

Review

Phytate in foods and significance for humans: Food sources, intake, processing, bioavailability, protective role and analysis

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The article gives an overview of phytic acid in food and of its significance for human nutrition. It summarises phytate sources in foods and discusses problems of phytic acid/phytate contents of food tables. Data on phytic acid intake are evaluated and daily phytic acid intake depending on food habits is assessed. Degradation of phytate during gastro-intestinal passage is summarised, the mechanism of phytate interacting with minerals and trace elements in the gastro-intestinal chyme described and the pathway of inositol phosphate hydrolysis in the gut presented. The present knowledge of phytate absorption is summarised and discussed. Effects of phytate on mineral and trace element bioavailability are reported and phytate degradation during processing and storage is described. Beneficial activities of dietary phytate such as its effects on calcification and kidney stone formation and on lowering blood glucose and lipids are reported. The antioxidative property of phytic acid and its potential anticarcinogenic activities are briefly surveyed. Development of the analysis of phytic acid and other inositol phosphates is described, problems of inositol phosphate determination and detection discussed and the need for standardisation of phytic acid analysis in foods argued.

Keywords: Absorption / Antioxidant / Degradation / Inositol phosphates / Phytic acid

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1 Introduction

The discovery of phytate dates from 1855 to 1856 when Hartig first reported small round particles in various plant seeds similar in size to potato starch grains [1, 2]. Using the iodine test he showed that the particles were free of starch and concluded that they must contain reserve nutrients for the germination of seeds. Later it was discovered that the isolated particles were rich in phosphorous, calcium and

magnesium but neither contained proteins nor lipids [3]. The name 'phytin' was created by virtue of the fact that this substance is of plant origin, having been detected neither in meat nor in dairy products, and it originally described the classical calcium–magnesium phytate deposits of plant seeds [3]. Winterstein [4], Schulze and Winterstein [5] and Posternak [6] showed that hydrolysing phytin by hydrochloric acid liberated phosphoric acid and inositol. To explain the high phosphorous, calcium and magnesium contents of phytin, paired phosphoric acids were discussed as possible structures [7, 8]. Other molecular structures, however, were also under controversial debate for many years [8, 9]. In 1914, Anderson [10] presented the molecular structure of *myo*-inositol-1,2,3,4,5,6-hexakis dihydrogen phosphate, also called phytic acid (Fig. 1), which is still valid and was confirmed by various modern analytical methods [11–13].

Phytate, the salt of phytic acid, is widely distributed in the plant kingdom. It serves as a storage form of phospho-

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Abbreviations: HAP, hydroxyapatite; ICP, inductively coupled plasma; PAR, 4-(2-pyridylazo) resorcinol; TBARS, thiobarbituric acid reacting substances

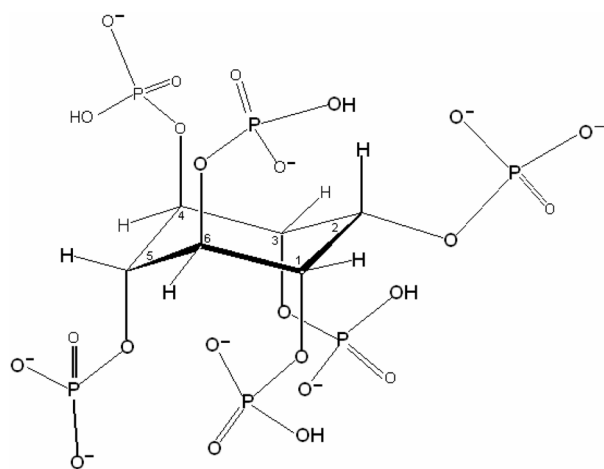


Figure 1. *myo*-inositol-1,2,3,4,5,6-hexakis phosphate at pH 6–7. Under physiological conditions the negative charges are counterbalanced most likely by sodium ions or by other cations. Conformation: 5 axial/1 equatorial (modified to Emsey and Niazi [11]).

rous and minerals and contains ~75% of total phosphorous of the kernels [14]. Other parts of plants such as roots, tubers and turions, however, are very low in phytate (~0.1% dw) [15]. Besides phytate, other inositol phosphates such as inositol pentaphosphates and inositol tetrphosphates are also present in seeds, however, to a much lower extent (<15%) [16]. During the germination of seeds, phytate is hydrolysed [17, 18] and phosphate along with minerals such as calcium and magnesium becomes susceptible for germination and development of the seedlings, explaining its significant role in plant metabolism.

Phytate is predominantly present in unprocessed food, but can be degraded during processing, so a broad range of inositol phosphates may be consumed. It has been estimated that the daily intake of phytate and other inositol phosphates on the basis of Western style diets varies from ~0.3 to 2.6 g [19] and in a global range from 0.180 to 4.569 g [20], strongly depending on the diet selected; low in normal Western diets and high in vegetarian diets. Under heat treatment up to ~100°C (home cooking, roasting, pressure cooking, *etc.*) phytate is quite stable [21, 22] while food processing with the aid of phytases may result in strong phytate hydrolysis [23].

For decades phytate has been regarded as an antinutrient, as, during gastro-intestinal passage, it may inhibit the absorption of some essential trace elements and minerals, which under certain dietary circumstances may lead to calcium, iron and zinc deficiencies [24–29]. Thus, intensive research has been carried out to remove phytate from food by proper processing, to improve the bioavailability and to avoid deficiencies of essential trace elements and minerals. In the last 20 years, however, beneficial properties of phytate have been observed and antioxidant [30] and anticancer activities [31] were reported. Inhibition of calcium salt crystallisation and prevention of renal stone formation

Table 1. Sources of dietary energy consumption according to the FAO (kcal/capita/day, 2001–2003)^{a)}

Sources	Developed countries	%	Developing countries	%
Fruits and vegetables	308	9.3	295	11.1
Cereals	1020	30.7	1391	52.4
Pulses	286	8.6	198	7.5
Sugar	427	12.9	194	7.3
Animal products	712	21.5	311	11.7
Oils and fats	566	17.1	267	10.1
Total	3319	100.0	2656	100.0

a) Adapted from [43].

through dietary phytate were described [32]. Reduction of starch digestion along with slowing down of the glycemic index of foods [33, 34] have also been reported, as well as positive effects on blood glucose and blood cholesterol [35, 36]. These findings have revived discussions about the significance of phytate and other inositol phosphates in human nutrition and for human health.

Of central interest therefore is to understand how phytate exerts its beneficial effects in organs and cells, what the fate of phytate is during the digestion in the gut and how phytate and its degradations products can be absorbed.

Under physiological pH (~6–7) phytate is highly negatively charged [13] (Fig. 1) and as no adequate carriers have been detected by now, it has long been assumed that phytate cannot cross the lipid bilayer of plasma membranes and in consequence, its absorption in the gut has been considered rather improbable. However, recent studies in humans and rats have shown increasing levels of phytate in plasma and enhanced urinary phytate excretion after application of sodium phytate [37, 38]. Studies with radioactive labelled phytate in rats also provided some evidence for the absorption of phytate or at least of parts of its degradation products [39, 40]. Cellular uptake studies with MCF-7 cells also provide evidence of phytate absorption [41] and recent experiments with HeLa show that cellular uptake of phytate might occur *via* pinocytosis [42]. Moreover, the great number of studies showing anticancer activity of phytate in skin, lung, liver, mammary, prostate, soft tissue, *etc.* also suggest that phytate or phytate degradation products have to be absorbed to a certain extent, even though the absorption mechanism still remains to be clarified [41].

This review gives an overview of the main dietary food sources of phytate and the estimated daily intake. It describes the degradation of phytate during gastro-intestinal digestion and the passage throughout the gut and discusses the present knowledge of cellular uptake, absorption and bioavailability of phytate and lower phosphorylated inositol phosphates. Moreover, it surveys the adverse and beneficial activities of phytate and reports the development and progress made in the analysis of phytate and other inositol phosphates in food.

Table 2. Content of phytic acid/phytate in cereals

<i>Cereals</i> Common names		Taxonomic names	Phytic acid/phytate ^{a)} g/100 g (dw)	References
Maize		<i>Zea mays</i>	0.72–2.22	[47–57]
	Maize germ		6.39	[20]
Wheat		<i>Triticum</i> spp. (~25 species)	0.39–1.35	[48, 49, 51, 57–60]
	Wheat bran		2.1–7.3	[20, 46, 51, 53, 59, 60, 61, 72]
	Wheat germ		1.14–3.91	[20, 46, 74]
Rice		<i>Oryza glaberrima/sativa</i>	0.06–1.08	[47–50, 52, 55, 62, 63, 114]
	Rice bran		2.56–8.7	[46, 51, 59, 60, 75]
Barley		<i>Hordeum vulgare</i>	0.38–1.16	[51, 53, 57, 58, 60, 64–66, 99]
Sorghum		<i>Sorghum</i> spp. (~30 species)	0.57–3.35	[50, 51, 55, 67, 73]
Oat		<i>Avena sativa</i>	0.42–1.16	[51, 54, 56–58, 60, 61, 64, 67, 68]
Rye		<i>Secale cereale</i>	0.54–1.46	[47, 48, 53, 56, 60, 64, 69]
Millet		<i>Pennisetum</i> sp., etc.	0.18–1.67	[50, 55, 70, 75, 114]
Triticale		<i>Triticale secale</i>	0.50–1.89	[70, 71]
Wild rice		<i>Zizania</i> sp.	2.20	[53]

a) Depending on the data published.

Table 3. Content of phytic acid/phytate in legumes

<i>Legumes</i> Common names		Taxonomic names	Phytic acid/phytate ^{a)} g/100 g (dw)	References
Kidney beans		<i>Phaseolus vulgaris</i>	0.61–2.38	[47–50, 53, 75–83]
Haricot beans				
Pinto beans				
Navy beans				
Blackeye beans				
Broad beans		<i>Vicia faba</i>	0.51–1.77	[79, 85–87, 100–105]
Peas		<i>Pisum sativum</i> var. <i>arvense</i>	0.22–1.22	[48, 65, 84–90]
Dry cowpeas		<i>Vigna unguiculata</i>	0.37–2.90	[48, 50, 55, 82, 91–98]
Black-eyed peas				
Chickpeas (Garbanzo/Bengal gram)		<i>Cicer arietinum</i>	0.28–1.60	[48, 50, 65, 83, 85, 86, 92, 95, 99]
Lentils		<i>Lens culinaris</i>	0.27–1.51	[50, 65, 82, 83, 86, 95, 106–111]

a) Depending on the data published.

2 Sources of phytate in foods

The main sources of phytate in the daily diet are cereals and legumes, including oil seeds and nuts. They are important for human nutrition and represent ~40 and ~60% of total caloric intake for humans in developed and in developing countries, respectively (Table 1) [43].

In cereals, phytate is located in the aleurone layer and the germ while the endosperm is almost free of phytate [44–46]. Approximately 80% of phytate is located in the aleurone layer of small grains (wheat, rice, etc.), which represents ~20% of this tissue's dry weight and demonstrates the enormous phytate reservoir which can be stored in special tissues [14, 44]. Cereals are rich in phytate and contain ~1% phytic acid on the dry matter basis, ranging from ~0.06 to ~2.2% (dw) (Table 2) [46–73]. Cereal food products, however, may show higher phytic acid contents [46]. For wheat germs, wheat bran and special wheat bran fractions the phytic acid concentrations of 1.1–3.9, 2.0–5.3

and 7.3%, respectively, were reported [46, 51, 72, 74] and for rice bran it ranged up to 8.7% [75].

In legume seeds phytate predominantly occurs in the protein bodies of the endosperm or the cotyledon, containing up to 90% of the total phytic acid. In the whole seed the phytic acid content varies from 0.2 to 2.9% (dw) (Table 3) and is higher in the cotyledons (<3.7%) [48–50, 53, 55, 65, 75–111].

In oilseeds such as sunflower kernels, soybeans, soybean products, sesame seeds, linseeds and rape seeds the phytic acid content ranges from ~1 to 5.4% (dw) (Table 4) [46, 49–52, 55, 58, 74, 85, 91, 112–117] and special food products such as dehulled sesame seeds or rape seed protein concentrates show phytic acid contents up to ~5.4 and 5.3–7.5%, respectively [74, 113]. In soy concentrates a maximum phytic acid content of 10.7% is reported [75].

In nuts, the forth group of phytate-rich food, such as hazelnuts, walnuts, almonds and cashew nuts, the phytic acid content varies of ~0.1–9.4% (dw) (Table 5) [47, 49,

Table 4. Content of phytic acid/phytate in oilseeds

<i>Oilseeds</i> Common names	Taxonomic names	Phytic acid/phytate ^{a)} g/100 g (dw)	References
Soybeans	<i>Glycine max</i>	1.0–2.22	[50–52, 55, 58, 91, 106, 112, 114–116]
Soy concentrate		10.7	[75]
Tofu		0.1–2.90	[20, 46, 49, 51, 112, 115]
Linseed	<i>Linum usitatissimum</i>	2.15–3.69	[20, 46, 85, 117]
Sesame seed	<i>Sesmun indicum</i>	1.44–5.36	[20, 46, 47, 49, 51, 74, 99, 113, 114, 117]
Rapeseed	<i>Brassica napus</i>	2.50	[117]
Rapeseed protein concentrate		5.3–7.5	[113]
Sunflower meal	<i>Helianthus annuus</i>	3.9–4.3	[51]

a) Depending on the data published.

Table 5. Content of phytic acid/phytate in nuts

<i>Nuts</i> Common names	Taxonomic names	Phytic acid/phytate ^{a)} g/100 g (dw)	References
Peanuts	<i>Arachis hypogaea</i>	0.17–4.47	[47, 49, 76, 117, 118]
Almonds	<i>Prunus dulcis</i>	0.35–9.42	[47, 49, 76, 117, 118]
Walnuts	<i>Juglans regia</i>	0.20–6.69	[47, 49, 76, 103, 117, 118]
Cashew nuts	<i>Anacardium occidentale</i>	0.19–4.98	[47, 49, 76, 103, 118]
Brazil nuts	<i>Bertholetia excelssa</i>	0.29–6.34	[47, 117–119]
Pistachios	<i>Pistachia vera</i>	0.29–2.83	[49, 110, 117]
Hazelnuts	<i>Corylus avellana</i>	0.23–0.92	[110, 117]
Macadamia nuts	<i>Macadamia integrifolia</i>	0.15–2.62	[49, 76, 118]
Pecans	<i>Carya illinoensis</i>	0.18–4.52	[47, 49, 76, 118]
Pine nuts	<i>Pinus pinea</i>	0.20	[118]

a) Depending on the data published.

76, 103, 116, 118, 119]. Especial high phytate contents of 9.4, 6.7 and 6.3% (dw) are found in almonds, walnuts and Brazil nuts, respectively. However, these results require confirmation by specific methods for phytic acid analysis.

Table 2–5 show huge variations of the phytic acid or phytate content in different raw and unprocessed foods. For millet, rice, almonds, walnuts, peanuts, *etc.* variations of about one order of magnitude exist. For most legumes half this variation is present. These enormous ranges of phytic acid or phytate concentrations published do not only reflect the great number of botanical varieties of seeds, various environmental or climatic conditions of growing but also the different stages of seed maturation. All these factors influence the phytic acid contents presented, along with differences resulting from various unspecific and specific methods for the determination of phytic acid or phytate in food.

Confusion may arise if both phytic acid and phytate contents are mixed in food tables. Analysing phytate in food, all analytical procedures determine phytic acid following acidic extraction from food. This, however, eliminates any information on the phytate cations and in consequence no exact molecular weight of phytates occurring naturally can be calculated. Therefore, results should be given correctly as phytic acid even though it is not present in plants. However, if food tables present ‘phytate’ contents of food, this ‘phytate’ is mostly calculated on the basis of dodecasodium

phytate which also does not exist in nature but is widely used as calibration standard and should not be confounded with the naturally occurring phytates in foods.

To avoid confusion and to compare results on a common basis, international standardisation of specific methods for the determination of phytic acid/phytate and other inositol phosphates in foods and of listing either the phytic acid or the phytate content in food tables is absolutely essential.

3 Estimation of dietary phytate intake

Systematic studies on the mean daily dietary phytic acid or phytate intake in humans of various countries are very rare. Thus, the available intake data from different countries were collected and the daily dietary phytic acid intakes estimated (Table 6).

3.1 Europe

In the UK earlier studies showed phytate intake varying from 504 to 844 mg for adults [82, 120, 121], while a recent study calculated the mean daily phytate intake in men (aged 40 years) at 1436 ± 755 mg [122].

