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Phytase for Food Application

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Summary

Phytase [*myo*-inositol(1,2,3,4,5,6)hexakisphosphate phosphohydrolase], a phytate-specific phosphatase, is already used as a supplement in diets for monogastric animals to improve phosphate utilisation from phytate [*myo*-inositol(1,2,3,4,5,6)hexakisphosphate], the major storage form of phosphate in plant seeds. In recent years, this class of enzymes has also been found increasingly interesting for use in processing and manufacturing of food for human consumption, particularly because the decline in food phytate results in an enhancement of mineral bioavailability. Different strategies could be applied to optimise phytate degradation during food processing and digestion in the human alimentary tract such as adjustment of more favourable conditions during food processing for the phytases naturally occurring in the raw material, addition of isolated phytases to the production process, use of raw material with a high intrinsic phytate-degrading activity either naturally present or introduced by genetic engineering and the use of recombinant food-grade microorganisms as carriers for phytate-degrading activity in the human gastrointestinal tract. Furthermore, phytases may find application in the production of functional foods or food supplements with health benefits. Last but not least, technological improvements are expected to occur due to phytate degradation during processing as shown for breadmaking, production of plant protein isolates, corn wet milling and the fractionation of cereal bran.

Key words: digestion, enzymatic phytate dephosphorylation, food processing, functional food, *myo*-inositol phosphates, phytase, phytate

Introduction

Phytases [*myo*-inositol(1,2,3,4,5,6)hexakisphosphate phosphohydrolases] have been identified in plants, microorganisms, and in some animal tissues (*1*). They represent a subgroup of phosphatases which are capable of initiating the stepwise dephosphorylation of phytate [*myo*-inositol(1,2,3,4,5,6)hexakisphosphate], the most abundant inositol phosphate in nature. This classification is irrespective of their *in vivo* function, which remains usually unknown. Based on the catalytic mechanism, phytases can be referred to as histidine acid phytases, β-propeller phytases, cysteine phytases or purple acid phytases (*2,3*). Depending on their pH optima, phytases have been divided into acid and alkaline phytases and based on the carbon in the *myo*-inositol ring of phytate at which dephosphorylation is initiated into 3-phytases (E.C. 3.1.3.8), 6-phytases (E.C. 3.1.3.26) and 5-phytases (E.C. 3.1.3.72).

Up to now, phytases have been mainly, if not solely, used as animal feed additive in diets largely for swine and poultry, and to some extent for fish. The first commercial phytase products were launched into market in 1991. Meanwhile, the market volume is in the range of 150 million euro (*4*). Numerous animal studies have

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shown the effectiveness of supplemental microbial phytase in improving the utilisation of phosphate from phytate (*5–10*). Therefore, including adequate amounts of phytase in the diets for simple-stomached animals reduces the need for orthophosphate supplementation of the feed. As a result, the environment is protected from pollution with excessive manure phosphorus runoffs because the faecal phosphate excretion of the animals is reduced by up to 50 %.

There is also a great potential for the use of phytases in processing and manufacturing of food for human consumption, but up to now, no phytase product for a relevant food application has found its way to the market. Research in this field focuses on the improvement of the nutritional value of plant-based foods as well as on the technical improvement of food processing. A diet rich in phytate leads to a considerably reduced absorption of dietary minerals (*11,12*) and the dephosphorylation of phytate during food processing results in the formation of only partially phosphorylated *myo*-inositol phosphate esters with a lower capability to impair with the intestinal uptake of dietary minerals (*13–15*). Individual *myo*-inositol phosphate esters have been shown to have several important physiological functions in man (*16*). Therefore, phytases may find application in food processing to produce functional foods (*17*), if such biochemically active *myo*-inositol phosphate esters could be generated by phytases and absorbed in the alimentary tract of humans. Technical improvements by adding phytases during food processing have been reported for breadmaking (*18*), production of plant protein isolates (*19,20*), corn wet milling (*21,22*) and the fractionation of cereal bran (*23*).

Nutritional Impact of Phytates

Salts of phytic acid, designated as phytates, are regarded as the primary storage form of both phosphate and inositol in plant seeds and grains. Phytate is formed during maturation of the plant seed and in dormant seeds it represents 60–90 % of the total phosphate (*24*). Phytate is therefore a common constituent of plant-derived foods (Table 1). Depending on the amount of plant-derived foods in the diet and the grade of food processing, the daily intake of phytate can be as high as 4500 mg (*25*). On average, daily intake of phytate was estimated to be 2000–2600 mg for vegetarian diets as well as diets of inhabitants of rural areas in developing countries and 150–1400 mg for mixed diets (*25*).

Phytate as an antinutrient

Phytate behaves in a broad pH range as a highly negatively charged ion and has therefore a tremendous affinity for food components with positive charge(s), such as minerals, trace elements and proteins (*11,26*). This interaction does not have only nutritional consequences, but also affects yield and quality of food ingredients such as starch, corn steep liquor or plant protein isolates (*19–23*).

The major concern about the presence of phytate in the human diet is its negative effect on mineral uptake. Minerals of concern in this regard include zinc, iron, calcium, magnesium, manganese and copper (*11,12*). The formation of insoluble mineral-phytate complexes at physiological pH values is regarded as the major reason for the poor mineral bioavailability, because these complexes are essentially nonabsorbable from the human gastrointestinal tract. Furthermore, the human small inte-

Table 1. Phytate content per mass of dry matter in plant-derived foods (Greiner, unpublished results)

Food	*w*(phytate)/(mg/g)	Food	*w*(phytate)/(mg/g)
Cereal-based		*Legume-based*	
french bread	0.2–0.4	chickpea (cooked)	2.9–11.7
mixed flour bread (70 % wheat, 30 % rye)	0.4–1.1	cowpea (cooked)	3.9–13.2
mixed flour bread (70 % rye, 30 % wheat)	0–0.4	black beans (cooked)	8.5–17.3
sourdough rye bread	0.1–0.3	white beans (cooked)	9.6–13.9
whole wheat bread	3.2–7.3	lima beans (cooked)	4.1–12.7
whole rye bread	1.9–4.3	faba beans (cooked)	8.2–14.2
unleavened wheat bread	10.6–3.2	kidney beans (cooked)	8.3–13.4
maize bread	4.3–8.2	navy beans (cooked)	6.9–12.3
unleavened maize bread	12.2–19.3	soybeans	9.2–16.7
oat bran	7.3–2.1	tempeh	4.5–10.7
oat flakes	8.4–12.1	tofu	8.9–17.8
oat porridge	6.9–10.2	lentils (cooked)	2.1–10.1
pasta	0.7–9.1	green peas (cooked)	1.8–11.5
maize	9.8–21.3	peanuts	9.2–19.7
cornflakes	0.4–1.5	*Others*	
rice (polished, cooked)	1.2–3.7	sesame seeds (toasted)	39.3–57.2
wild rice (cooked)	12.7–21.6	soy protein isolate	2.4–13.1
sorghum	5.9–11.8	soy protein concentrate	11.2–23.4
		buckwheat	9.2–16.2
		amaranth grain	10.6–15.1

stine has only a very limited capability to hydrolyse phytate (*27*) due to the lack of endogenous phytate-degrading enzymes and the limited microbial population in the upper part of the digestive tract.

