interactions in food systems. *CRC Crit. Rev. Food Sci. Nutr.* **1980**, *13* (4), 297–335.

(5) Rossander, L.; Sandberg, A.-S.; Sandström, B. Dietary fibre – a component of food. In *Nutritional Function in Health and Disease*; Schweizer, T. F., Edwards, C. A., Eds.; Springer Verlag: London, 1992; pp 197–216.

(6) Sandberg, A.-S.; Brune, M.; Carlsson, N.-G.; Hallberg, L.; Rossander-Hultén, L.; Sandström, B. The effect of various inositol phosphates on iron and zinc absorption in humans. In *Bioavailability' 93. Nutritional, Chemical and Food Processing Implications of Nutrient Availability* Sclemmer, U., Ed.; Bundesforschunganstalt für Ernährung: Germany, 1993; pp 53–57.

(7) Sandberg, A.-S.; Brune, M.; Carlsson, N.-G.; Hallberg, L.; Skoglund, E.; Rossander-Hultén, L. Inositol phosphates with different numbers of phosphate groups influence iron absorption in humans. *Am. J. Clin. Nutr.* **1999**, *70*, 240–246.

(8) Carnovale, E.; Lugaro, E.; Lombardi-Boccia, G. Phytic acid in faba bean and pea: Effect on protein availability. *Cereal Chem.* **1988**, *65*, 114–117.

(9) Lott, J. N. A.; Goodchild, D. J.; Craig, S. Studies of mineral reserves in pea (*Pisum sativum*) cotyledons using low-water-content procedures. *Aust. J. Plant Physiol.* **1984**, *11*, 459–469.

(10) Prattley, C. A.; Stanley, D. W. Protein–phytate interactions in soybeans. Localization of phytate in protein bodies and globoids. *J. Food Biochem.* **1982**, *6*, 243–252.

(11) Reddy N. R.; Pierson M. D.; Sathe S. K.; Salunkhe D. K. *Phytates in Cereals and Legumes*; CRC Press: Boca Raton, FL, 1989.

(12) Okubo, L.; Waldrop, A. B.; Iacobucci, G. A.; Myers, D. V. Preparation of low-phytate soybean protein isolate and concentrate by ultrafiltration. *Cereal Chem.* **1975**, *52*, 263–271.

(13) Omosaiye, O.; Cheryan, M. Low-phytate, full fat soy protein product by ultrafiltration of aqueous extracts of whole soybeans. *Cereal Chem.* **1979**, *56*, 58–62.

(14) Rham, de O.; Jost, T. Phytate-protein interactions in soybean extracts and low-phytate soy protein products. *J. Food Sci.* **1979**, *44*, 596–600.

(15) Sørensen, A. D.; Hansen, A. B.; Sørensen, S.; Barkholt, V.; Frøkiær, H. Influence of industrial processing of peas on the content of antinutritional factors and the *in-vitro* digestibility. In *Proceedings of the 3rd European Conference on Grain Legumes, Valladolid, Spain*; AEP: Paris, 1998; pp 348–349.

(16) Sandberg, A.-S.; Ahderinne, R. HPLC method for determination of inositol tri-, tetra-, penta-, and hexaphosphates in foods and intestinal contents. *J. Food Sci.* **1986**, *51* (3), 547–550.

(17) Sandberg, A.-S.; Carlsson, N.-G.; Svanberg, U. Effects of inositol tri-, tetra-, penta-, and hexaphosphates on *in-vitro* estimation of iron availability. *J. Food Sci.* **1989**, *54* (1), 159–161, 186.

(18) Carlsson, N.-G.; Karlsson, H.; Sandberg, A.-S. Determination of Oligosaccharides in Foods, Diets, and Intestinal Contents by High-Temperature Gas Chromatography and Gas Chromatography/Mass Spectrometry. *J. Agric. Food Chem.* **1992**, *40*, 2404–2412.

(19) Fredrikson M.; Larsson Alminger, M.; Carlsson N.-G.; Sandberg A.-S. Phytate content and Phytate degradation by endogenous phytase in pea (*Pisum sativum*). *J. Sci. Food Agric.* **2001**, in press.