Early studies from Italy, evaluating 13 Italian diets, also reported a broad range of 112–1367 mg phytic acid *per day*

Table 6. Daily intake of phytic acid/phytate^{a)} in different countries

Country	Sex, age and conditions	Daily intake of phytic acid/phytate mean value, mean $\pm$ SD or range (mg)	References
<i>Europe</i>			
United Kingdom	Male (>40 years)	1436 $\pm$ 755	[122]
	Male–female	600–800	[82]
	Male–female	504–848	[120, 121]
Italy	Male–female	219 (112–1367)	[123]
	Male–female, average Italian diet	293 (265–320)	[124]
	Male–female, north-west	288	[124]
	Male–female, north-east	320	[124]
	Male–female, south	265	[124]
Sweden	Male–female	180	[125]
	Male–female (35–76 years) Western type diets	369 (230–532)	[126]
	Male–female (35–76 years) vegetarian diets	1146 (500–2927)	[126]
	6–8 m, commercial milk-based cereals and porridge	124 $\pm$ 82 (μ mol phytate/day)	[127]
	6–8 m, infant formula and porridge	26 $\pm$ 18 (μ mol phytate/day)	[127]
	9–11 m, commercial milk-based cereals and porridge	189 $\pm$ 78 (μ mol phytate/day)	[127]
Finland	9–11 m, infant formula and porridge	62 $\pm$ 47 (μ mol phytate/day)	[127]
	Male–female	370	[128]
<i>North and Central America</i>			
USA	Infants (<1 years)	166 $\pm$ 167	[129]
	Children (1–3 years)	390 $\pm$ 231	[129]
	Children (4–5 years)	501 $\pm$ 271	[129]
	African-American male–female (median intake)	538 (253–1352)	[131]
	African-American females (median intake)	512	[131]
	African-American males (median intake)	608	[131]
	Male–female (19–35 years)	1293 $\pm$ 666	[133]
	Female (18–24 years)	395 $\pm$ 334	[134]
	Female omnivorous (self-selected)	631 (590–734)	[135]
	Male omnivorous (self-selected)	746 (714–762)	[135]
	Female vegetarians	~1250 $\pm$ 450	[135]
	Male vegetarians	~1550 $\pm$ 550	[135]
	Male lacto-ovo-vegetarian	5577	[136]
	Male lacto-ovo-vegetarian	972	[137]
	Average American (bw, 75 kg)	750	[136]
Canada	Female (4–5 years)	250 (132–318)	[130]
	Male (4–5 years)	320 (203–463)	[130]
Mexico	Lacto-ovo diets – Asian immigrants	1487 $\pm$ 791	[132]
	Male–female (18–30 m)	1666 $\pm$ 650	[138]
Guatemala	Male–female (7–9 years)	3380 $\pm$ 1070	[139]
	Female (15–37 years)	2254	[140]
<i>Asia</i>			
India	Male–female	670	[141]
	Male–female (4–9 years)	720–1160	[142]
	Male–female (10–19 years)	1380–1780	[142]
	Male–female (20–45 years)	1560–2500	[142]
	Male–female (>45 years)	1290–2080	[142]
	Females (16–20 years)	~840	[143]
Thailand	Females nonurban	1139 $\pm$ 481	[144]
	Males nonurban	1104 $\pm$ 965	[144]
	Females urban	997 $\pm$ 435	[144]
	Males urban	1304 $\pm$ 956	[144]
Peoples Republic of China	Male–female	1186 (823–1603)	[145]
	Male–female urban	781 (443–1205)	[145]
	Male–female nonurban	1342 (970–1757)	[145]
Republic of China	Females	690 $\pm$ 189	[146]
	Males	915 $\pm$ 330	[146]

Table 6. Continued

Country	Sex, age and conditions	Daily intake of phytic acid/phytate ^{a)} mean value, mean $\pm$ SD or range (mg)	References
South Korea	Male (21–70 years)	839 $\pm$ 400	[114]
	Female (21–70 years)	752 $\pm$ 407	[114]
	Female (20–24 years)	322 $\pm$ 220	[147]
	Female (64–75 years)	496 $\pm$ 252	[147]
<i>Africa</i>			
Egypt	18–30 months	796 $\pm$ 248	[138]
	7–9 years	1270 $\pm$ 280	[139]
Kenya	18–30 months	1066 $\pm$ 324	[138]
	7–9 years	2390 $\pm$ 480	[139]
Malawi	Preschool girls (4–6 years)	1621 $\pm$ 660 to 1729 $\pm$ 592	[148]
	Preschool boys (4–6 years)	1857 $\pm$ 530 to 2161 $\pm$ 684	[148]
Gambia	1–17 months	10–560	[149]
Ghana	children	578 $\pm$ 161	[150]
Nigeria	Male–female	2200	[77]
Ethiopia	Female pregnant	1033 $\pm$ 843	[99]

a) Depending on the data published.

with an estimated mean intake of 219 mg/day [123], while a later report stated the mean phytic acid intake of the national Italian diet at 293 mg, defined as the average of typical diets from the north-west (288 mg), from the north-east (320 mg) and from the south of Italy (265 mg) [124]. The study indicates, moreover, that cereals, contributing the highest portion to the phytic acid intake (52–57%), are lowest in the south and highest in the north-east, resulting in the highest phytic acid intake in the north-eastern part of Italy.

In Sweden, the mean phytic acid intake of adults was reported at 180 mg [125]. However, a later study from Sweden in adults (35–76 years) showed a phytic acid intake of 230–532 mg (mean value 369 mg) and of 500–2927 mg (mean value 1146 mg) for Western type diets and vegetarian diets, respectively [126], whereas the phytic acid intake was recalculated on the basis of the phytate phosphorus, reported in the paper. In Swedish infants (6–8 m), nourished with two different diets, one being an infant formula and porridge and the other one a commercial milk-based cereal diet, the mean daily phytate intake was 26 $\pm$ 18 and 124 $\pm$ 82 mg, respectively. With increasing age (9–11 m), enhanced daily phytate intake of 62 $\pm$ 47 and 189 $\pm$ 87 mg, for both diets, respectively, was reported [127].

For Finland the average *per capita* intake of phytic acid from cereal products, the main dietary phytate source, was estimated at 370 mg [128].

3.2 North and Central America

Preschool children in the US showed a mean daily phytate intake of 166 $\pm$ 167 mg for up to 1 year of age, 390 $\pm$ 231 mg for 1–3 years, and 501 $\pm$ 271 mg for 4–5 years [129] while Canadian girls and boys of the same age (4–5 years) showed lower phytate intakes of 250 and 320 mg, respectively [130].

In African-American adults in the US, the median of the daily phytate intake was 538 mg, with strong differences for females (512 mg) and males (608 mg) [131].

In adult Asian immigrants to Canada, consuming predominantly lacto-ovo vegetarian diets, high mean daily phytate intake of 1487 $\pm$ 791 mg were also found [132]. A study in American students and university faculty staff members (19–35 years) showed high mean daily phytate intake of 1293 $\pm$ 666 mg, ranging from 198 to 3098 mg [133] and in a study with young American women (18–24 years) a low mean daily phytate intake of 395 $\pm$ 334 mg was estimated [134]. In self selected diets of omnivorous females and males the phytate intake was found to be 631 mg (590–734 mg) and 746 mg (714–762 mg) and in female and male vegetarians ~1250 $\pm$ 450 and ~1550 $\pm$ 550 mg, respectively [135]. The maximum phytate intake ever reported in humans was 5.770 mg for lacto-ovo vegetarians (Trappist monks) in 1978 [136] which, however, was much lower with 972 mg when the study was repeated 10 years later in the same group of Trappist monks [137]. For average Americans with a body weight of 75 kg a study of 1976 calculated the daily phytate intake at 750 mg [136].

Infants in Mexico (18–30 m) showed the daily phytate intake of 1666 $\pm$ 650 mg [138] and pupils (7–9 years) of 3380 $\pm$ 1070 mg [139]. Studies from Guatemala also indicated a high daily phytate consumption of 2254 mg for females (15–37 years) [140].

3.3 Asia

In India the mean daily phytate intake ranges from 670 to 2500 mg [141, 142]. For children (4–9 years) it is 720–1160 mg, for adolescents (10–19 years) 1350–1780 mg, for adults (20–45 years) 1560–2500 mg and for elderly people (>45 years) 1290–2080 mg [142]. Another study,

however, reported comparable lower daily phytate intake of ~ 840 mg for females (16–20 years) [143].

In Thailand the mean daily phytate intake for women was 1139 ± 481 mg and for men 1104 ± 965 mg, reported from Ubon Ratchathani in the northern part of Thailand. For the city of Bangkok, however, it was 997 ± 435 mg (♀) and 1304 ± 956 mg (♂) [144].

In the Peoples Republic of China the median daily phytate intake was 1186 mg, ranging from 823 to 1603 mg, and strong differences in the median daily phytate intake for urban population (781 mg, ranging from 443 to 1205 mg) and rural population (1342 mg, ranging from 970 to 1757 mg) were described [145]. For students and university members in the Republic China (Taiwan), the mean phytate intake *per day* for females was 690 ± 189 mg and for males 915 ± 330 mg [146], fitting well with the data of the phytate intake of urban population in the Peoples Republic of China [145].

In the Republic of Korea the daily phytate intake for males (21–70 years) was 839 ± 400 mg and for females 752 ± 407 mg [114]. Differences for young (mean 23 years) and aged females (mean 70 years) were 322 ± 220 and 496 ± 52 mg, respectively [147].

3.4 Africa

Infants in Egypt (18–30 m) showed daily phytate intake of 796 ± 248 mg [138] and pupils (7–9 years) of 1270 ± 280 mg [139]. For children from Kenya of the same age a daily phytate intake of 1066 ± 324 mg (18–30 m) [138] and of 2390 ± 480 mg (7–9 years) was described [139]. The mean daily phytate intake for girls and boys (4–6 years) from Malawi ranged from 1621 to 1729 mg and from 1857 to 2161 mg, respectively [148]. Infants in Gambia (1–17 m) showed a daily phytate intake of 10–560 mg [149] and children in Ghana a phytic acid intake of 578 ± 161 mg [150]. A mean daily intake of phytate for pregnant women in Ethiopia was 1033 ± 843 mg [99] and for Nigerian people an average phytate intake of 2200 mg *per day* and person was estimated [77].

3.5 Conclusions

The data collected give an overview of the mean daily phytate intake in humans of countries from all over the world, strongly varying in sex and ages.

For infants (<1 years) in Sweden the mean daily phytate intake reaches 26–189 mg [127]. Whether this infant formula is representative and comparable to other industrialised countries remains to be clarified but it conveys an idea of the level of phytate intake in infants in Europe. Children in the US and in Canada (1–5 years) show a mean daily phytate intake of 166–501 mg [129, 130] which is much lower compared to Egypt, Kenya and Mexico, where infants ($\sim 1\frac{1}{2}$ – $2\frac{1}{2}$ years) show a mean daily phytate intake of 796,

1066 and 1666 mg and pupils (7–9 years) show one of 1270, 2390 and 3380 mg, respectively [138, 139]. For children from Malawi (4–6 years) a very high mean daily dietary phytate intake of 1622–1729 mg (♀) and 1857–2161 mg (♂) was also reported [148].

For adults, studies from Sweden, Finland and Italy report a low mean daily phytate intake of 180–370 mg, if Western style diets are consumed [123–126, 128], and for vegetarians a much higher one of 1146 mg (Sweden) [126]. In the UK two levels of phytate intake are also present, one in the range of 504–848 mg [120, 121] and another one of 1436 mg [122]. Similar data exist for the US, indicating for women a low range of the mean daily phytate intake of 395 mg (18–24 years) [134] and a high one of 1250 mg (19–35 years) [135]. In 1971 the mean phytate intake of average Americans was estimated at 750 mg [136]. In Nigeria and Guatemala much higher daily phytate intakes of 2200 and 2254 mg, respectively, were described [77, 140] and the maximum daily phytate intake ever reported in humans was 5770 mg for lacto-ovo vegetarians (Trappist monks) [136].

The data suggest that for adults different levels of the daily dietary phytate intake exist:

(i) at a low level of ~ 200 to ~ 350 mg, probably due to Western style diets low in phytate rich plant foods.

(ii) At a higher level of ~ 500 – ~ 800 mg, probably due to primarily Western style diets with enhanced portions of cereals, whole grain products and other phytate rich foods.

(iii) At a high level of >1000 mg, probably due to diets rich in plant and phytate containing foods such as vegetarian diets.

In developing countries, due to the high content of cereals and legumes in the traditional diet, obviously quite high levels of phytate intake of up to 2000 mg and more can be assumed, although only a few studies are available [77, 139, 140, 150]. Strong differences between the mean phytate intake in rural population (1342 mg) and urban population (781 mg) are evident in the Republic of China, probably reflecting the changing of dietary habits from traditional to more Western type diets in cities and metropolises [145].

Evaluating the results of the daily phytate intake in humans, strong differences are apparent between developing and industrialised countries, between urban and rural areas, between females and males, between young and old and between omnivores and vegetarians (Table 6). These differences certainly derive from differences in the phytate content of cultivated plant foods, different contents in plant foods in the daily diet, different amounts of foods consumed and different processing and preparation of vegetables, legumes, pulses, cereals and whole grain products, *etc.* Moreover, different methods for the determination of phytic acid may also play a role in the high variation of reported phytate intake. To obtain a reliable overview of the phytic acid intake in different countries, further studies with detailed documentation of the social and nutritional back-

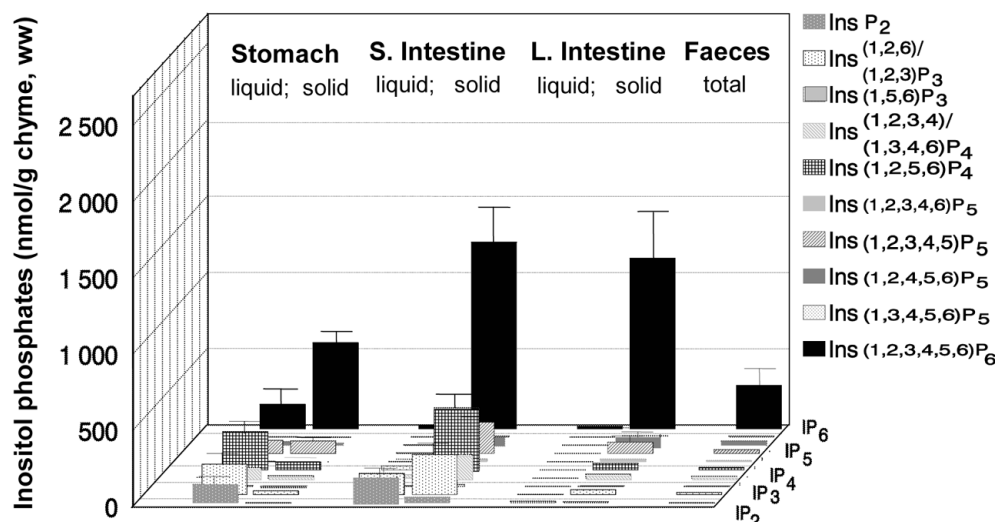


Figure 2. Hydrolysis of phytate from a diet rich in intrinsic feed phytases during the passage throughout the stomach, small intestine and large intestine as well as in the faeces of pigs 5 h after feeding [159]. Inositol phosphates are listed from the front to the back in the following row:

Ins_{P2}, Ins(1,2,3)P₃/Ins(1,2,6)P₃, Ins(1,5,6)P₃, Ins(1,2,3,4)P₄/Ins(1,3,4,6)P₄, Ins(1,2,5,6)P₄, Ins(1,2,3,4,6)P₅, Ins(1,2,3,4,5)P₅, Ins(1,2,4,5,6)P₅, Ins(1,3,4,5,6)P₅ and Ins_{P6}.

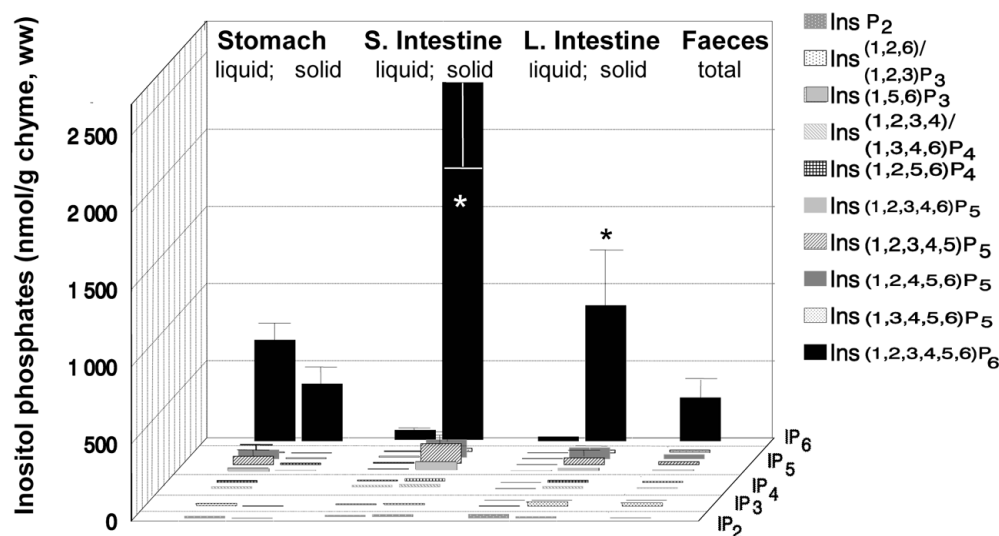


Figure 3. Hydrolysis of phytate from an extruded diet with inactivated phytases, during the gastro-intestinal passage throughout the stomach, small intestine and large intestine as well as in the faeces of pigs 5 h after feeding [159]. *Ins_{P6} concentrations were different ($p < 0.05$). Inositol phosphates are listed from the front to the back in the following row:

Ins_{P2}, Ins(1,2,3)P₃/Ins(1,2,6)P₃, Ins(1,5,6)P₃, Ins(1,2,3,4)P₄/Ins(1,3,4,6)P₄, Ins(1,2,5,6)P₄, Ins(1,2,3,4,6)P₅, Ins(1,2,3,4,5)P₅, Ins(1,2,4,5,6)P₅, Ins(1,3,4,5,6)P₅ and Ins_{P6}.

ground and specific, evaluated methods for the analysis of phytic acid and lower phosphorylated inositol phosphates in food are required (see discussion in Section 2).