Solubility and stability of *myo*-inositol phosphate-mineral complexes have been found to decrease as the number of phosphate residues on the *myo*-inositol ring decreases. Therefore, removal of phosphate residues from phytate results in a reduced impairment of intestinal uptake of essential dietary minerals (*13–15*). In isolated form only *myo*-inositol pentakisphosphate suppressed absorption of iron, zinc and calcium in humans, while *myo*-inositol tetrakis- and trisphosphates had no effect in the concentrations under investigation. In the presence of higher phosphorylated *myo*-inositol phosphates, however, *myo*-inositol tetrakis- and trisphosphates were shown to contribute to the negative effect of phytate on iron absorption (*13*). Because a strong negative correlation was found between zinc absorption and the sum of *myo*-inositol tris- through hexakisphosphate from cereal and legume meals (*28*), such a contribution is probably also true for zinc absorption.

Phytate is known to form complexes with proteins at both acidic and alkaline pH (*26*). This interaction may affect changes in protein structure that can decrease enzymatic activity, protein solubility and proteolytic digestibility. However, the significance of protein-phytate complexes in nutrition is still under scrutiny. Strong evidence exists that phytate-protein interactions negatively affect protein digestibility *in vitro* and the extent of this effect depends on the protein source (*26*). A negative effect of phytate on the nutritive value of protein, however, was not clearly confirmed in studies with monogastric animals (*29*). While some have suggested that phytate does not affect protein digestibility, others have found an improvement in amino acid availability with decreasing levels of phytate. This difference may be at least partly due to the use of different protein sources. Of nutritional significance might also be the inhibition of digestive enzymes such as α-amylase (*30,31*), lipase (*32*) or proteinases (*33–35*), such as pepsin, trypsin and chymotrypsin, by phytate as shown in *in vitro* studies. The inhibitory effect increases with the number of phosphate residues per *myo*-inositol molecule and the *myo*-inositol phosphate concentration. This inhibition may be due to the nonspecific nature of phytate-protein interactions, the chelation of calcium ions which are essential for the activity of trypsin and α-amylase, or the interaction with the substrates of these enzymes. The inhibition of proteases may be partly responsible for the reduced protein digestibility. Phytate has also been considered as an inhibitor of α-amylase *in vivo* as indicated by a negative relationship between phytate intake and blood glucose response (*36*). Therefore, food rich in phytate has been considered to have great nutritional significance in the prevention and management of diabetes mellitus, one of the most common nutrition-dependent diseases in Western society.

The most severe effects attributable to phytate have occurred in populations with unrefined cereals and/or pulses as a major dietary component. Especially zinc and iron deficiencies were reported as a consequence of high phytate intakes (*37,38*). To reduce the risk for mineral deficiency in vulnerable groups such as child-bearing women, strictly vegetarians, inhabitants of developing countries, especially fast growing children, different strategies have been developed. The most widely recognised strategies for reducing micronutrient malnutrition are supplementation with pharmaceutical preparations, food fortification, dietary diversification and disease reduction (*39*). For various reasons, none has been very successful. An alternative approach would be to increase the total level of micronutrients in the edible parts of staple crops while at the same time increasing the concentration of compounds which promote their uptake and/or decreasing the amount of compounds which inhibit their absorption either by plant breeding or by genetic engineering. Recently, low phytate mutants in maize, barley, rice and soybeans have been isolated (*40*) and their potential for improving the absorption of iron, zinc and calcium has been shown (*41*). To improve rice as a source of iron, three proteins were expressed in the central endosperm of the rice seed: a *Phaseolus* phytoferritin, an endogenous cysteine-rich metallothionein-like protein, and an *Aspergillus fumigatus* phytase (*42*). If properly targeted, overexpression of phytase during seed development can result in reduced phytate levels in the mature seed (*43*). Enhanced levels of seed phytase may also contribute to an improvement in mineral absorption by reducing phytate levels in plant-based food during processing and digestion in the human stomach once a meal is consumed. In addition, phytate degradation during food processing could be optimised by adding exogenous phytases or by adjusting favourable conditions for the native plant or microbial phytases. Besides enzymatic degradation, nonenzymatic hydrolysis of phytate during food processing or physical separation of phytate-rich parts of the plant seed could result in reduced levels of phytate in the final foods. In general, the lower phytate levels must be paid for by a loss of valuable nutrients which are either removed together with the phytate-rich parts of the plant or destroyed by the strong acids or high temperatures needed for nonenzymatic phytate dephosphorylation. Enzymatic phytate degradation, however, occurs also under mild conditions and does not affect other food components.

Potential health benefits of phytate-rich diets

Consumption of phytate, however, does not seem to have only negative aspects on human health. Dietary phytate was reported to prevent kidney stone formation (*44*), and to protect against atheriosclerosis and coronary heart disease (*45*) as well as against a variety of cancers (*46*). The levels of phytate and its dephosphorylation products in urine, plasma and other biological fluids are fluctuating with ingestion or deprivation of phytate in the human diet (*47*). Therefore, the reduction in phytate intake in developed compared to developing countries might be a factor responsible for the increase in diseases typical for Western societies such as diabetes mellitus, renal lithiasis, cancer, atheriosclerosis and coronary heart diseases. It was suggested that phytate exerts the beneficial effects in the gastrointestinal tract and other target tissues through its chelating ability, but other mechanisms have also been discussed. Because several *myo*-

-inositol phosphates, including phytate, are present as intracellular molecules and because the second messenger D-*myo*-inositol(1,4,5)trisphosphate is bringing about a range of cellular functions including cell proliferation *via* mobilising intracellular Ca^{2+} (*16*), phytate was proposed to exert its anticancer effect by affecting cell signalling mechanisms in mammalian cells (*46*). An effect of extracellular phytate on the concentration of several intracellular *myo*-inositol phosphate esters has already been demonstrated in human erythroleukemia cells (*48*). Furthermore, it has recently been reported that highly negatively charged *myo*-inositol polyphosphates can cross the plasma membrane and be internalised by cells. *Myo*-inositol hexakisphosphate was shown to enter HeLa cells followed by an intracellular dephosphorylation to partially phosphorylated *myo*-inositol phosphates (*49*), whereas turnover of *myo*-inositol(1,3,4,5,6)pentakisphosphate was quite slow after internalisation by SKOV-3 cells (*50*). In addition, individual *myo*-inositol phosphate esters have been proposed to be metabolically active. D-*myo*-inositol(1,2,6)trisphosphate, for example, has been studied in respect to prevention of diabetes complications and treatment of chronic inflammations as well as cardiovascular diseases (*51,52*) and due to its antiangiogenic and antitumour effects *myo*-inositol(1,3,4,5,6)pentakisphosphate was suggested as a promising compound for anticancer therapeutic strategies (*50*).