4 Mechanism of phytate hydrolysis in the gut

Studies on the phytate degradation in the human gut are scarce. Early experiments showed that some phytate is

degraded in the human gut. In 1935 McCane and Widowson already observed 36–63% of phytate in the stool of adults after applying diets, rich in alimentary phytate, and calculated the mean phytate degradation of $54 \pm 8\%$ [151]. This was confirmed by a later experiment, showing phytate phosphorus absorption of 50% [24]. Similar results were described by Hoff-Jørgensen *et al.* [152] for infants (1–11 m) and children (10 years), indicating a gastro-intestinal phytate hydrolysis of ~30–47%. However, in 1945 Cruick-

shank *et al.* [153] reported an almost complete phytate phosphorus digestibility in adults, implying an almost total phytate hydrolysis, and in 1947 Walker *et al.* [154] pointed out that the dietary calcium level effects phytate degradation in the gut (43–90%). Although it was not clear at that time to which extent phytate might be hydrolysed exactly in the human gut – which was also due to the lack of an adequate and specific method for determining phytate – these early studies already showed that phytate is degraded strongly during the gastro-intestinal digestion in humans.

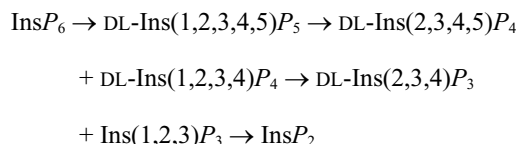
4.1 Stomach

In ileostomy patients Sandberg *et al.* in 1986 [155–158] found phytate degradation of 56–66% for the total passage through the stomach and small intestine when the diet contained active food phytases. If the dietary phytases were inactivated either by extrusion or heat treatment of the diet, the total phytate degradation in the upper part of the gut decreased to 0–28% [155–158].

As studies in ileostomy patients allow only limited information on the phytate degradation in the gut and as for ethical reasons detailed studies in humans on the phytate hydrolysis in the different parts of the digestive tract are nonfeasible, Schlemmer *et al.* [159] studied the phytate hydrolysis in detail in the stomach, the small intestine, the large intestine, the faeces and the enzymes involved in pigs which is an excellent model for simulating digestion processes of humans [160]. Figures 2 and 3 show the distribution of inositol phosphates in the chyme of the different parts of the gut and of the faeces of pigs fed a diet rich in intrinsic feed phytases (control diet) (Fig. 2) and the same but extruded diet with inactivated feed phytases (extruded diet) (Fig. 3). The two diets were applied to differentiate between phytases from dietary and from endogenous origin, possibly involved in the gastro-intestinal hydrolysis of phytate. Moreover, the inositol phosphates in the liquid and solid phase of the chyme were separated to differentiate between soluble, degradable and possibly physiologically active inositol phosphates and insoluble, nondegradable and probably physiologically inactive ones. Figure 3 shows that no phytate degradation occurs in the stomach if no active feed phytases are present. This confirms earlier findings in ileostomy patients [157, 158]. In the presence of active food phytases, however, strong hydrolysis of soluble phytate occurs and lower phosphorylated inositol phosphates are formed by stepwise inositol phosphate degradation (Fig. 2). From the inositol phosphate isomers determined in the gastric chyme and the respective cleaving specificity of the enzymes, it can be concluded that intrinsic feed phytases of plant origin (6-phytases) degrade phytate in the stomach, as the main inositol pentaphosphate formed is DL-Ins(1,2,3,4,5) P_5 which is specific for 6-phytases (EC 3.1.3.26) (see Section 7.2). The same findings are reported from ileostomy patients, also showing DL-Ins(1,2,3,4,5) P_5

as the predominant inositol pentaphosphate in the gastro-intestinal content [161]. As no Ins(1,2,4,5,6) P_5 , specific for 3-phytases (EC 3.1.3.8), is formed during gastric digestion, it can be excluded that endogenous phytases, *e.g.* described as 3-phytases in human and rat small intestinal mucosa cells [162–165], are involved in the phytate degradation in the pigs' stomach.

In order to confirm these findings an additional *ex vivo* experiment was carried out and Na-phytate was hydrolysed by enzymes purified from the pigs feed and from the stomach chyme of pigs fed the same diet. The kinetic of the phytate hydrolysis for both enzymes was the same and the inositol phosphate isomers formed were identical [159]. This shows clearly that the enzymes, active in hydrolysing phytate in the gastric chyme, are phytases of feed origin and no evidence is given that endogenous phytases (3-phytases) take part in the phytate hydrolysis to a physiologically relevant degree. Based on the quantitative evaluation of the inositol phosphate isomers, a major pathway of phytate hydrolysis by 6-phytases of plant origin in the stomach was proposed [159]:



(see Fig. 5).

This is similar to the pathway reported for cereal phytases from wheat, barley, rye and oat [161].

Due to the fact that only ~2/3 of total phytate is soluble and present in the liquid phase of the gastric chyme of the pigs (Fig. 2), this means that ~1/3 of the phytate is still bound to the feed matrix and should be hardly available for enzymatic hydrolysis. Thus, incomplete phytate liberation from feed matrix during digestion in the stomach consequently results in incomplete gastric phytate hydrolysis. This explains why neither in ileostomy patients [155–158] nor in pig studies [159, 166, 167] complete phytate degradation during passage throughout the stomach and small intestine has ever been detected. Moreover, it confirms earlier assumptions of Kemme *et al.* [168] that the gastro-intestinal phytate hydrolysis might be governed by the velocity of phytate liberation from feed.

In order to study the maximum phytate hydrolysis in the upper part of the gut, phytase activity in the diets of pigs was enhanced by adding microbial phytases to the pig's diets (<1800 FTU *Aspergillus niger* phytase/kg feed) and the phytate phosphorous availability was determined. With increasing phytase activity phytate phosphorous availability in pigs increased to a plateau at 60–66% [169, 170]. This implies that there is not only a maximum of phytate phosphorous availability but also a maximum of phytate hydrolysis of ~60–66% during digestion in the stomach and small intestine which indicates that total phytate degradation in

Table 7. Solubility of inositol phosphates in the intestinal chyme of pigs^{a)}

	Small intestine pH 6.6 ± 0.2 [%]	Large intestine pH 6.2 ± 0.2 [%]
InsP ₂	75	24
InsP ₃	31	6
InsP ₄	8	0
InsP ₅	7	3
InsP ₆	2	2

a) Chyme was removed from the small and large intestine of pigs (jejunum and colon, respectively) fed a normal pigs diet with active feed phytases (control diet) [159]. Chyme was centrifuged and the inositol phosphates of the liquid and solid phase separated [173]. Inositol phosphates were determined by HPLC on Mono-Q (HR 5/5) and quantified by Fe³⁺-detection [159]. Inositol phosphate isomers with the same numbers of phosphate groups were summed. Results are mean values on the basis of double determinations. Solubility of inositol phosphates was defined as the ratio of soluble to total inositol phosphates (%) determined in the chyme. The pH given is the mean pH of the respective homogenised chyme sample ($n = 3$).

the upper part of the gut of pigs is unavailable even at very high dietary phytase activity. It confirms the assumption mentioned above that most probably incomplete liberation of phytate from the feed matrix is responsible for the incomplete phytate hydrolysis in the upper part of the gut.

Gastric degradation of dietary phytate by splitting phosphate groups from phytate is not only relevant for monogastric animals, such as pigs and broilers, to enhance their phosphorous supply, but is also significant for humans. By hydrolysing phytate to lower phosphorylated inositol phosphates (InsP₅, InsP₄) inositol phosphates with higher solubility are formed [47], improving on the one hand the susceptibility for further enzymatic hydrolysis and accelerating on the other hand the stepwise degradation to low phosphorylated inositol phosphates, mainly to InsP₃ but also to InsP₂. As lower inositol phosphates in comparison to phytate are assumed to show lower affinity to minerals [171, 172] and higher solubility of the complexes with metal ions [47], phytate hydrolysis in the stomach by this reduces the inhibition of the intestinal absorption of essential trace elements and minerals in humans and animals.

4.2 Small intestine

In the small intestine most inositol phosphates are precipitated and present in the solid phase of the chyme (Fig. 2). Only InsP₂ shows higher contents in the liquid than in the solid phase and the solubility of InsP₃ in the chyme is higher than that of the other inositol phosphates such as InsP₄, InsP₅ and InsP₆. Due to interactions with multivalent cations such as iron, zinc, calcium, *etc.* and increasing pH during the passage from the stomach (pylorus, pH ~ 2) to the

small intestine (pH ~ 5–7), inositol phosphates with increasing phosphorylation degrees precipitate, especially phytate (Fig. 2). By precipitation, inositol phosphates reduce the availability of these metal ions in the chyme and thus interfering in the intestinal absorption of these essential minerals and trace elements.

By means of an additional *ex vivo* experiment, the distribution of soluble and insoluble inositol phosphates in the intestinal chyme was studied due to the physiological significance for the understanding of the mechanism of inositol phosphates interfering in the intestinal absorption of metal ions. The results show that the solubility of the inositol phosphates in the chyme, defined as the ratio of soluble to total inositol phosphates (%), varies with the degree of phosphorylation. The higher phosphorylated the inositol phosphates are, the lower is their solubility in the intestinal chyme and vice versa (Table 7) [173]. Earlier discussions on the role of phytate in the small intestine, assuming that the more phytate is degraded the more inositol phosphates with lower metal affinity are formed [171], seems to be insufficient to fully explain the inhibitory effect of phytic acid on mineral intestinal absorption. It seems more probable, that the inhibition of the metal intestinal absorption by phytate is the direct consequence of reduced solubility of minerals and trace elements, interacting with phytates in the small intestinal chyme.

The results presented in Figs. 2 and 3 on the soluble inositol phosphates in the gastro-intestinal chyme show that by phytate degradation in the stomach the concentration of soluble phytate in the gastric chyme decreases, reducing the interactions with metal cations in the gastric chyme. In parallel the concentration of lower phosphorylated inositol phosphates in the gastric chyme increases (pH 4.6) and even at pH 6.2 in the small intestine the concentration of soluble inositol-di- and inositol-triphosphates remains considerably high. High concentration of soluble inositol-di- and inositol-triphosphates in the small intestinal chyme seems to be relevant to avoid precipitation of metal-inositol phosphates and keeping trace elements and minerals in solution in the small intestine, the predominant location of the metal absorption in the gut. As iron uptake by Caco-2 cells is not shown to be impaired by lower phosphorylated inositol phosphates such as InsP₃ and InsP₄ at a molar ratio of 2:1 (inositol phosphates/iron) [174], it can be assumed that the higher the concentration of the soluble InsP₂ and InsP₃ is in the small intestinal chyme, the less affected is the intestinal absorption of minerals and trace elements. This might best explain why the degradation of phytate improves the intestinal absorption of minerals and trace elements in humans and animals.

During the passage from the stomach to the small intestine, constant digestion and absorption of absorbable nutrients lead to increased concentration of non- and low-absorbable compounds such as inositol phosphates in the chyme. Consequently, the concentration of inositol phos-

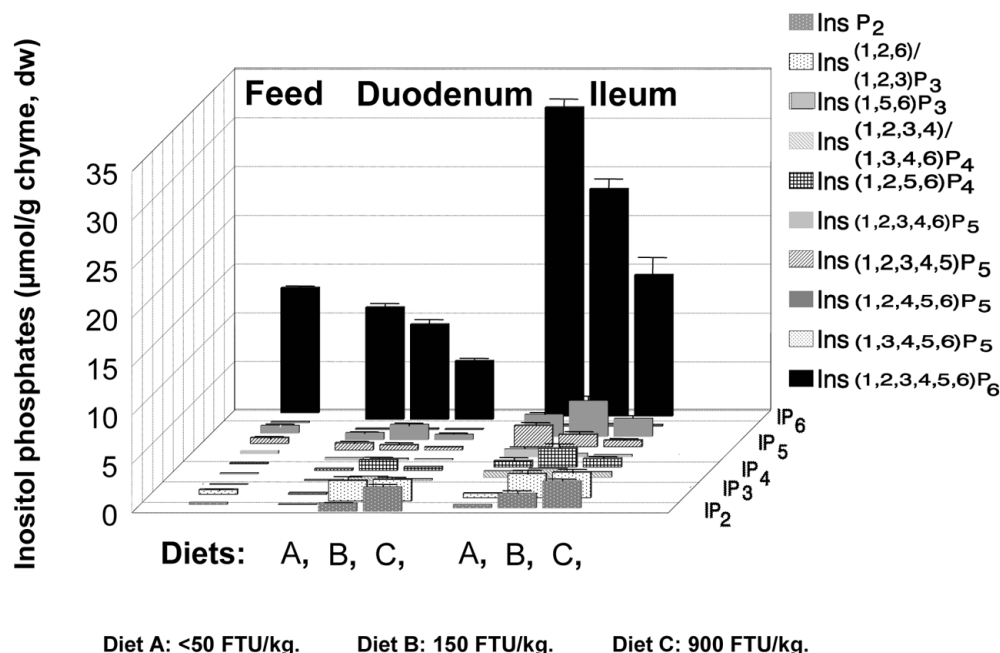


Figure 4. Hydrolysis of phytate from extruded feed with addition of defined *Aspergillus niger* phytases activities (diet A: <50 FTU/kg, diet B: 150 FTU/kg and diet C: 900 FTU/kg) during the passage through the stomach, duodenum and the ileum of pigs [177]. Inositol phosphates are listed from the front to the back in the following row: InsP₂, Ins(1,2,3)P₃/Ins(1,2,6)P₃, Ins(1,5,6)P₃, Ins(1,2,3,4)P₄/Ins(1,3,4,6)P₄, Ins(1,2,5,6)P₄, Ins(1,2,3,4,6)P₅, Ins(1,2,3,4,5)P₅, Ins(1,2,4,5,6)P₅, Ins(1,3,4,5,6)P₅ and InsP₆.

phates increases during the passage from the stomach to the small intestine and is higher in the small intestinal than in the stomach chyme (Figs. 2 and 3). In pigs, fed a diet free of active phytases, the phytate concentration in the small intestine is higher than in pigs fed the diet with active feed phytases, due to the lack of any relevant gastric phytate degradation (Figs. 2 and 3). Moreover, the absence of any phytate hydrolysis products in the intestinal chyme clearly indicates that no endogenous phytases are involved in the intestinal phytate hydrolysis (Fig. 3). This also confirms that only phytases of food or feed origin are relevant for the phytate degradation in the small intestine of humans and pigs.

In pigs fed a diet with active phytases no change of the inositol phosphates pattern (the relation of the different inositol phosphate isomers to each other) shows that obviously no further degradation of phytate and the other inositol phosphates occurs (Fig. 2). This is certainly the consequence of strong inactivation of the intrinsic plant feed phytases by the peptic digestion in the pylorus [175] and by an unfavourable pH in the duodenal chyme (pH ~ 6.5–7) at which plant phytases show only very low activity [176]. If, however, the pigs' diet is enriched with microbial phytases such as *Aspergillus niger* phytases, to improve phytate hydrolysis, the degradation of inositol phosphates continues in the small intestine (Fig. 4) [177]. Similar results were also described by Rapp et al. [178]. As DL-Ins(1,2,4,5,6)P₅ is the major inositol pentaphosphate formed in the duodenum and ileum and typical for *Aspergillus Niger* phytase (3-phytase, EC 3.1.3.8), this

clearly shows that when feeding a diet supplemented with *Aspergillus Niger* phytases, these microbial phytases are obviously responsible for continuing phytate hydrolysis in the small intestine (Fig. 4). These results show, moreover, that microbial phytases are more stable than intrinsic plant feed phytases towards peptic digestion and inactivation in the pylorus and duodenum in pigs.

4.3 Large intestine

In the large intestinal chyme the inositol phosphates are almost completely present in the solid phase with high concentrations of phytate and very low ones of the lower phosphorylated inositol phosphates (InsP₂–InsP₅). This is true for both diets (Figs. 2 and 3). The most prominent phytate degradation products are DL-Ins(1,2,3,4,5)P₅ and DL-Ins(1,2,4,5,6)P₅, indicating that 6- and 3-phytases are involved in the phytate hydrolysis in the large intestine. As feed phytases of plant origin (6-phytases) are no longer active in the intestine (see Section 4.2), the DL-Ins(1,2,3,4,5)P₅ detected must have been formed by *Escherichia coli* phytases, which are also 6-phytases [179] and prominent in the micro flora, while the DL-Ins(1,2,4,5,6)P₅ is generated by 3-phytases of other microbial origin.

Evaluating the inositol phosphate isomers formed during the phytate hydrolysis in the large intestine, two major pathways of the phytate hydrolysis by bacterial 6-phytases and microbial 3-phytases were established [159]:

Table 8. Activity of phytases and alkaline phosphatases in feed and gastro-intestinal chyme of pigs fed the control and the extruded diet

	Feed	Stomach [mU/mg protein]	Small intestine	Colon
<i>Control diet</i>				
Phytases	43.1 ± 2.1 ^{a)}	3.7 ± 0.6 ^{b)}	0.9 ± 0.3	2.2 ± 0.1
Alkaline Phosphatases	–	–	146 ± 37 ^{d)}	29.9 ± 6.9 ^{e)}
<i>Extruded diet</i>				
Phytases	0.2 ± 0.1 ^{c)}	0.3 ± 0.2	0.7 ± 0.2	1.8 ± 0.1
Alkaline Phosphatases	–	–	153 ± 43 ^{f)}	39.3 ± 5.8 ^{g)}

means ± SD, *n* = 3a vs. b, d vs. e and f vs. g are significantly different *p* < 0.125.a vs. c is significantly different *p* < 0.05.

Adapted from [159].

(i) $\text{InsP}_6 \rightarrow \text{DL-Ins}(1,2,4,5,6)\text{P}_5$ $\rightarrow \text{DL-Ins}(1,2,5,6)\text{P}_4 \rightarrow \text{DL-Ins}(1,2,6)\text{P}_3 \rightarrow \text{InsP}_2$.(ii) $\text{InsP}_6 \rightarrow \text{DL-Ins}(1,2,3,4,5)\text{P}_5$ $+ \text{DL-Ins}(1,2,4,5,6)\text{P}_5 \rightarrow \text{DL-Ins}(1,2,3,4)\text{P}_4$ $+ \text{DL-Ins}(1,2,5,6)\text{P}_4 \rightarrow \text{DL-Ins}(1,2,3)\text{P}_3/$ $\text{DL-Ins}(1,2,6)\text{P}_3 \rightarrow \text{InsP}_2$

(see Fig. 5).

As in pigs fed the diet with active phytases (control diet) the concentration of InsP_6 in the large intestinal chyme was as high as in the small intestine, it demonstrated that no phytate degradation could have been taken place during the passage from the small to the large intestine (Fig. 2). This is in contrast to the phytase inactivated diet (extruded diet), showing only half of the phytate concentration in the large intestine in comparison to the small intestine, indicating strong phytate degradation in the large intestine for the phytase free diet (Fig. 3). Applying the concentration of free inorganic phosphate and inositol monophosphates as a marker for intensive phytate hydrolysis, the high concentration of free inorganic phosphate and inositol monophosphates confirmed that phytate was degraded more intensively in pigs fed the diet free of phytase than in the diet with active phytases [159]. Even if the reason for this stronger InsP_6 hydrolysis remains to be clarified, the results allow the assumption, that the higher the concentration of InsP_6 is in the small intestinal chyme, the stronger is the InsP_6 hydrolysis in the large intestine.

Experiments in rats also focussing on phytate hydrolysis in the large intestine [180] reported 56% of phytate hydrolysed in conventional rats while in germfree rats almost no phytate hydrolysis was detected. This finding also emphasises the significance of microbial phytases for phytate degradation during the digestion in the gut and may explain the strong phytate hydrolysis in the large intestine.

4.4 Faeces

In the faeces very low phytate concentrations are present, which demonstrate continuing strong phytate hydrolysis (Figs. 2 and 3) [159]. This is true for both diets. As the main phytate hydrolysis products are $\text{DL-Ins}(1,2,3,4,5)\text{P}_5$ and $\text{DL-Ins}(1,2,4,5,6)\text{P}_5$, it indicates that the same bacterial 6-phytases and microbial 3-phytases as in the large intestine are responsible for the continuation of the phytate hydrolysis in faeces.

Phytate degradation in the gut depends on the calcium level in the diet. This was already shown in humans by Walker *et al.* in 1947 [154]. Sandberg *et al.* [181] found in pigs that the total phytate degradation throughout the gut decreased significantly 97, 77 and 45% (*p* < 0.001) when the dietary calcium intake increased by 4.5, 9.9 and 15 g/day, respectively. As the phytate degradation in the stomach and small intestine was almost unaffected, different supplementation of calcium carbonate predominantly affected the hydrolysis of phytate in the large intestine. Similar results were reported for rats by Pileggi *et al.* [182] and Wise and Gilbert [180]. Most probably, high dietary calcium content affects the phytate solubility in the gastro-intestinal chyme and thereby reduces the accessibility of phytate for enzymatic hydrolysis. A similar effect on phytate degradation was also assumed for magnesium [46]. The phytate hydrolysis in the gut and the role of calcium in the intestinal phytate degradation were previously discussed by Wise [183] and by Sandberg [161].

4.5 Enzymes

To assess the contribution of enzymes involved in the gastro-intestinal degradation of phytate, enzyme activities of phytases and alkaline phosphatases were determined in the feed and the chyme during the passage throughout the gut.

In the diet with active phytases (control diet), the phytase activity was high (43.1 mU/mg protein), decreased to ~1/10 in the stomach chyme (3.7 ± 0.6 mU/mg protein) and

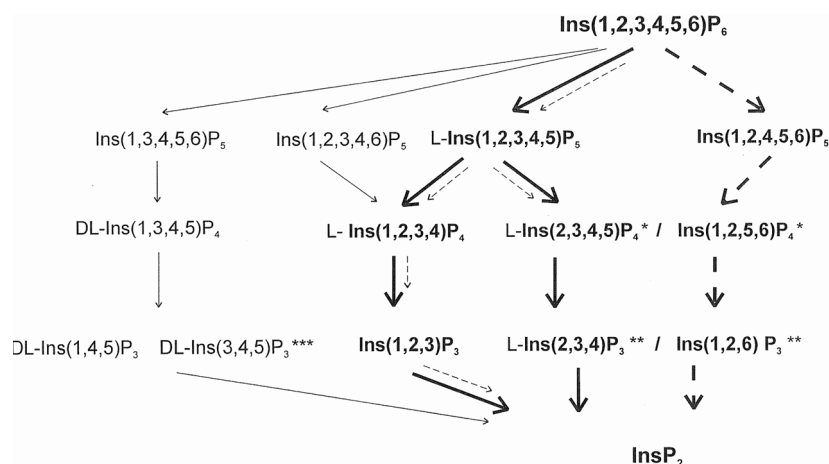


Figure 5. Phytate hydrolysis during the gastro-intestinal digestion in pigs – pathways of the inositol phosphates. Plant phytases —; microbial phytases ---; major pathways →; minor pathways →: *, **, and ***: enantiomers of inositol phosphates are not distinguishable by HPLC, *: Ins(1,2,5,6)P₄ is equivalent to L-Ins(2,3,4,5)P₄, **: Ins(1,2,6)P₃ equivalent to L-Ins(2,3,4)P₃, and DL-Ins(3,4,5)P₃ equivalent to DL-Ins(1,5,6)P₃ (with permission from Taylor & Francis, London, UK, <http://www.informaworld.com> [159]).

was only $\sim 1/40$ in the small intestine (0.9 ± 0.3 mU/mg protein) (Table 8). This means that strong inactivation of feed phytases occurs during digestion in the stomach and the small intestine. Lower phytase activity in the small intestine than in the stomach was also observed by Jongbloed *et al.* [167] and Rapp *et al.* [178] and reduced phytase activity as a consequence of proteolytic digestion in the proximal small intestine was already described by Scheuermann *et al.* [176]. In rats Miyazawa and Yoshida [184] also observed lower phytase activity in the colorectal than in the upper small intestinal chyme.

In the diet free of phytases (extruded diet) the phytase activity was very low (0.2 ± 0.1 mU/mg protein) and did not change significantly in the stomach chyme (0.3 ± 0.2 mU/mg protein). A slight increase of the phytase activity in the small intestine was probably due to phytases of microbial origin and of detached mucosal cells (0.7 ± 0.2 mU/mg protein) and was the same in both diets (Table 8). This phytase activity in the small intestine, however, is too low to contribute to any physiologically relevant degradation of inositol phosphates in the small intestine (Figs. 2 and 3). If microbial phytases are added to the diet, phytase activity is higher and phytate hydrolysis also occurs in the small intestine (Fig. 4, diets B and C) [167, 177]. Low phytate degradation in the small intestine is also in accordance with Poin-tillart *et al.* [185] Hu *et al.* [186], Iqbal *et al.* [165] and Davies and Flett [162] who reported low phytase activity in mucosa cell homogenates of pigs, humans and rats, respectively. The lack of detectable inositol phosphates degradation in the small intestine, however, is not only due to the low activity of endogenous phytases but also to the low solubility of inositol phosphates at intestinal pH 6.6 ± 0.2 (Table 7) [159] which may restrict their susceptibility to enzymatic hydrolysis.

Activity of alkaline phosphatases in the diet and also in the stomach is nondetectable, but high in the small and large intestine and is independent of the diet fed (Table 8). It indicates that alkaline phosphatases are of endogenous and not of dietary origin. Compared to phytases, the activity of

alkaline phosphatases in the small intestine is about two orders of magnitude higher (~ 150 mU/mg protein) (Table 8). This is in accordance with Iqbal *et al.* [165] who found in small intestinal mucosal homogenates of humans 1000 times higher activity of alkaline phosphatase than that of phytases. In the colon, the alkaline phosphatase activity declines (~ 35 mU/mg protein) (Table 8), probably due to subsequent inactivation in the large intestine and the lower secretion of alkaline phosphatases in the colon rather than in the small intestine. Similar results were also reported for rats [184].

As the activity of alkaline phosphatases is much higher than that of phytases in the intestine, the question was raised whether or not the high alkaline phosphatase activity explains the strong hydrolysis of the inositol phosphates in the large intestine. Thus, in an *ex vivo* experiment [173] aliquots of small intestinal chyme of the pigs fed a diet with active phytases [159] were either incubated at pH 6.2 or 8.7 to differentiate between phytases and alkaline phosphatases under the intestinal conditions [173]. The pH 6.2 was selected as it is almost optimal for phytases but nonoptimal for alkaline phosphatases, while pH 8.7 is nonoptimal for phytases but almost optimal for alkaline phosphatases [187]. The results show that hydrolysis of phytate and the lower phosphorylated inositol phosphates was high at pH 6.2, while at pH 8.7 no significant changes of the inositol phosphate concentrations ($p < 0.05$) occurred but the concentration of InsP₆ only decreased by trend [173]. These findings indicate that only microbial phytases are involved in the hydrolysis of inositol phosphates while alkaline phosphatases obviously do not contribute to a relevant degree to the degradation of inositol phosphates in the large intestinal chyme. This is true even if the phytase activity is low (Table 8), but the residual time of the chyme in the large intestine is obviously sufficient long for the strong phytate degradation observed in the large intestine and faeces. Bitar and Reinhold [163] studied the pH dependence of phytases and alkaline phosphatases from human mucosal homogenates

and found pH optima for phytases and alkaline phosphatases of pH ~ 7.5 and ~ 9.5 , respectively. Since at pH 8.7, selected for the incubation of small intestinal chyme, the degradation of inositol phosphates in the pigs' chyme was very low, it indicates that phytases and alkaline phosphatases from human mucosa cells also might not be involved in relevant degree in the intraluminal hydrolysis of inositol phosphates in the small intestinal chyme in pigs [173].

4.6 Pathway of the stepwise phytate degradation in the gut

Based on the analysis of the inositol phosphates isomers in the gastro-intestinal chyme, Schlemmer *et al.* [159] elucidated the pathway of the stepwise phytate degradation in the different parts of the gut (Fig. 5). The inositol phosphates analysis does not only show that phytate is hydrolysed by 6- and 3-phytases from feed and microbial origin, respectively, but that further inositol phosphates, such as DL-Ins(1,4,5) P_3 and DL-Ins(1,3,4,5) P_4 , which are not formed within the known pathway of the 3- and 6-phytases, are also present in the gastric chyme. As Ins(1,4,5) P_3 and Ins(1,3,4,5) P_4 possess second messenger activity and are highly important for the intracellular metabolism, it has been assumed earlier that most probably these inositol phosphates will not exist extracellular to prevent disordered cell metabolism after their absorption. Thus, it is highly remarkable that their existence could be proven extracellular in the gastric chyme of pigs [159]. Even though their concentration is low, their physiological role in the gut requires to be clarified.

4.7 Phytate balance during the passage throughout the gut

Phytate degradation for the passage through the digestive tract was nearly complete for both diets with high (control diet: $97.4 \pm 2.3\%$) and low intrinsic feed phytases (extruded diet: $97.7 \pm 2.2\%$) [159]. This shows that during digestion in the gut phytate will be almost completely degraded, independent of the intrinsic feed phytase activity. If active phytases are present in the diet, strong phytate hydrolysis then occurs in the stomach. If phytases are inactivated by processing, phytate degradation then mainly occurs in the large intestine by microbial phytases.

This is comparable with humans, as most food containing phytate is heat treated in one way or the other, thus inactivating phytases. If diets contain high amounts of wheat or rye bran, strong phytate hydrolysis can then be assumed in the stomach and the rest of phytate will be degraded almost completely in the large intestine.

Similar phytate degradation in the whole gut (~ 92 – 100%) has also been reported by other groups [167, 181, 188]. It should be pointed out, however, that phytate degradation in the lower part of the intestine depends on the diet-

ary calcium level. The redistribution of inositol phosphates from solid to liquid phase is probably affected by increasing calcium content in the feed and as a result enzymatic hydrolysis is subsequently reduced.

Brune *et al.* [189] studied the effect of high phytate intake over a long period of time on the intestinal absorption of nonheme iron. In vegetarians with a high phytate intake (~ 1100 mg phytic acid/day) and in control subjects with low phytate intake (~ 370 mg phytic acid/day) over several years, they found an almost identical inhibition of iron absorption from wheat rolls with iron labelled bran (^{55}Fe , ^{59}Fe). This indicates that in the course of high phytate consumption, no adaptation to reduced iron absorption occurs over time and allows the assumption that high phytate intake does not induce endogenous or microbial phytase activity in the intestine.

4.8 Conclusions

Studies in humans showed that 37–66% of dietary phytate is degraded during digestion in the stomach and small intestine when the diet is rich in plant food phytases [151, 156, 158]. As most plant food such as whole grain products, cereals and legumes – the main sources of dietary phytate intake – are processed or heat treated either during food production or preparation in one way or the other, phytases in prepared food should probably be inactivated to a large extent. This means that in humans, consuming Western style diets with low phytase activity, phytate degradation in the stomach and the small intestine by food phytases is very limited. From studies in pigs, shedding light on phytate degradation in different parts of the gut, can be concluded that in humans the main phytate hydrolysis occurs in the large intestine by means of microbial phytases [159]. Studying the stepwise phytate degradation in the gut, clarity could be gained on the pathway of the gastro-intestinal inositol phosphate degradation in different parts of the gut as well as the enzymes involved [159]. Even though the activity of alkaline phosphatases is much higher than that of phytases in the large intestine, the *ex vivo* studies let assume that alkaline phosphatases do not contribute to the inositol phosphate degradation to a relevant degree and microbial phytases are responsible for the strong phytate hydrolysis in the large intestine [173].

During the stepwise degradation of dietary phytate in the gut, large numbers of inositol phosphates are formed. Among the inositol phosphates typically formed by 3- and 6-phytases, other inositol phosphates such as DL-Ins(1,3,4,5) P_4 and DL-Ins(1,4,5) P_3 , showing intracellular signal transduction function, are detected in the gastric chyme [159]. Even though their concentration is low, their extracellular existence is remarkable. As their physiological properties in the gut are not yet understood, their physiological role in the gastric chyme needs to be clarified. This is also true for the major inositol phosphates formed during

phytate digestion in the gut. The higher phosphorylated inositol phosphates are, the lower the solubility of their metal complexes in the gastro-intestinal chyme. Consequently, high phosphorylated inositol phosphates, and especially phytic acid, bind strongly to minerals and trace elements under the acidic conditions of the gastric chyme and form soluble complexes. During the passage from the stomach to the small intestine and with increasing pH they precipitate. Thus, the availability of bound trace elements and minerals is reduced, the intestinal absorption of these elements affected and under certain dietary conditions and imbalanced nutrition this may lead to serious deficiencies of these elements in humans and animals. The changing of the inositol phosphate solubility in the gastro-intestinal chyme during the passage throughout the gut confirms the long assumed mechanism for the phytate inhibition of the bioavailability of trace elements and minerals in the gut [159].

For optimum physiological benefit of phytate, the phytate content in the upper part of the gut has to be low to avoid adverse mineral interactions but high to use its anti-oxidative and anticancer activity as well as its contribution of preventing kidney stone formation in humans. Thus, the fate of phytate during digestion in the gut is of principle significance to assess its role in human nutrition.

5 Absorption and bioavailability of phytate and other inositol phosphates

Under physiological pH (~6–7), phytic acid is highly charged and eight of twelve hydroxyl groups carry negative charges (Fig. 1). Due to the small size of the inositol molecule, phytic acid shows an extremely high negative charge density. For these reasons, it has been assumed that phytic acid or phytate in all probability cannot cross the lipid bilayer of plasma membranes. As adequate carriers have not yet been detected in the gut, the gastro-intestinal absorption of phytate was considered rather improbable.

Recent studies in cell lines, rats and humans, however, give some evidence to the gastro-intestinal absorption of phytate. Due to various difficulties in the sensitive determination of phytate and other inositol phosphates in body fluids, such as blood plasma or urine, only a few studies have been carried out so far.