Enzymatic Phytate Dephosphorylation During Food Processing in Order to Improve Mineral Bioavailability

Various food processing and preparation methods result in a reduction in the phytate content of the processed material. Regarding enzymatic phytate dephosphorylation during food processing and preparation, adjustment of optimal conditions during food processing for the native plant or microbial phytases has to be distinguished from the addition of exogenous ones. Phytate hydrolysis during, for example, germination, soaking, cooking and fermentation is a result of the phytate-degrading activity naturally present in plants and microorganisms. The capability to dephosphorylate phytate differs greatly among different plant and microbial species due to differences in their intrinsic phytate-degrading activities (*53–56*) and the properties of the enzymes such as protein stability and pH as well as temperature optimum for phytate degradation (*1*). Most plant grains and seeds exhibit phytate-degrading activity over a wide pH range (pH=3–10) (Greiner, unpublished results) with maximal activity at pH values from pH=5–5.5 (*1*). In addition, a second lower activity peak from pH=7–8 could be observed in most cereals and legumes (Greiner, unpublished results). Compared to legumes, cereals exhibit, in general, a significantly higher phytate-degrading activity in the pH range from pH=5–5.5 (*53–56*), whereas phytate-degrading activity at pH=8.0 was slightly lower in cereals compared to legumes (Greiner, unpublished results). Performing activity assays by incubation of flours of grains and seeds at pH=5.5 and defining 1 phytase unit (U) as equivalent to the enzymatic activity liberating 1 μmol of phosphate per minute, phytate-degrading activity ranges from 0 to 450 U/kg in legume grains and from 100 to 7000 U/kg in cereal seeds (*54–56*). Phytate-degrading activity is intermediate in wheat (1200–3000 U/kg) and barley (1000–2300 U/kg) and high in rye (5000–7000 U/kg). Maize (70–150 U/kg), rice (150–350 U/kg) and oat (100–500 U/kg) represent cereals with a low intrinsic phytate-degrading activity. At pH=8, phytate-degrading activity ranges from 150 to 600 U/kg in legumes and from 100 to 300 U/kg in cereals (Greiner, unpublished results). To understand phytate hydrolysis it is important to recognise and account not only for phytase activity, but also for activities of further phosphatases present in the plant material. Per definition all enzymes capable of dephosphorylating phytate are classified as phytases. However, *myo*-inositol pentakis-, tetrakis-, tris-, bis-, and monophosphates, the products of phytase action on phytate, might be further dephosphorylated during food processing by phytases as well as phosphatases which do not accept phytate as a substrate.

During food processing or preparation phytate is, in general, not fully hydrolysed by the phytases naturally occurring in plants and microorganisms. It was found, however, that phytate must be reduced to very low levels to strongly increase mineral bioavailability, especially of iron (*57*). To optimise food processing and preparation in respect to phytate degradation, it is essential to know the properties of the natural occurring phytases. In recent years several phytases from cereals (*58–64*), legumes (*65–69*) and microorganisms used for food fermentation (*70,71*) have been purified and their enzymatic properties have been determined. The properties of a purified enzyme, however, are not necessarily identical to the properties of the same enzyme in a food matrix. Temperature optimum for phytate dephosphorylation by a phytase of black beans (*Phaseolus vulgaris* var. Preto), for example, was determined to be 50 °C for the isolated enzyme and 65 °C for the enzyme in the bean matrix (*72*). In addition, the bean matrix had a stabilising effect on the bean phytase at higher temperatures (*72*).

Soaking

Soaking is often used as a pretreatment to facilitate processing of legume grains and cereal seeds. Soaking may last for a short period, about 15 to 20 minutes, or for a very long period, usually 12 to 16 hours. In household situations cereals and legumes are typically soaked in water at room temperatures overnight. Because phytate is water soluble, a significant phytate reduction can be realised by discarding the soak water. In addition, action of endogenous phytases contributes to phytate reduction. Temperature and pH value have been shown to have a significant effect on enzymatic phytate hydrolysis during soaking (*72–74*). If the soaking step is carried out at temperatures between 45 and 65 °C and pH values between pH=5.0 and 6.0, which are close to the optimal conditions for phytate dephosphorylation by the intrinsic plant phytases, a significant percentage of phytate (26–100 %) was enzymatically hydrolysed (*73,74*).

Cooking

Because phytate is heat-stable, significant heat destruction of phytate during cooking is not expected to occur. Therefore, considerable phytate dephosphorylation during cooking only takes place either by discarding the cooking water or by enzymatic phytate hydrolysis due to the action of the intrinsic plant phytases during the early part of the cooking phase (*72*). Prolonged times at elevated temperatures lead to a progressive inactivation of the endogenous enzymes. Thus, providing plants with heat-stable phytases or addition of exogenous heat-stable phytases are seen as possibilities to improve phytate dephosphorylation during cooking.

Germination

Germination is a process widely used in legumes and cereals to increase their palatability and nutritional value, particularly through the breakdown of certain anti-nutrients, such as phytate and protease inhibitors. In non-germinated legume grains and cereal seeds, with the exception of rye and to some extent wheat, triticale and barley, only little intrinsic phytate-degrading activity is found (*53–56*), but during germination a marked increase in phytate-degrading activity with a concomitant decline in phytate content was observed (*65,66,75,76*). Phytate is hydrolysed during germination in a stepwise manner by phytases or a concerted action of phytases and phosphatases which do not accept phytate as a substrate to supply the nutritional needs of the plant without an accumulation of less phosphorylated *myo*-inositol intermediates. Phytate levels resulting in a strong increase in mineral uptake could be achieved after 6 to 10 days of germination. Because long time periods are needed to improve mineral bioavailability through germination, this approach is meant to be useful for household applications, but it does not appear to be an economical industrial method for food processing.