5.1 Cellular uptake of phytate and phytate absorption in rats

Nahapetian and Young [39] observed in 1980 that the radioactivity of ^{14}C labelled phytate was almost quantitatively absorbed in rats and recovered in blood, organs, bones, urine and expired CO_2 . This was one of the first reports indicating absorption of phytate or of its degradation products in rats. Sakamoto *et al.* [40] also administered radiolabelled phytate to rats and found broad distribution of radio-

Table 9. Phytate concentration in organs and fluids of rats fed either a diet free of phytate (AIN 76 A) or the same diet with added calcium–magnesium phytate (AIN 76 A + 1% Ca–Mg–phytate) for 12 wk^{a)}

Organs/ liquids	Phytate content	Diets	
		Phytate free diet (AIN 76 A)	Phytate free diet + 1% Ca– Mg–Phytate
Brain	µg/g (dw)	2.55 ± 0.30	24.89 ± 1.14
Kidney	µg/g (dw)	0.048 ± 0.005	1.71 ± 0.06
Bone	µg/g (dw)	n.d.	1.79 ± 0.06
Urine	mg/L	0.06 ± 0.04	3.20 ± 0.06
Plasma	mg/L	0.021 ± 0.004	0.22 ± 0.01

Values in each line were significantly different ($p < 0.05$).

a) Adapted from [191].

activity in organs such as the liver, kidneys and gut. In blood and urine, however, only traces of radioactivity were measured. Quite a lot of radioactivity was detected in the GI-tract and the chromatographic analysis revealed that InsP_3 was possibly the main inositol phosphate in the gastric epithelial cells along with traces of other low phosphorylated inositol phosphates while high phosphorylated inositol phosphates like InsP_5 and InsP_6 were absent. Whether or not InsP_3 really was present in this tissue as the predominant inositol phosphate remains to be clarified as also other organic phosphates such as nucleotides, being not removed during the sample preparation, may coelute during chromatographic separation from the column under the same conditions as some of the inositol triphosphate isomers (see Section 9.4 and Fig. 9). The same experimental uncertainties also exist for a cellular uptake study using ^3H - InsP_6 in different cells (YAC-1, K562 and HT-29 cells), showing major radioactivity in the chromatic fractions of InsP_1 , InsP_2 and InsP_3 [190] which, however, could also derive from other compounds such as nucleotides. These findings do not prove beyond any doubt the cellular uptake of low phosphorylated inositol phosphates formed by phytate hydrolysis. Recent studies on cellular uptake of radiolabelled InsP_6 by MCF-7 breast cancer cells, however, showed 86% of the absorbed radioactivity in the cytosol, 7.4% in the nuclear or membrane pellet and 58% localised chromatographically in the InsP_6 fraction. Besides InsP_6 , also InsP_4 was detected, giving evidence to cellular uptake of InsP_6 and its metabolic degradation product InsP_4 [41].

Ferry *et al.* [42] also found some evidence for the internalisation of InsP_6 into HeLa cells *via* pinocytosis as colchicine, a probable pinocytosis inhibitor, blocked the cellular uptake of phytate completely and as the chromatographical analysis of the cells after incubation with ^3H - InsP_6 showed most radioactivity in the InsP_6 and other high phosphorylated inositol phosphate fractions. Even if the procedure of washing the cells to separate intracellular from extracellular inositol phosphates was not described in detail, these find-

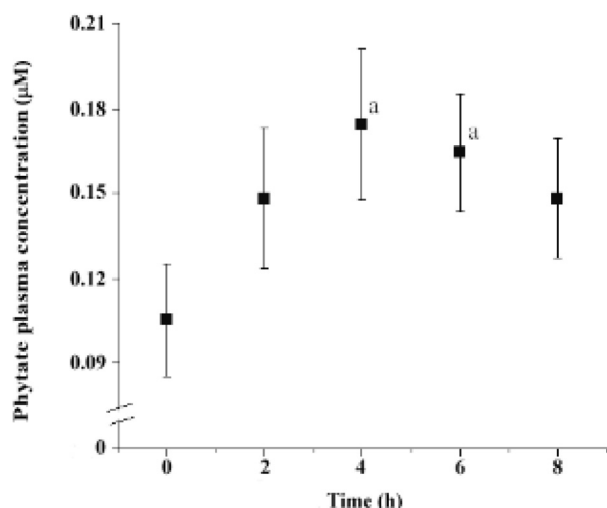


Figure 6. Phytate plasma concentration after ingestion of a single dose of 1400 mg dodecasodium phytate (1515 μmol of InsP_6) in humans; (a) significantly different from phytate concentration at time 0 h ($p < 0.05$) (with permission from IOS Press BV, Amsterdam, NL [195]).

ings may give further evidence to cellular uptake of InsP_6 by cultivated HeLa cells.

Grases *et al.* [191] fed a purified control diet free of phytate and the same diet with addition of 1% sodium phytate to rats for 7 days. The plasma concentration of phytic acid was significantly higher ($p < 0.05$) for rats receiving the sodium phytate containing diet ($0.022 \pm 0.01 \text{ mg/L}$) than for the rats fed the phytate free control diet ($0.21 \pm 0.04 \text{ mg/L}$). The same was observed in the urine. After feeding the phytate diet, significant ($p < 0.05$) higher phytic acid concentration in the urine was determined ($3.20 \pm 0.06 \text{ mg/L}$) compared to the control diet ($0.06 \pm 0.04 \text{ mg/L}$). In the organs of rats, fed the phytate containing diet, significant higher phytic acid concentrations ($p < 0.05$) compared to the control diet were also observed (Table 9), with ten times higher phytic acid content in the brain ($24.89 \pm 1.14 \mu\text{g/g}$) than in the kidney ($1.71 \pm 0.06 \mu\text{g/g}$). The study indicates that the level of phytic acid in body fluids and organs varies strongly with the phytate content of the diet applied.

Another study on rats followed the phytic acid concentration in the urine after application of a liquid diet with different contents of phytic acid (61, 182 and 425 mg/L) for 100 days [192]. With increasing dietary phytic acid content, enhanced phytic acid excretion in the urine was detected. Maximum urinary excretion of phytic acid was found for the applied phytic acid concentration of 182 $\text{mg InsP}_6/\text{L}$, corresponding to a daily intake of 6.9 mg InsP_6 per rat or to 20.9 $\text{mg InsP}_6/\text{kg}$ body weight. When phytate was omitted from the diet, the InsP_6 concentration in the urine declined immediately and became undetectable ($<0.1 \text{ mg/L InsP}_6$) after 22 days [192]. These findings show varying urinary excretions of phytate in rats depending on different dietary phytate levels and also indicate absorption of phytate in the

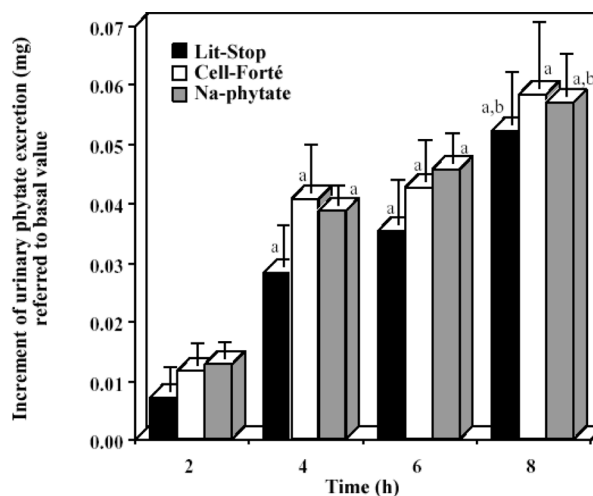


Figure 7. Urinary phytate excretion in humans 2–8 h after application of three different phytate supplements: 400 mg Lit-Stop (Ca-Mg-Phytate, 467 $\mu\text{mol InsP}_6$), 3200 mg Cell-Forté' (Ca-Mg-Phytate, 3734 $\mu\text{mol InsP}_6$) and 1400 mg Na-phytate (1515 $\mu\text{mol InsP}_6$) (a) vs. 2 h ($p < 0.05$); (b) vs. 4 h ($p < 0.05$). (with permission from IOS Press BV, Amsterdam, NL [195]).

rat's gut. Other studies in rats also show strong effects of high dietary phytate intake on phytic acid concentration in plasma and brain [193, 194].

5.2 Phytate absorption in humans

Grases *et al.* [195] showed in three women and four men that the plasma concentration of phytic acid was low ($0.106 \pm 0.015 \mu\text{mol InsP}_6/\text{L}$) when the diet was low in phytate ($<100 \text{ mg phytate intake per day}$) and reached a maximum of $0.181 \pm 0.030 \mu\text{mol InsP}_6/\text{L}$ 4 h after a bolus application of 1.4 g sodium phytate (1.5 mmol InsP_6) (Fig. 6). When a diet with an average phytate content ($\sim 700 \text{ mg phytate per day}$) was consumed for 16 days, the plasma concentration of phytic acid reached a level of $0.393 \pm 0.045 \mu\text{mol/L}$. Applying 0.4 g calcium–magnesium phytate (0.46 mmol InsP_6) or 1.4 g sodium phytate (1.5 mmol InsP_6) after a 15-day period on a low phytate diet, the urinary excretion of phytic acid was 0.008 mg (12.1 nmol InsP_6) and 0.013 mg (19.7 nmol InsP_6) after 2 h, respectively, and reaching after 8 h a significant ($p < 0.05$) higher urinary phytic acid excretion of 0.052 mg (79 nmol InsP_6) and 0.058 mg (88 nmol InsP_6), respectively (Fig. 7) [195]. As higher applications of calcium–magnesium phytate such as 3.2 g (3.7 mmol InsP_6) did not result in significantly higher urinary phytic acid excretion compared to 0.4 g calcium–magnesium phytate (0.46 mmol InsP_6) or 1.4 g sodium phytate (1.5 mmol InsP_6), this lets assume that obviously limited capacity of phytic acid absorption and/or urinary phytic acid excretion in humans exists [195]. This study shows varying phytate concentrations in plasma and different phytate excretion in urine due

to changing dietary phytate consumption, giving some evidence to phytate absorption in humans depending on the dietary phytate applied. Phytic acid was measured in this study by a nonspecific but highly sensitive method, determining phytic acid on the basis of the inositol content after anion exchange separation on AG 1 X 8 which may include also other high phosphorylated inositol phosphates and hence may result in some higher phytic acid content but with the same error for all plasma and urine samples [195].

Other studies also indicate a strong relation between dietary phytate intake and phytate absorption in humans. One study showed significantly lower phytate excretion ($p < 0.05$) when phytate intake in healthy subjects and in calcium oxalate kidney stone formers was changed from freely chosen phytate containing diets to an experimental diet free of phytate [196]. Another study showed a strong increase of urinary phytate excretion up to 440 μg phytate (<667 nmol InsP_6) within 8 h after application of 400 mg of calcium–magnesium phytate (0.46 mmol InsP_6) [197].

5.3 Conclusions

From the different studies it can be concluded that in cultured cells, in rats and humans, phytate absorption may occur even though the mechanism of absorption remains to be clarified. Support for phytate absorption in humans comes from the tight correlation of phytate consumed and increasing phytate concentration in plasma and enhanced urinary excretion [195–197]. Similar results were found in rats [191–194]. As the absorption of dietary phytate is independent of the filling stage of the stomach [197], it is assumed that phytate absorption probably takes place in the small intestine. The studies, moreover, suggest that the phytate absorption in the gut is very low and does not exceed a few percent ($\leq 2\%$) [192], which was also concluded from pig studies, determining the disappearance rates of phytate and other inositol phosphates from the gastro-intestinal tract [177].

The great number of *in vitro* experiments and animal studies, showing anticancer or anticalcification activities after application of sodium phytate or calcium–magnesium phytate, moreover, strongly supports the absorption of phytate or of its degradation products in the gut, as without gastro-intestinal absorption the observed effects are unexplainable [32, 41]. The assumptions that phytate might be hydrolysed completely in the gut and be rephosphorylated intracellularly after absorption of *myo*-inositol and phosphate requires to be proven. The studies in rats and humans, applying phytate and detecting phytic acid in plasma and urine after comparably short time [191–197], do not seem to support these hypotheses. Whether phytate or other inositol phosphates might be absorbed *via* active transports, pinocytosis or other ways of absorption or by a combination of these, also remains to be clarified.

A recent study of Irvine and coworkers [198], however, found no detectable InsP_6 either in human serum and plate-

let-free plasma (<1 nM InsP_6) or in human urine (<5 nM), using an enzymatic method, which determines InsP_6 specifically and with high sensitivity after ^{32}P -labelling and HPLC separation. As the statistical basis of these findings is vague and detailed information on the dietary phytate level of the persons is missing, it is difficult to assess whether or not these results really may contradict the earlier reports in humans and rats, discussed above.

6 Influence of phytate on intestinal mineral absorption

Health authorities from all over the world universally recommend increasing consumption of whole grains and legumes for health promoting diets. Whole grain foods are valuable sources of carbohydrates, dietary fibre, numerous bioactive compounds, vitamins, minerals and trace elements which are in short supply in many countries. Mineral malnutrition is a global problem affecting industrialised and developing countries as well. Children, infants and women at childbearing age are primarily affected [199, 200]. Under nonvaried and nonbalanced dietary conditions, phytate may affect the bioavailability and in consequence the status of iron, zinc and calcium [24–29, 201]. It should be stressed that in many countries whole grain cereals and legumes are among the most important food sources for minerals and trace elements but also contain high amounts of phytate and polyphenols. Thus, when advice is given for good dietary sources of minerals and trace elements, various interactions between the minerals and trace elements and phytic acid have to be taken into consideration to ensure high bioavailability and adequate supply.

6.1 Phytic acid: Chelating properties, binding capacity for minerals and inhibition of mineral absorption

Phytate occurs in all edible plant seeds such as grains, legumes, oilseeds and nuts but also in lower content in roots, tubers and vegetables (Tables 2–5). As phytic acid is strongly negatively charged under physiological conditions (Fig. 1) [13], it shows great potential for complexing positively charged multivalent cations, especially of iron, zinc, magnesium and calcium [202]. These complexes are soluble under the acidic conditions in the stomach and precipitate at neutral pH in the intestine, resulting in poor absorption of minerals and trace elements (see Section 4). They also may affect peptides and proteins, leading to reduced protein bioavailability [155, 156, 203–206] and impaired enzymatic activity [205, 207]. Even if phytate seems to be most effective for impairing the mineral absorption other components like inorganic phosphate, polyphenols and nondigestible dietary fibre reduce the absorption of mineral and trace elements as well [208, 209].

Studies, both in animals and humans, have shown that foods or diets rich in dietary fibre may alter mineral metabolism in the presence of phytate [210, 211] while other studies indicate no effect of dietary fibre on mineral absorption [212, 213]. Numerous studies have described the negative effect of phytate on the bioavailability of minerals and trace elements [171, 205, 214–221] which recently was extensively reviewed [222–226].

There are, however, a number of dietary components which counteract the inhibitory effects of phytate on mineral absorption. Some evidence exists from human studies for improving calcium absorption after application of fermentable carbohydrates [227–229]. Other studies, which first showed decreased calcium absorption in the presence of phytate, reported enhanced calcium availability after degradation of phytate [230, 231]. This is due to the reduced formation of the low soluble Ca–phytate which not only affects the mineral availability of calcium in the intestine but is also poorly susceptible to enzymatic dephosphorylation to lower phosphorylated inositol phosphates [23, 181].

Organic acids obtained by food fermentation also counteract the inhibitory effects of phytate and enhance zinc absorption in the presence of phytate [232]. The same effect was reported for dietary protein, whereby the content and type of protein along with the content of zinc are important for the improvement of zinc absorption [233, 234]. Diets low in animal protein result in low zinc absorption in the presence of phytate [235] and high calcium content increases the inhibitory effect on zinc bioavailability by forming calcium–zinc–phytates. Insufficient zinc intake, however, is the main cause for zinc deficiencies in humans [236].

Iron deficiency is one of the most prevalent deficiencies in the world and mainly caused by insufficient iron intake [237]. Moreover, the sources of iron, hem or nonhem iron and the total composition of the diet are of great importance for iron bioavailability. The content of phytate in food has been closely related to iron absorption and high phytate content results in lower iron absorption [126]. Phytic acid decreases the solubility of iron by forming low soluble iron–phytate [238] and for this reason iron availability from the chyme is affected and the intestinal iron absorption inhibited. This inhibition can be counteracted by complexing agents like proteins, peptides, beta-carotene, organic acids and ascorbic acid [239–246]. Ascorbic acid, moreover, stops the oxidation of ferrous to ferric iron and thus preventing the formation of the very low soluble Fe^{3+} –phytates.

6.2 Phytic acid interactions with toxic trace elements (Cd, Pb)

Due to the high binding affinity of phytic acid to metal ions the question was raised whether or not phytate could be applied to affect the bioavailability of toxic trace elements such as cadmium or lead. In infant cynomolgus monkeys (*Macaca fascicularis*) strong reduction of the blood lead

concentration following the addition of phytate to the diet was observed [247]. In adult human volunteers, who ingested ^{203}Pb as lead acetate, the absorption of lead was affected by a meal high in calcium and in phytate [248]. In rats the effect of calcium and phytate was studied regarding the absorption of lead and cadmium. The addition of calcium or phytate significantly reduced the lead accumulation in bones ($p < 0.001$) and in blood and liver ($p < 0.05$) [249] and applying phytate and calcium together, the strongest inhibition of the tissue lead retention was reported. For cadmium a significant increase in the liver and kidney accumulation by calcium ($p < 0.05$) was observed which after supplementation by phytate, was reduced again and no further effect of phytate on the cadmium tissue levels was detected [249]. Applying a fish-meal diet in rats either with or without sodium phytate, Yannai and Sachs found no effect on the cadmium, lead and mercury absorption in this diet after addition of sodium phytate [250]. In a study with albino rats, Rimbach *et al.* [251] fed three diets based on egg white and corn starch and supplemented by zinc (15 mg/kg) and cadmium (5 mg/kg). The control diet (diet 1) was free of phytate and active phytases while the both experimental diets contained either 0.5% sodium phytate (diet 2) or 0.5% sodium phytate plus microbial phytases (2000 U/kg) (diet 3). Liver cadmium concentration in rats fed diet 2 was significantly higher than in rats fed diet 1 (control diet) or diet 3. By trend similar results were found for kidney cadmium accumulation. These results show that the high cadmium accumulation in liver and kidneys were due to the sodium phytate added as after phytate degradation by phytases the high cadmium accumulation was reduced [251]. The results were confirmed by the same authors in another rat study, showing also significantly higher cadmium liver and kidney accumulation after application of a sodium phytate containing diets (0.5%) and also a reduction of the accumulated cadmium by supplementation of microbial phytases (2000 U/kg) [252]. A later rat study by Rimbach and Pallauf [253] showed, however, only a slight increase in the cadmium accumulation in the kidneys and no significant alteration of the liver cadmium content with increasing dietary phytate up to 1.4% (3.5–14.0 g Na–phytate/kg). As the zinc content of these diets was more than twice as much (225 mg Zn) as that of the earlier ones (100 mg Zn) [251, 252], the authors concluded that under the conditions of high dietary zinc contents, phytate might have only little effect on the carry over of cadmium in growing rats [253]. In mice Lind *et al.* [254] studied the accumulation of cadmium from fibre rich diets based on wheat bran, sugar-beet fibre and carrots in comparison to a semisynthetic control diet supplemented with CdCl_2 . All diets contained a cadmium content of 0.05 mg Cd/kg. The wheat-bran diet showed significantly lower fractional cadmium accumulation in the liver and kidneys (% of total Cd intake), indicating a lower absorption of cadmium. The authors discussed that most probably the higher dietary content of inositol hexa- and inositol pentaphosphates, forming with cadmium low

soluble Cd–phytate complexes, contributed more to the lower cadmium absorption than the elevated Zn level [254]. Similar results were reported by Wing [255] who determined the fractional cadmium accumulation (^{109}Cd) from different diets based on either whole wheat, bran or endosperm with different levels of phytate (4.2, 7.6 and 0.3 mmol IP₆/kg, respectively). The fractional accumulation of ^{109}Cd in the liver and the kidneys was significantly lower ($p < 0.001$) in rats fed the phytate rich whole wheat and bran diets compared to the low phytate endosperm diet (control diet).