Fermentation

Food fermentation covers a wide range of microbial and enzymatic processing of food and ingredients to achieve desirable characteristics such as prolonged shelf life, improved safety, attractive flavour, nutritional enrichment, elimination of anti-nutrients and promotion of health. Many cereals, legumes and vegetables are extensively used in the preparation of a variety of fermented foods. Microorganisms used for food fermentation may be part of the natural microflora found in the raw material that is fermented or specially cultivated cultures designed to bring about specific changes in the material that is being fermented. Today, defined starter cultures and controlled conditions are generally used in food fermentation. The type of microorganism, the fermentation conditions used, and the starting amount of phytate present in the raw material significantly affect the extent of phytate removal during the fermentation process. Major fermentation microorganisms include lactic acid bacteria, moulds and yeast. Yeast and/or lactic acid bacteria are, for example, used to produce bread, a staple food in many countries. Phytate reduction occurs throughout the different stages of breadmaking and obviously depends on the type of bread being made. The phytase present in the cereal flour is mainly responsible for phytate dephosphorylation during bread fermentation, whereas the contribution of microbial phytate-degrading activity from baker's yeast and lactic acid bacteria is very low or even not existing (*77–79*). In particular, the capability of lactic acid bacteria to produce a phytate-degrading enzyme is still in some dispute. Some studies seem to establish the capability of lactic acid bacteria to hydrolyse phytate (*71,80*), whereas others failed to identify a phytate-degrading enzyme (*78*). Therefore, lowering the pH value of the dough to a more favourable one for the activity of the endogenous cereal phytases is very likely the contribution of the microorganisms to phytate hydrolysis during fermentation. In oriental food fermentation, however, convincing evidence exists that phytases of the microorganisms used for fermentation contribute significantly to phytate degradation (*81–84*). Food products such as tempeh, miso, koji and soy sauce are produced by fermentation of soybeans with *Rhizopus oligosporus* and *Aspergillus oryzae*, respectively. Both moulds have been shown to produce intra- as well as extracellular phytate-degrading activity (*82–84*).

Addition of isolated phytases during food processing

Addition of a phytase preparation during food processing was shown to be an alternative to the optimisation of phytate dephosphorylation by enzymes already present in the raw material used for food processing. Effectiveness of supplemental phytase in reducing phytate content during food processing was demonstrated for cereal as well as for legume-derived food products (*73,85*) and even a complete degradation of phytate was shown to be feasible. The extent of phytate hydrolysis during food processing is affected by the raw material used, the manufacturing process, the source of phytase and the amount of enzyme activity added. An ideal phytase for all food applications does not exist. The added phytase has to be highly active during food processing or preparation. Because temperature and pH value are the major factors determining enzyme activity, favourable properties for phytase that should be used in food processing are high phytate-degrading capability even at room temperature, acceptable heat resistance and a high activity over a broad pH range.

According to pH optimum, phytases could be classified into acid phytases with a pH optimum from pH=3.5–6.0 and alkaline phytases with a pH optimum from pH=7.0–8.0 (Tables 2–3). The majority of the so far characterised phytases showed maximal phytate-degrading activity in the acid pH range. Alkaline phytases have been purified from different *Bacillus* species (*109–111*), lily pollen (*115*) and the rat intestine (*117*) only. Almost all the phytases characterised so far exhibit a single sharp pH optimum. One exception is the phytase from *Aspergillus fumigatus*, which has a broad pH optimum with at least 80 % of the maximal activity at pH values from pH=4.0–7.3 (*87*). Enzyme activity increases with temperature up to a maximal temperature. A further increase in temperature results in a heat-induced denaturation of the enzymes. Depending on the enzyme source, optimal temperature for phytate hydrolysis varies from 35 to 80 °C (Tables 2–3).

Table 2. Some properties of microbial phytases

Phytase source	pH optimum	Temperature optimum	Specific activity at 37 °C	Reference
		°C	U/mg	
A. niger	2.2, 5.0–5.5	55–58	50–103	(*86,87*)
A. terreus	5.0–5.5	70	142–196	(*87*)
A. fumigatus	5.0–6.0	60	23–28	(*87–89*)
A. oryzae	5.5	50	11	(*90*)
A. caespitosus	5.5	80	–	(*91*)
E. nidulans	6.5	–	29–33	(*87*)
M. thermophila	5.5	–	42	(*87*)
T. lanuginosus	6.0	65	110	(*92*)
P. simplicissimum	4.0	55	3	(*93*)
P. lycii	5.5	58	1080	(*94,95*)
Cladosporium	3.5	40	909	(*96*)
S. castellii	4.4	77	418 (70 °C)	(*97*)
P. anomala	4.0	60	–	(*98*)
C. krusei	4.6	40	1210	(*99*)
E. coli	4.5	55–60	811–1800	(*100,101*)
K. terrigena	5.0	58	205	(*102*)
K. pneumoniae	5.0, 5.5	50, 60	224, 297	(*103,104*)
K. aerogenes	4.5, 5.2	68	–	(*105*)
P. agglomerans	4.5	60	23	(*106*)
C. braakii	4.0	50	3457	(*107*)
P. syringae	5.5	40	769	(*108*)
L. sanfranciscensis	4.0	45	–	(*71*)
B. subtilis	6.5–7.5	55–60	9–15	(*109,110*)
B. amyloliquefaciens	7.0–8.0	70	20	(*111*)

Table 3. Some properties of phytases from plants and the rat intestine

Phytase source	pH optimum	Temperature optimum	Specific activity at 37 °C	Reference
		°C	U/mg	
wheat PHY1	6.0	45	127	(*64*)
wheat PHY2	5.0	50	242	(*64*)
spelt D21	6.0	45	262	(*62*)
rye	6.0	45	517	(*60*)
oat	5.0	38	307	(*58*)
barley P1	5.0	45	117	(*59*)
barley P2	6.0	55	43	(*59*)
maize root	5.0–5.1	35–40	5.7	(*112*)
maize seedling	4.8	55	2.3	(*63*)
soybean	4.5–5.0	55–58	2.4	(*69,113*)
mung bean	7.5	57	0.5	(*68*)
scallion leaves	5.5	51	500	(*114*)
faba bean	5.0	50	636	(*65*)
lupine L11	5.0	50	539	(*66*)
lupine L12	5.0	50	607	(*66*)
lupine L2	5.0	50	498	(*66*)
lily pollen	8.0	55	0.2	(*115*)
tomato root	4.3	45	205	(*116*)
rat intestine	7.0, 7.5–8.0	–	–	(*117*)