The bioavailability of cadmium from cow's milk formula, soy formula, wheat/oat/milk formula, whole meal/milk formula and water were compared in rat pups [256]. The pups received a single oral dose of one diet labelled with ^{109}Cd , 0.1 or 0.3 mg Cd/kg body weight. The lowest cadmium bioavailability was found in the cereal-based formulas, explained by the cadmium binding to dietary fibre and phytate [256].

6.3 Conclusion

Dietary phytate probably affects the bioavailability and retention of lead in rats, monkeys and humans [247–249]. Inhibition of the cadmium bioavailability and retention by phytate in mice was reported [254, 255], while under certain dietary conditions, such as low dietary zinc content, the cadmium absorption and retention in kidneys was even improved by addition of sodium phytate [251, 252], and normalised after phytate degradation or at high dietary zinc content [253]. Thus, the concentration and relation of various minerals, trace elements and phytate in the diet seems to be important for the effects of phytate on the cadmium bioavailability and retention. From the different results it can be concluded that in respect to the bioavailability and retention of cadmium, phytate as an endogenous part of the food matrix probably reacts differently than sodium phytate supplemented to the diet. Moreover, as the study with a fish-meal based diet shows no inhibitory effect of phytate on the intestinal absorption of cadmium, lead and mercury [250], it can be assumed that the kind of proteins consumed also might play an important role in phytate effecting the bioavailability and retention of toxic trace elements.

6.4 Determinants of the phytate – mineral interaction

Several factors are present which govern the inhibitory effect of phytate on mineral bioavailability: pH, content of minerals and phytate, solubility of phytates and concentration of enhancers or inhibitors. Moreover, great variations exist for the affinity of phytate complexes with different valent cations showing increasing binding strength from mono- to multivalent cations (e.g. Na^+ , Ca^{2+} , Fe^{3+}) [257]. Furthermore, different phytate–mineral ratios in foods and

diets seem to be also important for phytate inhibition of the intestinal absorption of minerals and trace elements and the phytate concentration in the diet has to exceed a certain level to have a substantial effect on the bioavailability of minerals and trace elements [258]. To predict the bioavailability of minerals from diets just based on the phytate content in foods is not reliable as all other factors involved in the phytic acid–mineral interaction have to be taken into consideration [223]. The same is true for the quotient of Zn/Ca/phytic acid, widely applied for assessing the mineral bioavailability [132, 135, 137], which also disregards the complexing agents in the diet, competing with phytic acid for the binding of metal ions.

6.5 Conclusions

Growing interest in whole grains and whole grain products in developing and industrialised raised the questions on these foods on the mineral status. High content of phytate in these products has been considered a major factor for limited mineral bioavailability, resulting in iron, zinc and calcium deficiencies. The inhibition of the intestinal metal absorption, however, can be counteracted by many food compounds such as organic acids and complexing agents, ascorbic acid, food fermentation products, *etc.* competing with phytic acid in the binding of minerals and trace elements. Thus, it will be assumed that in well balanced diets the inhibitory effects of phytic acid is low and little evidence exist from nutritional surveys that in well nourished population groups dietary phytate may seriously effect the status of iron, zinc and calcium.

Under malnutrition and nonbalance diets low in minerals and essential trace elements but high in phytate, however, the situation is completely different. Vulnerable groups in developing and developed countries with inadequate intake or deficiencies of minerals and trace elements need to increase total intake of these elements *via* the daily diet or to improve the bioavailability of these elements under consideration of all factors inhibiting or enhancing the bioavailability of the minerals and trace elements in the diet. Adequate strategies to prevent deficiencies of these essential elements adjusted to the specific situation are required and different approaches are possible either by supplementation of the respective elements, by increasing the contents of competing and complexing agents or by removing phytate from food.

7 Effects of preparation, processing and storage on phytate and other inositol phosphates

Preparation and processing can make food healthier, tastier and safer and increase its storage stability. Preparation and processing, however, can also affect the nutritional value by

destroying labile nutrients like vitamins or by removing phytate to improve the bioavailability of mineral and trace elements.

As phytate is quite heat stable up to $\sim 100^{\circ}\text{C}$ [22], it cannot be easily removed by conventional heat treatment like cooking. Enzymatic degradation of phytate, however, either by phytases occurring naturally during food processing or by adding phytases, effectively degrades phytate in food [23].

7.1 Thermal hydrolysis of phytate

During boiling and heat treatment, phytate in food shows a high stability up to $\sim 100^{\circ}\text{C}$ for a boiling time of 1 h [22]. Only 9% of phytate is degraded under these conditions in soybeans (*Soja hispida* Max.). Table 10 shows the stepwise phytate hydrolysis in brown beans (*Phaseolus vulgaris* L.) at various temperatures and boiling times, applied for legume processing and household preparation (U. Schlemmer, 1996 unpublished results). To cook brown beans well, a temperature of 110°C for 1.5 h is needed after soaking the beans over night [22]. Under these conditions InsP_6 is degraded from 86 to 66% and lower phosphorylated inositol phosphates (InsP_5 – InsP_3) are formed. The total sum of inositol phosphates (InsP_3 – InsP_6), however, does not decline but increases slightly, probably due to a better release of phytate from the bean matrix. This shows that preparing beans for consumption under normal household conditions $\sim 1/4$ of phytate in raw beans is degraded and quantitatively transformed to InsP_5 – InsP_3 . If the temperature is raised very high, e.g. to 140°C , which is beyond any practical conditions of household preparation but relevant for certain industrial processing at a quite extended boiling time of 45 min, phytate is only reduced from 86 to 36% along with a total loss of inositol phosphates (InsP_6 – InsP_3) of only 28%. These results demonstrate the high stability of phytate during thermal preparation of brown beans. Table 10 gives an overview on how boiling temperature and time govern the thermal hydrolysis of phytate in legumes during processing and preparation and confirms earlier reports [17, 18, 125, 222, 259, 260].

7.2 Phytases

Phytases (*myo*-inositol hexaphosphate phosphohydrolase, EC 3.1.3.8 and EC 3.1.3.26) are found in plants, microorganisms and in animal tissues as well [163, 261–263]. They hydrolyse phytate as well as other organic phosphates like lower inositol phosphates, nucleotides, etc., however, with different affinity and efficacy [187, 264]. The stepwise cleaving of phosphate groups leads to lower phosphorylated inositol phosphates and inorganic phosphate and finally stops at *myo*-inositol. Different phytases show different cleaving specificities for hydrolysing phosphate groups form phytate, resulting in different inositol pentaphosphate

Table 10. Stepwise hydrolysis of phytate (%) in brown beans by hydrothermal treatment^{a)}

	$\sum \text{InsPs}$	InsP_6	InsP_5 [%]	InsP_4	InsP_3
Raw brown beans	100	86	14	–	–
110°C					
30 min	113	85	23	2	3
60 min	107	72	24	5	3
90 min	104	66	26	10	6
120°C					
30 min	111	68	28	10	5
60 min	102	58	27	11	6
90 min	91	47	23	11	10
130°C					
15 min	113	68	29	11	5
45 min	96	50	25	13	8
90 min	79	38	21	10	10
140°C					
15 min	90	54	24	9	3
45 min	72	36	21	10	5
90 min	58	21	21	10	6

a) Brown beans (*Phaseolus vulgaris* L., 20 g dw/50 mL H_2O) were heated under pressure in a pressure cooker and the thermal degradation of inositol phosphates determined [22]. After heating, the brown beans were freeze dried, ground (<1 mm, particle size) and extracted with HCl (2 g/40 mL 2.4% HCl, 3 h, 22°C). Samples were prepared and inositol phosphates with different numbers of phosphate groups were determined by RP HPLC in according to Sandberg and Ahderinne [398]. Total inositol phosphates ($\sum \text{InsPs}$) of the respective samples were summed to 100% and the total loss of InsP_6 – InsP_3 during the heat treatment was calculated. Results are mean values of double determination.

isomers [187, 265–267]. There are two internationally classified phytases: 3-phytase (EC 3.1.3.8) and 6-phytase (EC 3.1.3.26), named after the position of the first phytate phosphorester bond hydrolysed [209, 268]. The 3-phytases are primarily of microbial origin [261–263] while the 6-phytases are mainly of plant sources [269]. There are, however, exceptions: *Escherichia coli* phytases are 6-phytases and soybean phytases 3-phytases. One predominant inositol phosphate seems to be formed at any stage of the stepwise inositol phosphate degradation and the rate of hydrolysis decreases with the degree of phosphorylation [187, 202, 265–267].

The optimal temperature for phytase in cereals is 45°C , with an approximate stability limit at 55°C and a pH optimum at 5.0–5.6 [269]. Microbial phytases produced by fungi often show two different pH optima, one at 2.5 and one at 5.0 [187, 270–272] with a temperature optimum of 58°C , and with nearly no activity $>68^{\circ}\text{C}$. Bacterial phytases such as *Bacillus subtilis* show a neutral to alkaline pH opti-

mum (pH $\sim$ 7.0–7.5) [270] and most of them have optimum activity at $\sim$ 50°C.

Phytases have been studied in a great number of plants and have been isolated and characterised from different plant sources. High phytase activity is described in seeds [273] where they seem to be mainly associated with the aleurone tissue but to a minor extent also with the endosperm and scutellum. Cereal phytases are present with varying activity in all unprocessed cereal grains, with the highest activity found in rye and with lower activity in wheat, oat, spelt and corn [274]. Phytase activity also varies with the harvest year and cultivars [275]. Unprocessed oat shows similar phytase activity as wheat [276] but due to the conventional heat treatment of oat to avoid lipid rancidity, oat phytases are largely inactivated [277, 278]. During food processing and preparation, predominantly during soaking, malting, germination, fermentation and bread making, phytases of plant or microbial origin are widely applied to reduce phytate content in foods to improve the bioavailability of minerals and trace elements.

7.3 Soaking, malting and germination of cereals

Soaking is a commonly used method as pretreatment of germination, malting, *etc.* Soaking may last for short periods (15–20 min) or long periods (12–16 h). The soaking medium used depends upon the type of seed. In the household, cereals and legumes are most often soaked in water overnight. At optimal conditions for phytases (55°C, pH 4.5–5.0) phytate might be reduced effectively by soaking [188]. As phytate is water soluble, some phytate removal can be obtained by discarding the soak water [172].

Phytase activity increases considerably during germination [279], but there are great differences between different cereals. Barley has shown an increase of phytase activity up to 11 times of the original one [280], while phytases in wheat, rye and oats increased 4.5, 2.5 and 9 times from the original activity [277]. The phytate content was reduced by 16% in barley [280], and 30% in wheat and rye and 17% in oats [277]. In malted wheat, rye and barley, ground and soaked for 2 h, almost total hydrolysis of phytate was obtained. Oats needed a longer time (up to 17 h) to reach complete reduction [281]. Optimal conditions for hydrothermal processes of whole kernels of barley to hydrolyse phytate up 95–96% have been developed, using two wet steps and two dry steps, followed by drying [282]. Degradation seems to be highest in the scutellum cells and less in the aleurone layer due to changes in the microstructure of the phytate globoids in the barley during hydrothermal processing [283].

7.4 Soaking and germination of legumes

Supplementation of cereals with legumes rich in protein is considered to be effective to fight protein-calorie malnutri-

tion in many developing countries. A common method of processing legumes to reduce phytate is soaking and germination. As these foods are the main sources for protein in many developing countries, it is important that phytate is reduced to a minimum to obtain bioavailability as high as possible for both protein and minerals. Before pulses and legumes are consumed they are dehulled and prepared with subsequent soaking, germination, fermentation, roasting and autoclaving [284].

The decrease in phytate content due to germination has shown to be highest in pigeon peas (65.8%), followed by chickpeas (64.1%), bean curd (40.6%), soybeans (38.9%) and mung beans (37.2%) [285]. Other studies on beans [17, 286] have shown considerably lower reduction than reported in this study. Even if fermentation has been shown to result in a decrease of phytate content, this method was less effective than germination. It should however be pointed out that these two methods are the most effective ways to lower the phytate content in legumes and pulses.

Soaking of peas for up to 12 h decreased the content of phytate no more than 9% [287] while other studies showed no effect of soaking (16 h, 22°C) on the phytate content in peas, lentils or beans [22]. A small reduction could be caused by leaching phytate into the soaking water [288]. The most effective way of reducing phytate degradation was at pH 7.0 and 45°C [289]. Germination results in the reduction of phytate in peas – the longer the germination period, the greater the phytate degradation. While a loss of 6–8% of phytic acid was observed after 12 h, a loss of 67–83% occurred after 48 h [288]. It is recommended to dehull and soak legumes before consumption to reduce the phytate content and thereby increase the nutritional quality of proteins in these foods. With quinoa seeds it was also demonstrated that soaking, germination and lactic acid fermentation resulted in reduced phytate content up to 98% [290].

7.5 Fermentation and bread making

Fermentation has been used for processing and preservation of foods for a long time in history. Due to production of lactic acid and other organic acids in the dough pH is lowered, phytases activated and phytate degraded. Part of the phytate reduction is due to the action of endogenous phytases, but exogenous microbial phytases may also be active in phytate degradation during fermentation [265]. It seems as if phytases normally present in cereals are of greater importance for phytate reduction, than yeast phytases added to cereals [291]. Different yeast species have been identified as possible sources of phytase [292–294], and certain bacteria [295, 296] may also provide viable sources of exogenous phytase. Fermentation of maize, soybeans and sorghum has been shown to reduce the phytate content in foods [296, 297]. It has been demonstrated that combined germination and lactic acid fermentation of white sorghum and maize crusels can result in almost complete degradation of phytate [298].

Phytate hydrolysis occurs during the different stages of bread making, depending of the flour used for the bread, both type and extraction rate being of importance. The acidity of the dough is of utmost importance for the degradation of phytate during both scalding and sourdough fermentation of the bread [299, 300]. Sourdough fermentation has been shown to reduce the phytate content more effectively than yeast fermented bread [301, 302]. A slight acidification of pH to 5.5 in the bread dough, obtained either by sourdough or by lactic acid, resulted in increased phytate breakdown [303, 304]. It is remarkable that this minor acidification is sufficient to degrade the phytate effectively. Even if a lower pH due to higher addition of sourdough seems to be more effective for endogenous phytases in the grain, together with the added microbial phytases, pH at 5.5 seems to be more accepted by consumers who dislike the acidic taste. In rye bread, phytic acid is almost totally degraded to lower inositol phosphates and free inorganic phosphate during bread making when long fermentation times are used, but the degradation is lower when whole grain is included in the recipe [305].

7.6 Addition of exogenous phytases

Phytases can be added to food during processing to reduce the phytate content. Commercial sources of the enzyme are available from wheat bran, yeast or microbial sources. Phytases seem to have a broad specificity and when isolated, *e.g.* from wheat, they also degrade phytate in oats and other cereals [276, 306]. Phytases from fungal origin may achieve complete phytate degradation, while endogenous phytases just reduce phytate by 73–80% [307].

7.7 Extrusion cooking

Extrusion cooking is a high-temperature, short time process using high shear forces at elevated pressure and temperature. As this method gives desirable texture to the foods, it has become a widely used technique in food manufacture, especially for cereals and legumes, like breakfast cereals, weaning foods, crispbread, snacks, sweets and vegetable proteins. Extrusion is extremely versatile with respect to ingredients and production capacity, in addition to supplying variety to shapes and textures. An increase in dietary fibre has been reported after extrusion cooking [308], but little information has been given regarding the fate of phytate. One might expect hydrolysis of phytate during this process, however, the time of exposure is too short for significant reduction.

For cereals there were only slight changes in total inositol phosphates *via* extrusion cooking [154, 303, 309] and only a small decrease in phytate accompanied by a small increase in inositol pentaphosphates were observed [310]. Similar observations were made in oilseeds, and soybean meal was least affected by extrusion cooking [311]. The

observed phytate degradation during extrusion cooking is due to the high temperature and pressure occurring during the process. The effect of extrusion on phytate hydrolysis, however, seems to be strongly dependent on the extrusion conditions applied, as studies extruding total animal feed did not show any change in the inositol phosphate pattern of the extruded diet [159].