In general, plant phytases exhibit maximum activity at lower temperatures compared to their microbial counterparts. The higher pH and thermal stability as well as higher specific activity of microbial compared to plant phytases make the former more favourable for an application in food processing. Specific activity is one key factor for commercial exploitation of an enzyme because it has an impact on the economy of the intended use. Specific activity of the phytases characterised so far ranges from <10 U/mg (lily pollen, mung bean, soybean, maize, *Penicillium simplicissimum*) to >1000 U/mg (*Citrobacter braakii*, *Candida krusei*, *Peniophora lycii*) at 37 °C and their individual pH optimum (Tables 2–3). The highest specific activities were reported for *Citrobacter braakii* (3457 U/mg), *Candida krusei* (1210 U/mg), and *Peniophora lycii* (1080 U/g). Thus compared to plant phytases, microbial phytases seem to exhibit higher specific activities. The stability of most of the plant phytases decreased dramatically at pH values below pH=4 and above pH=7.5, whereas the majority of the corresponding microbial enzymes are rather stable even at pH values above pH=8.0 and below pH=3.0. A phytase from *Escherichia coli*, for example, did not lose any activity at pH=2.0 and pH= 10.0 when exposed to 4 °C for 2 hours (*101*). In addition, the majority of the plant phytases are irreversibly inactivated at temperatures above 70 °C within minutes, whereas most of the corresponding microbial enzymes retain significant activity even after prolonged incubation times. The phytases most resistant to high temperatures reported so far have been isolated from *Pichia anomala* (*98*), *Schwanniomyces castellii* (*97*) and *Lactobacillus sanfranciscensis* (*71*). Incubation of these enzymes at 70 °C for 10 minutes did not result in a significant loss of activity and the phytase of *Pichia anomala* was even reported to tolerate a 30-hour treatment at 70 °C without any loss of activity (*98*). For a technical application of phytases in food processing it is of practical interest that a crude enzyme preparation as well as an enzyme present in a food matrix is more pH and heat resistant than the corresponding highly purified enzyme. Although microbial phytases are better suited for an application in food processing, cereal and legume phytases are thought to be an alternative due to their higher acceptance among consumers and their assumed low allergenic potential. Phytases present in cereals and legumes are already part of the human diet and none of them has been reported to be an allergen. In contrast, the phytase from *Aspergillus niger* was assumed to be a high risk factor for occupational asthma and rhinitis. The enzyme was shown to cause specific IgE immune responses among workers exposed to powered phytase preparation (*118–120*). Enzyme preparations with the phytases from *Aspergillus niger* (Natuphos^{TM}, BASF), *Peniophora lycii* (Ronozyme^{TM}, DSM), *Schizosaccharomyces pombe* (Phyzyme^{TM}, Diversa/Danisco A/S), and *Escherichia coli* (Quantum^{TM}, Diversa/Syngenta) are available commercially, which makes their use in food processing technically feasible.

Because phytases with the required properties for food processing applications have not been found in nature so far, engineering of phytases in order to optimise their catalytic features is seen as a promising strategy. Enhancement of thermal tolerance and increase in specific activity are two important issues not only for animal feed, but also for food processing applications of phytases. Different strategies have been used to obtain an enzyme capable of withstanding higher temperatures. A shift in temperature optimum of the *Escherichia coli* phytase from 55 to 65 °C and a significant enhancement in its thermal stability at 80 and 90 °C was achieved by expression of the enzyme in the yeast *Pichia pastoris* after introduction of three glycosylation sites into the amino acid sequence of the *Escherichia coli* phytase by site-directed mutagenesis (*121*). Gene site saturation mutagenesis technology was a further approach used to optimise the performance of the *Escherichia coli* phytase (*122*). A library of clones incorporating all 19 possible amino acid changes in the 431 residues of the sequence of the *Escherichia coli* phytase was generated and screened for mutants exhibiting improved thermal tolerance. The best mutant showed no loss of activity when exposed to 62 °C for 1 hour and 27 % of its initial activity after 10 minutes at 85 °C, which is a significant improvement over the parental phytase. In addition, a 3.5-fold enhancement in gastric stability was observed. By using the consensus approach, which is based on the comparison of amino acid sequences of homologous proteins and subsequent calculation of a consensus amino acid sequence using one of the available standard programmes, a fully synthetic phytase was generated, which exhibited a 21–42 °C increase in intrinsic thermal stability compared to the 19 parental fungal phytases used in its design (*123*). Furthermore, a 3-fold increase in specific activity was achieved by replacing a single amino acid in the sequence of a fungal phytase by site-directed mutagenesis (*124,125*).

Finally, a phytase will not be competitive if it cannot be produced in high yield and purity by a relatively inexpensive system. Because of the low amount of phytase, which is obtained from wild-type organisms and their tedious and cost-intensive purification, wild-type organisms are not a suitable enzyme source for industrial applications. Therefore, highly efficient and cost-effective processes for phytase production by recombinant microorganisms have been developed. High levels of phytate-degrading activity accumulating in the fermentation medium have been described by using economically competitive expression/secretion systems for *Escherichia coli* (*126*) as well as for the yeasts *Hansenula polymorpha* (*127*) and *Pichia pastoris* (*128*). If phytate-degrading capability is introduced into or increased in microorganisms used for food fermentation such as *Saccharomyces cerevisiae*, *Lactobacillus sanfranciscensis* or *Lactobacillus plantarum* there is no need for protein purification. Application of microorganisms improved in such a way in fermentation of plant-derived raw material is expected to result in food products with significantly lower phytate levels. Recently, a genetically modified phytase-secreting *Lactobacillus plantarum* strain was reported (*129*), but the secretion levels were far too low for an industrial application. Furthermore, a *Saccharomyces cerevisiae* strain producing high levels of extracellular phytase activity was constructed (*130*), but its capability to contribute significantly to phytate hydrolysis during fermentation needs to be studied first.

Further Applications of Isolated Phytases in Food Processing

Besides improving mineral and trace element bioavailability, addition of phytase during food processing was reported to affect economy of the production process as well as yield and quality of the final products. Technical improvements by adding phytase during food processing have been reported for breadmaking (*18*), production of plant protein isolates (*19,20*), corn wet milling (*21,22*) and the fractionation of cereal bran (*23*).

Breadmaking

Phytase was shown to be an excellent breadmaking improver (*18*). Besides reduction in phytate content in doughs and fresh breads, fermentation time was shortened by phytase addition without affecting the dough pH. An increase in bread volume and an improvement in crumb texture were also observed. In all formulations the hardness or firmness of the bread crumbs was reduced, so softer crumbs were obtained with phytase supplementation. Other texture parameters such as gumminess and chewiness were also decreased. These improvements in bread quality were suggested to be associated with an indirect impact of phytase on α-amylase activity. Addition of phytase during breadmaking results in lower phytate levels in the final breads. Even so, a complete removal of phytate was not achievable. This, in turn, releases calcium ions, which are essential for α-amylase activity, from calcium-phytate complexes. In the final breads no phytase activity could be detected. Thus intrinsic cereal as well as supplemented microbial phytases were inactivated during baking.