7.8 Removal of phytate by mechanical processes

Mechanical removal of phytate is dependent on the type of seed processed, but also on the morphological distribution of phytate in seeds. For a large number of oil seeds and cereals, the main part of the phytic acid appears to be located in the aleurone layer, but also to a minor degree in the germ [44–46]. This is, however, not the case for, *e.g.* soybeans, where it seems more evenly distributed in the whole seed. In millet and oat, phytate seems to be evenly distributed both in the bran, kernel and germ [303, 312]. Milling of cereals, in which phytate is located in the outer layer of the seed, can cause up to 90% reduction of phytate. In corn where phytate is mainly located in the germ, removing of this part of the grain will effectively result in strong reduction of phytate. In legumes, where the majority of phytate is located in the protein bodies in the endosperm [215, 303], dehulling will therefore remove phytate only to a minor degree. Mechanical separation of the phytate containing compartments of the seeds, however, will also lead to a loss of nutrients and valuable bioactive compounds.

7.9 Storage

Several studies have indicated a decrease in phytate content both for legumes and cereals [66, 116, 313–315] during long storage. This reduction depends on the storage conditions (especially humidity and temperature), the type of seeds and the age of the seeds [316, 317]. When stored under dry and cool conditions no decrease in phytate is assumed. In a study with cowpea flour, added to macaroni to increase phytate content, no change in phytate was observed during the six months storage under optimal conditions [318].

8 Beneficial properties

8.1 Preventing pathological calcification

Kidney stones are prevalent in humans. It is assumed that 5–10% of humans suffer from the formation of kidney stones in the urinary collecting system. The formation of kidney stones is dependant on the solubility of calcium salts in the urine. The solubility, the maximum content of calcium, remaining soluble in an aqueous solution at given temperatures, is an equilibrium parameter which is independent of time and thermodynamics. It mainly depends on the stability of the crystal lattice and the stability of the formed aqueous

solvates (soluble species). Solubility can also be affected by the composition of the media (mainly ionic strength) due to its influence on the reactivity (related to chemical potential) of the solvates. When a system contains higher content of a solute than that corresponds to the *solubility* (saturated system), the system remains in an unstable state (supersaturated) and sooner or later must evolve to the stable conditions (thermodynamic equilibrium) through the crystallisation of the excess of solute. Precisely, the driving force that pushes the crystallisation processes is the difference between the equilibrium conditions (solubility) and the actual ones. These time-dependent processes, studied by the kinetics, can last for seconds or years. The general mechanism of the formation of single crystals can be explained as a result of the combination of two independent steps: nucleation and crystal growth. The time necessary to generate a crystal mainly depends on the nature of the crystal, the supersaturation of the solution, the presence of performed solid particles (the so-called *heterogeneous nucleants*) and the concentration of crystallisation inhibitors. These latter ones, due to their structure, may interact with the nucleus or interfere in the crystallisation processes [319].

Most of the human fluids are supersaturated with regard to some substances. Thus, blood, interstitial liquid and intracellular liquid, due to their pH value ($\text{pH} > 7.0$), free calcium ion concentration and phosphate concentration, are supersaturated with respect to calcium phosphate (hydroxyapatite, HAP). Urine is always supersaturated with respect to calcium oxalate and depending on its pH value, is also supersaturated with respect to uric acid ($\text{pH} < 5.5$) or calcium phosphates ($\text{pH} > 6.0$). In spite of this, normal crystallisation processes only take place in biologically controlled situations, like the formation of bone and teeth. Nevertheless, uncontrolled pathological crystallisation is also frequent: *e.g.* in tissue calcification associated to cancer, calcification in cardiovascular system and calculi formation (renal, biliar, sublingual).

The question is, why crystallisation does not occur indiscriminately in all human fluids and only appears in pathological situations? The answer is clear: there are four main aspects which must be considered in explaining pathological crystallisation:

- (i) supersaturation higher than usual of the crystallising substance, and/or
- (ii) the presence of heterogeneous nucleants (crystallisation inducers), and/or
- (iii) deficit of crystallisation inhibitors, and/or
- (iv) a failure of the immune system.

The crystallisation inhibitors act by delaying the crystallisation of supersaturated substances, by avoiding the crystallisation before the renovation of the corresponding fluid or by permitting that the immune system eliminates cellular debris (crystallisation inducers) or even incipient calcifications [320].

It has been known since the 1930s that the presence of trace amounts of molecules, such as polyphosphates, can act as water softeners through their inhibition of the crystallisation of calcium salts, such as calcium carbonate. However, the use of such compounds as natural regulators of calcification under physiological conditions was not explored until the 1960s. During that decade, Fleisch *et al.* [321, 322] showed that pyrophosphate, a naturally occurring polyphosphate, is present in serum and urine and can prevent calcification by binding to HAP. However, studies in animal models found that pyrophosphate can inhibit ectopic calcification in blood vessels and kidneys only when injected rather than ingested. Oral administration causes hydrolysis and hence inactivation of pyrophosphate, resulting in a search for more stable analogues. Bisphosphonates, a group of polyphosphates, show high affinity for HAP and prevent calcification both *in vitro* and *in vivo*, even when administered orally to animals [323]. Crystallisation inhibitors bind to crystal nuclei or crystal faces and disturb crystal development. The adsorption of such compounds to crystal faces can also inhibit crystal dissolution. It was shown that bisphosphonates may inhibit HAP crystal dissolution [324, 325] and bone resorption [324, 326, 327]. Many studies, both *in vitro* experiments and clinical trials, have also shown that various bisphosphonates inhibit the osteoclast-mediated bone resorption [327–329]. It is well known that proteins are active in modulating calcification in mammalian tissues. These proteins can either enhance or inhibit the ability of macrophages to destroy HAP deposits (*i.e.* osteoclastic activity) [330–332]. A common characteristic of these proteins involved in the calcification is that they show high affinity to calcium ions. These proteins include osteopontin [333–336], osteoprotegerin [337–339], matrix Gla protein [340–343] and osteocalcin [332, 343]. They have shown some crystallisation inhibitor activity, however, under *in vitro* conditions with nonphysiological high protein, calcium and phosphate concentrations [344–346]. Moreover, these proteins have also calcification promoter activity due to their heterogeneous nucleant capacity [347–349]. It appears that the major calcification modulator role of these proteins is to regulate osteoclast/osteoblast cell activity [330, 331, 350]. Recently, it has been demonstrated *in vitro* that phytate exerts potent action as crystallisation inhibitor of calcium salts (oxalate and phosphate) [351–354].

8.1.1 Phytate and renal lithiasis

Beyond proteins, phytate has been shown to possess strong activity of inhibiting the crystallisation of calcium oxalates or calcium–phosphates [351–354]. Phytate is naturally present in urine at similar concentrations to those used in the *in vitro* studies [355] and urinary phytate concentration also depends on dietary intake [190, 191]. This demonstrates the potential therapeutic effects of phytate in the treatment of calcium renal lithiasis in preventing calculus development.

The effects of phytate on urolith development in a nephrolithiasis animal model using ethylene glycol were studied [356]. In the group of rats treated with phytate, the number of calcifications on the papillary tips and the total calcium amount of the papillary tissue was significantly reduced when compared with the control group treated exclusively with ethylene glycol.

A purified rodent diet (AIN-76 A), free of phytate, has shown to possess some activity to cause kidney calcifications in female rats which was absent with nonpurified rodent diets. This suggests a nutritional factor which avoids these kidney calcifications. One possible candidate was phytate. The effects of phytate, added to the AIN-76 A diet, was therefore studied in the calcification of kidney tissue in female Wistar rats. Rats were randomly distributed into three groups and fed the AIN-76 A diet, the AIN-76 A diet + 1% phytate and the standard nonpurified diet. No phytate was detectable in the urine of the rats fed the AIN-76 A diet, free of phytate. Urinary phytate levels of the rats fed the AIN-76 A + 1% Na-phytate diet and the standard diet were significantly higher than those of the rats fed the phytate free AIN-76 A diet. The concentrations of calcium and phosphorus in kidneys were greater in the AIN-76 A group than in AIN-76 A + 1% phytate and standard groups. Only rats of the AIN-76 A group displayed mineral deposits at the corticomedullary junction. These findings demonstrate clearly that renal calcification in female rats was low in the presence of phytate and high in the absence of phytate [357]. Hypertension, induced by nicotine, combined with hypercalcemia, induced by vitamin D was used to induce calcification in renal tissue in male Wistar rats which were fed a purified phytate free diet. These rats developed significant calcium deposits in kidneys and papillae, as well as in kidney tubules and vessels, whereas calcium deposits were absent when phytate was added to this phytate free diet. These findings show that phytate acts as crystallisation inhibitor both in the intrapapillary tissue and in urine [358].

Furthermore, the effect of phytate on dystrophic calcification, chemically induced by subcutaneous injection of a 0.1% KMnO₄ solution, was studied. Male Wistar rats were randomly divided into four groups treated for 31 days. A: Animals were given a purified diet free of natural phytate but with added 1% Na-Phytate. In this group, the phytate plasma levels ($0.393 \pm 0.013 \mu\text{M}$) were similar to those observed in rats consuming a standard diet. B: Animals consuming only the purified diet free of natural occurring phytate. In this case the phytate plasma levels decreased ($0.026 \pm 0.006 \mu\text{M}$); C: Animals consuming the same purified diet as group B but received daily subcutaneous injections of 50 $\mu\text{g/kg}$ etidronate (a crystallisation inhibitor) during the last 14 days. In this case the phytate plasma levels were also very low ($0.025 \pm 0.007 \mu\text{M}$); D: Animals consuming the same diet as group B but 6% of carob germ, rich in phytate, was added. The phytate plasma levels ($0.363 \pm 0.035 \mu\text{M}$) were also similar to those observed in

rats consuming a standard diet. After 21 days plaque formation was induced. Calcification plaques were allowed to proceed for 10 days, after which the plaque material present was excised, dried and weighed. The results show that the presence of phytate in plasma at normal concentrations ($0.3\text{--}0.4 \mu\text{M}$) clearly inhibits the development of dystrophic calcifications [359].

Recent studies demonstrated that phytate is naturally present in human urine and normal levels oscillated between 0.5 and 3 mg/L [355, 358]; the urinary concentrations found in a group of calcium oxalate active stone-formers were significantly lower than those found in a group of healthy people [196] (see also Section 5). Ingestion of dietary phytate significantly reduced the risk to develop calcium stones in humans [360, 361]. Thus, a clinical study in 36 calcium oxalate active stone-formers with positive urinary risk to develop calcium stones was performed. In a subgroup of 19 stone-formers the urinary risk to develop calcium stones was re-evaluated after 15 days. The other group of 17 stone formers was treated with phytate (120 mg of phytate/day as calcium–magnesium salt *phytin*) for 15 days and then the urinary risk was re-evaluated. Other urinary lithogen parameters were also determined. The obtained results show that whereas the ordinary urinary lithogen parameters were not modified by ingestion of phytate, the urinary risk to develop calcium stones was significantly reduced, demonstrating an interesting therapeutic efficacy in using phytate as a crystallisation inhibitor. The urinary risk factor was evaluated using a test specially developed and validated for this purpose [360].

During an 8-year period a prospective study examined the association between dietary factors and the risk of incident symptomatic kidney stones in 96 245 female participants. The study lets assume that a high intake of dietary phytate decreases the risk of kidney stone formation and dietary phytate might be a new and safe way to prevent kidney stone formation [362].

It is important to remark that phytate was early used in the treatment of renal lithiasis at an early stage. Thus, in 1958, Henneman *et al.* [363] used high doses of phytate as sodium salt (8.8 g/day) to treat stone-former patients with idiopathic hypercalciuria. Nevertheless, the objective and basis of such treatment was clearly different to that presented here. Thus, high doses of phytate were supplied to hypercalciuric patients with the aim to form nonsoluble complexes in the intestinal tract in order to prevent the absorption of dietary calcium and subsequently to decrease urinary calcium excretion. However, the low dose supplied in the newly proposed treatment aims at raising the urinary excretion of phytate, increasing the inhibitory capacity of urine towards calcium salts crystallisation (oxalate and phosphate).

Consequently, all *in vitro* and *in vivo* results clearly indicate that phytate plays a significant role as crystallisation inhibitor of calcium salts in biological fluids and is an alternative in the treatment of calcium oxalate renal lithiasis.

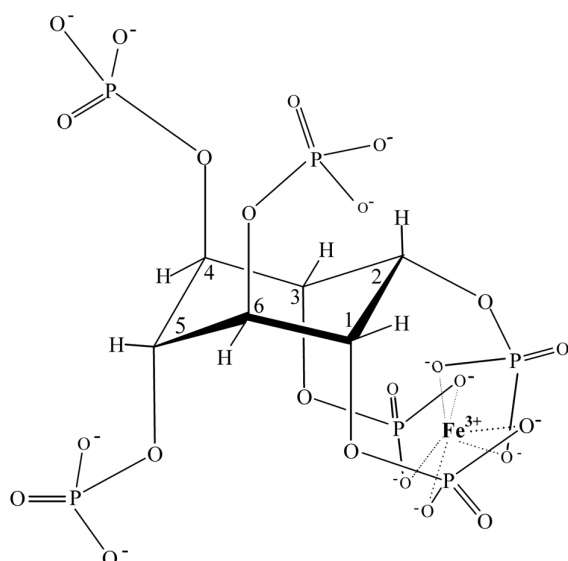


Figure 8. Molecular structure of mono ferric-phytate in which iron (Fe^{3+} -ion) is bound to phytic acid so that all six coordination sites of iron are occupied. Iron bound in this configuration (phosphate groups in position 1, 2 and 3 and in the axial, equatorial and axial position) does not take part in the formation of hydroxyl radicals from H_2O_2 via the Fenton reaction under assistance of the Haber–Weiss reaction in a two step reaction [368–370]. Under physiological conditions the negative charges are counterbalanced either by protons, sodium ions or other cations depending on pH and affinity.

8.1.2 Phytate and sialolithiasis

Sialolithiasis is a common disease of salivary glands. Little is known about the aetiology of these calculi and their exact mechanism of formation is unknown. In a study, the composition and structure of 21 sialoliths were studied and the composition of the saliva of each corresponding patient was determined (pH, calcium, magnesium, phosphorus, citrate and phytate). Eighteen sialoliths exhibited similar macro and microstructures, consisting of HAP and organic matter. The salivary Ca concentration of patients with HAP calculi was significantly higher and the salivary phytate concentration was significantly lower than those of the healthy subjects. It was concluded that a deficit of crystallisation inhibitors such as phytate is also an important aetiological factor of sialolith development [364].

8.1.3 Phytate and cardiovascular calcification

Calcification is an undesirable disorder, which frequently occurs in the heart vessels. In general, the formation of calcific vascular lesions involves complex physicochemical and molecular events. Calcification (HAP) is caused by injury and progresses through promoter factors and/or the deficit of calcification modulators. The capacity of phytate as a potential inhibitor of cardiovascular calcification was assessed in rats subjected to calcinosis induction by vitamin D plus nicotine [358] or by a macro dose of vitamin D [365]. In both cases phytate demonstrated significant

effects on decreasing calcifications of the cardiovascular system. From these results it can be concluded that phytate is important in preventing cardiovascular calcification and further human studies are needed to fully understand its role in this process.

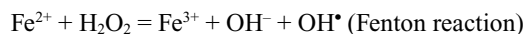
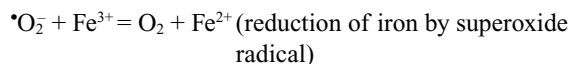
8.2 Blood glucose and lipid lowering effects

Under physiological conditions phytic acid interacts with proteins by forming low soluble complexes. Thus, enzyme activity might be inhibited by phytate. Thompson *et al.* [33] showed that *in vitro* formation of glucose from white bean flour was reduced in the presence of intrinsic dietary phytate and after addition of 1% sodium phytate. Administering the same flour to humans, the effect of phytate on the glycemic index was studied. Intrinsic dietary phytate as well as the addition of 1% sodium phytate to the flour significantly reduced ($p < 0.05$) blood glucose levels in six volunteers [33]. In a recently published study in diabetic KK-mice, Lee *et al.* [34] found reduced blood glucose levels depending on the dietary phytate contents (0–1.0%). In another study in diabetic KK-mice Lee *et al.* [366] reported decreased serum total cholesterol and LDL cholesterol by phytate (0–1.5%). No effects of phytate on the triglyceride concentration could be detected. The mechanism of these phytic acid effects remains to be clarified but it was assumed that the cholesterol lowering effects in blood caused by phytate might be in part related to an increase in faecal bile acid and lipid excretion [367] and a reduction in hepatic cholesterol synthesis.

These results let assume that dietary phytate may also beneficially affect the blood glucose and blood cholesterol levels.