Production of plant protein isolates

Due to their good nutritional and functional properties, the application of plant protein isolates and concentrates has been found increasingly interesting in food production. However, the relatively high content of phytate present in plant seeds and grains and its interaction with proteins under alkaline conditions, which are, in general, applied for protein extraction, negatively affects the yield and quality of the protein isolates obtained by using common production processes. By interacting with phytate, the solubility of the proteins decreases leading to a reduced protein content in the final concentrate. In addition, a considerable amount of the phytate ends up in the protein isolate affecting its nutritional as well as functional properties. Introducing an exogenous phytase into the production process, however, was reported to result in significantly higher protein yields and an almost complete removal of *myo*-inositolhexakis-, pentakis-, tetrakis-, and trisphosphates from the final plant protein isolate (*19,20*). Due to an improvement in mineral bioavailability, their amino acid composition as well as their *in vitro* protein digestibility, these phytate-reduced plant protein isolates were suggested as suitable protein sources for infant formulae. In addition, some phytate-reduced plant protein isolates are discussed as functional additives in food products, because of their good foaming, emulsifying and gelling properties.

Corn wet milling

Steeping is a process required in wet milling of maize to obtain the valuable corn steep liquor and to soften the maize kernel as well as to break the maize cell wall. The key issues of corn wet milling are starch yield, corn steep liquor quality and steeping time. Maize comprises phytate, which to a large extent ends up in the corn steep liquor and constitutes an undesirable component. Phytate-free corn steep liquor is easier to concentrate and this concentrate is used in the fermentation industry for the production of compounds such as enzymes, yeast, polysaccharides, antibiotics, and amino acids as well as a high-energy liquid animal feed ingredient. By adding phytases together with plant cell wall degrading enzymes to the steep liquor, corn steep liquor that was entirely free from phytate was obtained (*21,22*). In addition, the steeping time was reduced considerably and by facilitating the separation of starch from fibre and gluten, higher starch and gluten yields as well as lower energy consumption were achieved.

Fractionation of cereal bran

It is widely known and accepted that cereal bran, the by-product of producing flour, is the most nutritious part of a cereal grain. Very recently an industrial process was developed to economically separate the main fractions of the bran in order to produce high value protein, soluble non-starch carbohydrates, oil fractions, and insoluble fibre (*23*). First the bran is subjected to a combination of the enzymatic treatment using proteins of the group of starch- and phytate-hydrolysing enzymes and wet milling, followed by sequential centrifugation and ultrafiltration. The second step consists of fractionating the insoluble phase of the above-mentioned first step by enzymatic treatment with xylanase and/or β-glucanase and wet milling, again followed by sequential centrifugation and ultrafiltration. All obtained fractions have much broader market applications and greater value than the original bran.

Transgenic Plants

In order to increase phytate-degrading activity during food processing, incorporation of plants with a high phytase activity into the plant-derived raw material to be processed is seen as an alternative to the addition of exogenous phytases. The seeds of rye, triticale, wheat and barley are naturally high in phytase activity. In addition, the introduction and expression of microbial phytase-encoding genes into several different plants including tobacco (*131–136*), alfalfa (*137*), arabidopsis (*43,135,138,139*), subterranean clover (*140*), sesame (*141*), soybean (*142*), canola (*143*), potato (*144,145*), rice (*42,146–148*), wheat (*149*) and sugarcane (*150*) have been reported (Table 4). The introduced microbial phytase-encoding gene is mainly derived from *Aspergillus niger*, but also *Bacillus subtilis*, *Aspergillus fumigatus*, *Escherichia coli*, *Schwanniomyces occidentalis*, and *Selenomonas ruminantium* were used as the gene source (Table 4). Beside the proof of concept, two main objectives were followed; expression of the phytase in the plant seed and expression of a phytase secreted by the plant root. Phytase-expressing transgenic

Table 4. Phytase expression in genetically engineered plants

Host plant	Phytase source	Tissue	Reference
tobacco	*A. niger*	leaf (secreted)	(*131*)
tobacco	*A. niger*	leaf	(*132*)
tobacco	*A. niger*	seed	(*133*)
tobacco	*A. niger*	root (secreted)	(*134*)
tobacco	*B. subtilis*	root (secreted)	(*135*)
tobacco	*B. subtilis*	leaf	(*136*)
alfalfa	*A. niger*	leaf	(*137*)
arabidopsis	*A. niger*	root (secreted)	(*138,139*)
arabidopsis	*B. subtilis*	root (secreted)	(*135*)
arabidopsis	*E. coli*	seed	(*43*)
subterranean clover	*A. niger*	root (secreted)	(*140*)
sesame	*A. niger*	root (secreted)	(*141*)
soybean	*A. niger*	callus (secreted)	(*142*)
canola	*A. niger*	seed	(*143*)
potato	*A. niger*	leaf	(*144*)
potato	consensus	root (secreted)	(*145*)
rice	*A. fumigatus*	seed	(*42,146*)
rice	*S. occidentalis*	leaf	(*147*)
rice	*E. coli + S. ruminantium*	seed	(*148*)
wheat	*A. niger*	seed	(*149*)
sugarcane	*E. coli*	callus	(*150*)

seeds were discussed as a novel feed additive for improved phosphorus utilisation in animal agriculture, because it was shown that only a limited amount of transgenic seed is required in compound feeds to ensure proper degradation of the phytate present in animal diets during digestion in the stomach (*133*). Phytase secreted by the plant root is expected to improve acquisition of organic phosphorus by plants, which may reduce the environmental impacts of agricultural production systems by minimising the need for P-fertilisers (*138*).

So far, only one transgenic plant heterologously expressing a phytase has been developed in order to deal with the issue of dietary phytate in human nutrition (*42,146*). To improve white or polished rice as a source of iron, three proteins were expressed in its central endosperm; a *Phaseolus* phytoferritin, an endogenous cysteine-rich metallothionein-like protein, and an *Aspergillus fumigatus* phytase. Expression of the phytoferritin approximately doubled the endosperm iron content and the cysteine-rich peptides have been shown to improve iron absorption in the gut. The *Aspergillus fumigatus* phytase was selected because of its reported high thermal stability (*87*) and the hope of retaining activity following cooking. However, the enzyme was completely inactivated during cooking. Identification and/or development of phytases that retain thermotolerance following expression in plant tissues and cooking remain a target of active research.

Phytate Hydrolysis During Digestion

Hydrolysis of phytate in the gastrointestinal tract of humans may be carried out by the action of phytate-degrading enzymes from three sources: dietary phytases, mucosal phytases of the small intestine, and phytases from the bacterial flora in the colon. With the exception of calcium, phytate degradation in the colon is not expected to affect mineral absorption significantly, because minerals are mainly absorbed in the upper small intestine. Furthermore, it was demonstrated that only a very low phytate-degrading activity occurs in the human small intestine (*27*). Thus, the human small intestine has only very limited ability to hydrolyse phytate. Dietary phytases, in contrast, are an important factor for phytate degradation during digestion, since these enzymes are active in the human stomach (*151*). In general, the intrinsic phytate-degrading activity in plant-derived foods is not sufficiently high to hydrolyse dietary phytate during passage through the human stomach to such an extent that a significant improvement of, in particular, iron uptake occurs. Development of plants with higher phytase-degrading activities in the edible parts or the addition of phytase preparations to the raw materials or the final foods may result in a more extensive phytate degradation in the human stomach. These phytases should be effective in releasing phytate phosphate in the human stomach, stable to resist inactivation by storage, and the capability to withstand food processing and preparation might also be desirable. Thermal stability is a particularly important issue because food processing and preparation commonly involve exposure to elevated temperature. Phytases having the required level of thermal stability to withstand thermal treatments such as cooking in isolated form or within a certain food matrix have not been found in nature so far. Therefore, it comes as no surprise that isolation and characterisation of thermostable enzymes, as well as engineering phytases, to improve stability at elevated temperatures and the search for the determinants of thermal stability are hot spots of current phytase research (*88,92,111,121–123*). Likewise, a phytase that can tolerate long-term storage or transport at ambient temperatures is undisputedly attractive.

The ability of a phytase to hydrolyse phytate in the digestive tract is determined by its enzymatic properties. As the stomach is the main functional site of dietary and/or supplemental phytase, an enzyme with an acidic pH optimum, a high stability under acidic pH conditions and high resistance to pepsin is certainly desirable. In respect to their phytate-degrading capabilities in the human stomach, microbial phytases are thought to have advantages over their plant counterparts. Microbial phytases exhibit considerable enzymatic activity over a wide pH range and are active even below pH=3.5. In addition, the pH stability of some microbial phytases below pH=3.0 is remarkable. In addition, plant phytases are considered to be more susceptible to inactivation by gastrointestinal enzymes. Wheat phytase was reported to be less resistant to pepsin and pancreatin than the phytase of *Aspergillus niger* (*152*), and the phytases of *Escherichia coli* and *Citrobacter braakii* were shown to be even more resistant to pepsin and pancreatin than the *Aspergillus niger* phytase (*107,153*). In addition, the phytase of *Citrobacter braakii* was stable to trypsin (*107*). Compared to the phytase from *Escherichia coli*, the corresponding enzyme from *Bacillus subtilis* exhibited a similar sensitivity to pancreatin, but a much higher susceptibility to pepsin (*153*). It also has to be considered that recombinant enzymes may differ in proteolytic resistance compared to their wild-type counterparts, as recently reported for the *Escherichia coli* and *Aspergillus niger* phytase produced in *Pichia pastoris* (*154*).

A completely different approach to the improvement of phytate degradation in the stomach and upper small intestine of humans has been brought up very recently (*130,155*). Microorganisms safe for human consumption such as baker's yeast (*Saccharomyces cerevisiae*), lactobacilli or bifidobacteria were suggested as carriers for phytate-degrading activity in the gastrointestinal tract. Therefore, tolerance to the conditions in the stomach and small intestine as well as the capability to generate extracellular phytate-degrading activity under gastrointestinal conditions are features needed by the microorganisms for such an application. The capability to survive the passage through the human gastrointestinal tract was already shown for *Saccharomyces cerevisiae* (*156*) as well as for several *Lactobacillus* and *Bifidobacterium* strains (*157*). However, a sufficiently high extracellular phytate-degrading activity was demonstrated neither for *Saccharomyces cerevisiae* nor for any *Lactobacillus* or *Bifidobacterium* strains. To overcome this constraint, genetic engineering could be used for the construction of recombinant high phytase-producing strains. To be capable of hydrolysing dietary phytate, this phytase has to be secreted by the microorganisms or targeted to its outer cell wall. Two different approaches have been successfully applied to improve phytase production and secretion in the target microorganisms. A phytase-encoding gene from *Bacillus subtilis* was introduced into a *Lactobacillus plantarum* strain, but the secreted phytate-degrading activity was far too low for any application (*129*). In yeast, however, the regulation of phytase synthesis was modified by deletion of a gene encoding a negative regulator for the expression of the phytase-encoding genes. Compared to the corresponding wild-type yeast, the recombinant yeast exhibited a several-fold higher capability for phytate hydrolysis, both in the presence and absence of orthophosphate (*130*). In addition, the recombinant yeast was shown to degrade up to 40 % of the phytate present in wheat gruel under simulated gastric conditions, whereas no phytate hydrolysis was observed with the wild-type yeast. However, *in vivo* studies are still missing. Because yeast phytase is virtually inactive at pH values above pH=7, this phytase will be active only in the human stomach and no further phytate degradation is expected to occur in the human small intestine. Thus, using one or a mixture of food-grade microorganisms having both features, secretion of a phytase optimally active at acidic and another one optimally active at alkaline conditions, might improve phytate breakdown in the human stomach and upper small intestine during digestion with a concomitant improvement in mineral bioavailability.

Production of Metabolically Active Phytate Breakdown Products

Much scientific information has been reported in the last few years linking diet, specific foods, or individual food components with the maintenance of human health and the prevention of chronic diseases such as coronary heart disease, cancer or osteoporosis. Individual *myo*-inositol phosphate esters have been shown to have important physiological functions in man (*16*). Some of these compounds, in particular D-*myo*-inositol(1,4,5)trisphosphate and D-*myo*-inositol(1,3,4,5)tetrakisphosphate, have been demonstrated to play an important role as intracellular second messengers (*16*), and several isomers of *myo*-inositol phosphates have shown important pharmacological effects, such as prevention of diabetes complications and anti-inflammatory effects (*51,52*) as well as antiangiogenic and antitumour effects (*50*). In addition, dietary *myo*-inositol phosphates have been suggested to bring about benefits for human health, such as amelioration of heart disease conditions by controlling hypercholesterolemia and atheriosclerosis (*45*), prevention of renal stone formation (*44*), and protection against a variety of cancers, in particular colon cancer (*46*).

During food processing and digestion, phytate can be partially dephosphorylated to yield a large number of positional isomers of *myo*-inositol pentakis-, tetrakis-, tris-, bis-, and monophosphates. The number and distribution of the phosphate residues on the *myo*-inositol ring determines the metabolic effects triggered by the individual *myo*-inositol phosphate isomer. Different phytases may exhibit different phytate degradation pathways and therefore lead to the generation and accumulation of different *myo*-inositol phosphate intermediates. A summary of the so far established intermediates generated by the different phytases upon acting on phytate is given in Table 5.

Until now, the diversity and practical unavailability of the individual *myo*-inositol phosphate esters preclude their being tested for metabolic activity. Attempts to produce defined isomers of the different partially phosphorylated *myo*-inositol phosphates non-enzymatically have resulted in mixtures of *myo*-inositol pentakis-, tetrakis-, tris-, bis-, and monophosphate isomers. Purification of these isomers from the mixture is arduous and uneconomical. An alternative approach to make pure breakdown

Table 5. *Myo*-inositol phosphate intermediates generated through enzymatic phytate degradation

Enzyme	IP_5-isomer	IP_4-isomer	IP_3-isomer	IP_2-isomer	IP-isomer	Reference
barley P1; P2, spelt D21, wheat PHY1; PHY2, rye, oat, rice, lupine L2	D-Ins(1,2,3,5,6)P_5	D-Ins(1,2,5,6)P_4	D-Ins(1,2,6)P_3	D-Ins(1,2)P_2	Ins(2)P	(*158–161*)
wheat F2	D-Ins(1,2,3,5,6)P_5	D-Ins(1,2,3,6)P_4	Ins(1,2,3)P_3	D-Ins(1,2)P_2	Ins(2)P	(*162*)
mung bean	D-Ins(1,2,3,5,6)P_5	D-Ins(1,2,3,6)P_4	D-Ins(1,2,6)P_3/ Ins(1,2,3)P_3	D-Ins(2,6)P_2/ D-Ins(1,2)P_2	Ins(2)P	(*163*)
S. cerevisiae, *Pseudomonas*, lupine L11, lupine L12	D-Ins(1,2,4,5,6)P_5	D-Ins(1,2,5,6)P_4	D-Ins(1,2,6)P_3	D-Ins(1,2)P_2	Ins(2)P	(*161,164, 165*)
E. coli	D-Ins(1,2,3,4,5)P_5	D-Ins(2,3,4,5)P_4	Ins(2,4,5)P_3	Ins(2,5)P_2	Ins(2)P	(*166*)
Paramecium	D-Ins(1,2,3,4,5)P_5	D-Ins(1,2,3,4)P_4	Ins(1,2,3)P_3	D-Ins(2,3)P_2		(*167*)
lily	D-Ins(1,2,3,4,6)P_5	D-Ins(1,2,3,4)P_4/ D-Ins(1,2,3,6)P_4	Ins(1,2,3)P_3			(*168*)
B. subtilis	D/L-Ins(1,2,3,4,5)P_5/ D/L-Ins(1,2,4,5,6)P_5	Ins(1,2,3,5)P_4/ Ins(2,4,5,6)P_4	Ins(1,3,5)P_3/ Ins(2,4,6)P_3			(*169*)
B. subtilis, *B. amyloliquefaciens*	D-Ins(1,2,4,5,6)P_5/ D/L-Ins(1,2,3,4,5)P_5	Ins(2,4,5,6)P_4/ D-Ins(1,2,5,6)P_4	Ins(2,4,6)P_3/ D-Ins(1,2,6)P_3			(*170*)
Pantoea agglomerans	D-Ins(1,2,4,5,6)P_5					(*171*)

products of phytate available in sufficient quantities for physiological studies is the use of an immobilised enzyme-based bioreactor followed by anion-exchange chromatography of the hydrolysis mixture. The amount of the desired phytate degradation product could be controlled by the number of passages of the phytate-containing solution through the bioreactor (*172*).

If individual phytate degradation products are established to be metabolically active, phytases may find application in food processing to produce foods with improved nutritional value, health benefits and maintained sensory properties (functional foods). By adding phytase to the raw material, phytate will be degraded to metabolically active *myo*-inositol phosphates during food processing. To end up with foods with a reduced content of phytate and a regulated content and composition of partially phosphorylated *myo*-inositol phosphate esters with health benefits, phytate dephosphorylation during food processing has to be tightly controlled. An alternative could be to generate metabolically active *myo*-inositol phosphates as food supplements by using pure phytate as the source material. Because partially phosphorylated *myo*-inositol phosphate esters are subjected to degradation in the human gastrointestinal tract even if all dietary phosphatases including phytases are inactivated, it might be necessary to enrich foods with a precursor of the true active *myo*-inositol phosphate ester to trigger the desired physiological effects. That the human intestinal alkaline phosphatase exhibits activity towards lower *myo*-inositol phosphate esters has already been demonstrated in ileostomy patients (*173,174*) and the microflora in the human colon is also considered to be capable of degrading phytate as well as phytate breakdown products. To exert their metabolic effects in tissues far away from the alimentary tract, *myo*-inositol phosphates have to be absorbed in the gastrointestinal tract. There is some evidence of an uptake of *myo*-inositol phosphates from the human alimentary tract, because the levels of phytate and its dephosphorylation products in biological fluids are fluctuating with ingestion or deprivation of phytate in the human diet (*47*).

Conclusion

Up to now, phytases have only found application as an animal feed additive in diets for monogastric animals, but there is great potential for the use of this class of enzymes in processing and manufacturing of food for human consumption. Almost complete removal of phytate during food processing and/or food digestion in the human stomach and upper small intestine results in an improvement of the bioavailability of essential minerals such as iron and zinc. This is seen as a way to reduce the risk of running into mineral deficiency in vulnerable groups such as child-bearing women, strictly vegetarians and inhabitants of developing countries. In addition, phytate removal could affect economy of the production process as well as yield and purity of the final products as reported for breadmaking, production of plant protein isolates, corn wet milling and fractionation of cereal bran. Not a complete, but a controlled degradation of phytate is the aim of using phytase to produce food with a regulated content and composition of *myo*-inositol phosphate esters with health benefits or to generate these compounds as food supplements. Further work is needed to identify metabolically active *myo*-inositol phosphate isomers and phytases or a mixture of phytases and/or phosphatases without phytate-degrading activity capable of generating these isomers.

One ideal phytase for all food applications does not exist. Thus, screening nature for phytases with more favourable properties for food applications and engineering phytases in order to optimise their catalytic and stability features are suitable approaches to make a proper phytase available for a specific application in food processing. The phytases may be used in isolated form or produced in high levels in recombinant microorganisms

used for food fermentation and/or in the edible parts of a recombinant plant. If the intrinsic phytases present in the material to be processed should be used for phytate dephosphorylation during food processing, their catalytic properties have to be elucidated to be able to optimise phytate degradation in respect to the intended use of the food product.

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