8.3 Antioxidative property

The antioxidative property is one of the most impressive characteristics of phytate. It is mainly based on complexing iron between three phosphate groups in positions 1, 2 and 3 and in the axial, equatorial and axial position, respectively [368–370]. These phosphate groups are flexible and bind the iron ion in such a way that all six co-ordination sites of iron are occupied by $-\text{OH}$ groups (Fig. 8) and the labile bound water in the first coordination sphere of iron is removed. As the stability of this iron–phytate complex is high (Fe^{3+} –phytate complex $K_f 10^{17}$ [257]), other ligands with lower affinity cannot interact with iron in this ‘cage’. For the interaction with hydrogen peroxide (H_2O_2) at least one free coordination site of iron is required and thus, in the presence of phytate, the formation of hydroxyl radicals ($\text{OH}^\bullet$) from H_2O_2 via the Fenton reaction under assistance of the Haber–Weiss reaction in a two step reaction



is inhibited. It can be assumed that the molecular structure of mono ferrous-phytate (Fe^{2+} -ion) is similar to that shown for mono ferric-phytate [368–370] as the hydroxyl radical formation from H_2O_2 via the Fenton reaction is also inhibited when ferrous iron (Fe^{2+} -ion) is complexed by phytic acid [371]. For this reason phytic acid is an antioxidant unlike other antioxidants, such as ascorbic acid or β -carotene, which primarily act as radical scavengers. Even if phytate inhibits the oxidation of the Fe^{2+} -ion in the phytate complex by H_2O_2 , due to the lack of any free coordination sites and of any available aquo coordination sites of iron, this does not mean that any oxido-reduction process of the phytate bound iron is prevented. For example, the iron oxidation in the Fe^{2+} -phytate complex by molecular oxygen (O_2) is accelerated by a concentration dependent manner, whereas the reduction of the Fe^{3+} -ion in the mono ferric-phytate complex in the presence of ascorbic acid is inhibited by phytate [30].

However, for the inhibition of the hydroxyl radical formation certain ratios of phytic acid to iron are important. While Graf *et al.* [368] found a large range of 1:4 to 20:1 (InsP_6/Fe), Rimbach and Pallauf [371] pointed out that the inhibition of hydroxyl radicals via the Fenton reaction requires a ratio $>5:1$, confirming earlier results of Hawkins *et al.* [370]. For stoichiometric reasons a molecular ratio >1 seems to be more reasonable as phytic acid only interacts one iron ion in this specific configuration.

Beyond its antioxidative property, phytic acid also reduces the iron mediated lipid peroxidation and inhibits the formation of thiobarbituric acid reacting substances (TBARS) [30, 372]. This effect has been applied in meat processing to prevent the oxidation of myoglobin to met-myoglobin, which changes the colour in homogenised meat from fresh red to a brownish shade during preparation and storage [373–375].

Porres *et al.* [376] studied in pigs the effect of different dietary phytate on the protection of oxidative stress induced by moderately high iron intake in colon and liver. They found that colonic lipid peroxidation, measured as TBARS, was lowered by phytate to some extent in the colon mucosa, while it was unaffected in the liver. Highest lipid peroxidation was observed with high dietary iron content (FeSO_4) and in the absence of phytate. The authors concluded that phytate is protective against lipid peroxidation in the colon induced by a moderately high iron intake. However, no effect was observed with a normal iron intake.

In Sprague–Dawley rats, Rao *et al.* [377] studied the effect of phytate on the damage caused by experimentally induced ischemia-reperfusion injury. The results show that phytate, injected intravenously (7.5–15 mg/100 g BW) prior to cardiac excision, protects the myocardium from damage. This was measured by markers of muscle damage, heart function and lipid peroxidation.

Rimbach and Pallauf [371] studied the effect of iron, phytate and vitamin E on the antioxidative status in male albino rats for 28 days. At marginal dietary iron supply, phytate reduced the iron bioavailability, but no effect was observed on oxidative stress measured by determining reduced glutathione, TBARS and protein carbonyls. In a follow up study, also in male albino rats, Rimbach and Pallauf [378] fed diets with different levels of magnesium and sodium phytate (300 mg Mg and 0, 7.5 and 15 g Na-phytate/kg). With increasing dietary phytate, magnesium bioavailability and magnesium content in plasma and femur decreased. This was accompanied by an increase in hepatic TBARS and protein carbonyls along with a moderate decline of reduced glutathione levels in the liver. The results show a decreased antioxidative effect of phytate mediated by magnesium. A reversed effect of dietary phytate was observed by Shan and Davis [379] in chickens. Here phytate increased the selenium concentration in tissues and by this the activity of glutathione peroxidase (GSHPx) in blood and heart was increased, along with decreased GSHPx activity in the kidneys.

Comparing diets high and low in intrinsic dietary phytate in postmenopausal women, Engelman *et al.* [380] found only small and nonsignificant effects on oxidative stress measured by protein carbonyls, 8-iso-prostaglandin-F-2 alpha and oxidised LDL.

The assumption that oxidative stress and reactive oxygen species might be significant for the development of cancer, arteriosclerosis, neurodegenerative diseases, cirrhosis, irradiation damages and other civilisation diseases [381] raised the question on the role of phytate as antioxidant in human nutrition and disease prevention. *In vivo* studies reported, however, show an equivocal picture. On the one hand antioxidative activity of phytic acid is obvious while on the other hand no antioxidative activity can be observed. This might be due to the different modes of action by which phytate takes part in antioxidative processes in the metabolism, mainly mediated by binding various bi- and trivalent cations such as iron and copper. Although under clearly defined *in vitro* conditions, the antioxidative property of phytate, such as the inhibition of the hydroxyl radical formation, can be determined exactly, it is difficult to do this *in vivo* in complex body fluids, tissues and organs. Thus, future studies will have to elucidate the significance of the phytate for disease prevention by inhibiting hydroxyl radical formation. As the concentration of phytate in mammalian cells is comparable high, reaching a level of ~ 15 – $100 \mu\text{M}$ [382, 383], much has been speculated on its real role in the cellular metabolism. As H_2O_2 and reactive oxygen species such as $\cdot\text{O}_2^-$ are ubiquitous produced and iron is widespread in the metabolism, the intracellular significance of phytic acid could be to govern intracellular formation of hydroxyl radicals which is of vital interest to any living cell and organism.

8.4 Anticancer activity

The anticancer activity of phytic acid is one of the most important beneficial activities of phytic acid. It was demonstrated in various kinds of cancer, such as colon, liver, lung, mammary, prostate, skin and soft tissue cancer of mice and/or rats [384–387]. Moreover, different mechanistic steps of the anticancer activity of phytic acid in various, mostly human cell lines, were observed. Mainly sodium phytate was added either to the diet, to the drinking water or to the cell medium [384]. Intrinsic dietary phytate, however, has also shown to effect aberrant crypt foci (ACF) and silomucin-producing ACF, both early markers of colon cancer formation [388]. Thus, it can be concluded that dietary phytate also shows anticancer effects whereas applying high content of sodium phytate might show more pronounced effects. As most conclusions of the anticancer activity of phytic acid have been drawn from carcinogen-induced cancer models and cell line experiments, there is a strong need for evaluating the anticancer activity of phytic acid in humans. Given that excellent and extensive reviews on the anticancer activity of phytic acid have been published very recently [384–388], a further review here of this topic is not yet required.

9 Determination of phytic acid/phytate and other inositol phosphates in foods – development of analytical methods

9.1 Nonspecific methods

The analysis of phytic acid dates back to the method of Heubner and Stadler in 1914 [389]. Essentially this method is based on the extraction of phytic acid from ground cereal powder by hydrochloric acid and subsequent precipitation as Fe^{3+} -phytate from the filtrate after stepwise addition of FeCl_3 . The phytic acid content is deduced on the basis of the iron content of the Fe^{3+} -phytate precipitate. Due to problems detecting the exact endpoint of this titration, McCance and Widdowson [390] changed this procedure and determined the phosphorous content of the Fe^{3+} -phytate precipitate. Phytic acid content was calculated on the assumption that the total phosphorous originated from phytate. Due to inconsistent stoichiometric ratios of Fe^{3+} -ions or phosphorous to phytic acid in the Fe^{3+} -phytate precipitate, Harland and Obeleas [391] omitted the Fe^{3+} -phytate precipitation and purified the phytate-containing HCl extract by anion-exchange chromatography on AG 1 resin. Separating inorganic phosphate from organic phosphate by stepwise NaCl elution they gained an elution fraction containing mainly phytate. However, it included other organic phosphates present in the sample. The phosphate content of this fraction was used to calculate the phytic acid content or more correctly the content of 'phytic acid equivalents'. As this method does not discriminate between various organic

phosphates, the calculated phytic acid content is misleadingly enhanced when phytate and other inositol phosphates or nucleotides are present. As this method was mainly applied to raw and unprocessed food with a low content of lower phosphorylated inositol phosphates ($\sim <15\%$, InsP_1 – InsP_5) [16], this error is of minor practical relevance. In 1986 a modified version of this method became the official AOAC-method in determining phytate in food [392].

However, for processed food with a high content of phytate hydrolysis products or for biological samples rich in lower phosphorylated inositol phosphates (InsP_1 – InsP_5), the AOAC method is inadequate and specific methods for the precise determination of phytic acid and other inositol phosphates are required. For extensive discussion of the nonspecific analysis the reader is referred to the excellent reviews of Oberleas and Harland [393] and Xu *et al.* [394].

9.2 Specific methods

In 1952 Smith and Clark [395] were first able to separate phytic acid and other inositol phosphates from soil by means of anion-exchange chromatography on a weak-base exchange resin and stepwise elution with increasing HCl concentration. Inositol phosphates were determined by analysing the phosphorous content of the different peaks. In 1963 Cosgrove [396] applied AG 1 as a strong anion exchange resin to separate various inositol phosphates obtained after acidic hydrolysis of phytic acid by means of HCl gradients (0–1.5 N HCl). Quantification of the inositol phosphates in the different peaks occurred by analysing the phosphorous-inositol ratio.

In 1980 Tangendjaja *et al.* [397] tried to determine phytic acid of rice by RP chromatography on $\mu\text{Bondapak C}_{18}$ column and using sodium acetate (5 mmol/L) as mobile phase. Graf and Dintzis [74] improved this analysis by adding a preceding purification and concentration step on AG 1 resin. For phytic acid detection they favoured the refractive index detection rather than the UV absorption. This purification and concentration proved to be very helpful for a good separation and detection of inositol phosphates by different HPLC methods, especially in complex matrix, and was widely used. Due to the low retention of phytic acid (1.4 min) on $\mu\text{Bondapak C}_{18}$ columns, other inositol phosphates could hardly be discriminated [74]. By applying tetrabutylammonium hydroxide as an ion-pair reagent, Sandberg and Ahderinne [398] improved the retention of inositol phosphates on the stationary phase and separated inositol phosphates with different numbers of phosphate groups (InsP_3 – InsP_6). However, separation of the stereoisomers of the different inositol phosphates also remained impossible with this method.

In 1984 in the field of cell biology, Berridge and Irvine [399] observed that $\text{Ins}(1,4,5)\text{P}_3$, cleaved from membrane-bound phosphatidylinositol 4,5-diphosphate by phospholipase C, shows a second messenger function in mobilising intracellular calcium. This intensified the search to separate

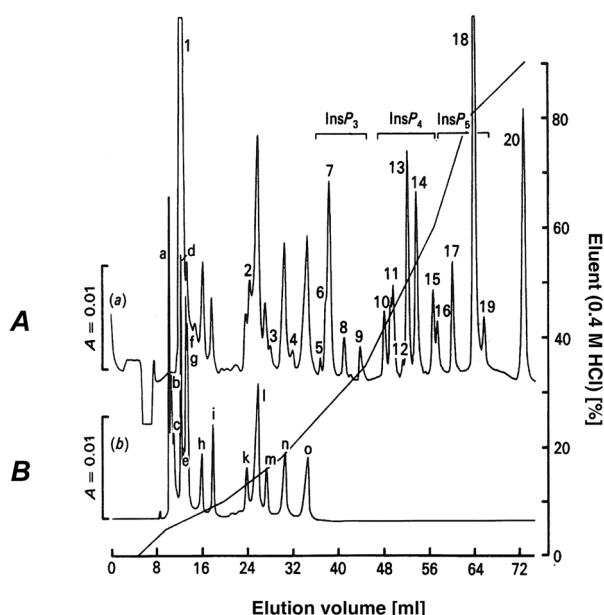


Figure 9. (A) Separation of inositol phosphates on a Mono-Q (250 mm) column by using a HCl gradient (0–0.4 M). Inositol phosphates were detected by using the Yttrium–PAR complex and measured at 546 nm [409]. 1, $\text{Pi} + \text{InsP}_1$; 2, $\text{Ins}(1,2)\text{P}_2 + \text{Ins}(1,6)\text{P}_2$; 3, PPI ; 4, unidentified; 5, $\text{Ins}(1,3,5)\text{P}_3 + \text{Ins}(1,4,6)\text{P}_3$; 6, $\text{Ins}(1,3,4)\text{P}_3$; 7, $\text{Ins}(1,2,6)\text{P}_3 + \text{Ins}(1,2,3)\text{P}_3 + \text{Ins}(1,4,5)\text{P}_3$; 8, $\text{Ins}(1,5,6)\text{P}_3$; 9, $\text{Ins}(4,5,6)\text{P}_3$; 10, $\text{Ins}(1,2,3,5)\text{P}_4/\text{Ins}(1,2,4,6)\text{P}_4$; 11, $\text{Ins}(1,2,3,4)\text{P}_4/\text{Ins}(1,3,4,6)\text{P}_4$; 12, $\text{Ins}(1,3,4,5)\text{P}_4$; 13, $\text{Ins}(1,2,5,6)\text{P}_4$; 14, $\text{Ins}(2,4,5,6)\text{P}_4$; 15, $\text{Ins}(1,4,5,6)\text{P}_4$; 16, $\text{Ins}(1,2,3,4,6)\text{P}_5$; 17, $\text{Ins}(1,2,3,4,5)\text{P}_5$; 18, $\text{Ins}(1,2,4,5,6)\text{P}_5$; 19, $\text{Ins}(1,3,4,5,6)\text{P}_5$; 20, $\text{Ins}(1,2,3,4,5,6)\text{P}_6$. (B) Nucleotides are also determined by this detection at 254 nm: a, $\text{AMP} + \text{CMP} + \text{NAD}$; b, cyclic AMP; c, GMP; d, NADH; e, UMP; f, NADP; g, ADP + ADP-ribose; h, ATP + CTP; i, GDP; k, IDP; m, UDP; n, ITP; o, UTP. They coelute with some InsP_1 – InsP_3 , interfering their determination. Nucleotides are measured at 254 nm with optimum sensitivity (B), but also can be detected at 546 nm. The figure was adapted from [409].

the different inositol phosphates by adequate HPLC anion-exchange chromatography methods. AG 1, silica based SAX, Partisil SAX 10 columns, *etc.* with aqueous mobile phases of ammonium formate, ammonium acetate or ammonium phosphate [400–402] were successfully used to differentiate radiolabelled inositol phosphates, especially lower phosphorylated inositol phosphates (InsP_1 – InsP_3). Using a modification of the ion-pair RP HPLC and varying the ACN concentration of the mobile phase, Sulpice *et al.* [403] were able to discriminate inositol phosphates such as $\text{Ins}(1,4,5)\text{P}_3$ from other organic phosphates such as ATP and 2,3-DPG. Irth *et al.* [404] later obtained distinct differentiation of $\text{Ins}(1,2,6)\text{P}_3$ from other low phosphorylated inositol phosphates.

Interestingly, the HPLC methods used in cell biology research have not been widely applied in food science or nutrition research. One reason for this might have been the

problems of adequate inline detection due to missing characteristic absorption spectra of and specific colorimetric reagents for inositol phosphates. As the detection of radio-labelled inositol phosphates in the mobile phase of highly concentrated aqueous ammonium formate or ammonium phosphate (maximum ~ 1 mol/L) was unproblematic, sensitive inline detection of nonradioactive inositol phosphates, such as from biological samples in the nano- or micromolar range, remained a challenge.

In 1985 Phillippy and Johnston [405] and later Phillippy *et al.* [15] showed good separation of a great number of different inositol phosphates on a strong anion exchange AS3 column (Dionex) by using an HNO_3 gradient (0–0.155 M HNO_3). This eluent offered reliable and sensitive inline detection of inositol phosphates by complexing the phosphate groups by means of Fe^{3+} -ions. This detection showed a linear calibration curve up to ~ 100 nmol phytic acid and a detection limit of 1–2 nmol phytic acid, measured at 290 nm [15]. It was based on earlier observations by Imanari *et al.* [406], who found Fe^{3+} -ions highly appropriated to form soluble complexes with inositol phosphates under acidic conditions, allowing for quantitative in-line determination by means of postcolumn derivatisation.

In 1986 Cilliers and Niekerk [407] and later Rounds and Nielsen [408] presented another method for inline detection of phytic acid by modifying the former phytic acid determination of Latta and Eskins [106]. This determination based on an exchange reaction of the ligand (Fe^{3+}) from the Fe^{3+} –sulfosalicylate complex (Wade reagent) to the Fe^{3+} –phytate, resulting in a bleaching of the intense purple colour of the ferric sulfosalicylate complex, measurable at 500 nm.

In 1988 Mayr [409] proposed the separation of inositol phosphates on a Mono-Q column (L: 250 mm; $10\ \mu\text{m}$ beads; strong anion exchanger; General Electric) by using an HCl gradient (0–0.4 M) with a comparable long running time of 60–90 min. This HPLC method provided very good separation of most inositol phosphates including their stereoisomers (Fig. 9). Good separation of the higher phosphorylated inositol phosphates (InsP_5 – InsP_6) as well as of the major lower phosphorylated inositol phosphates (InsP_3 – InsP_4) can also be achieved on a short Mono-Q column HR 5/5 (5×50 mm) in less than half an hour (Fig. 10, below), which is adequate for most food analysis and many biological samples as well [159]. Mayr [409] applied for the detection of inositol phosphates the coloured complex of Yttrium and 4-(2-pyridylazo)resorcinol (PAR). The Yttrium–PAR complex is bleached in the presence of inositol phosphates, due to the higher affinity of the Y^{3+} -ions to the phosphate groups of the inositol phosphates and the detection is called the metal-dye detection (MDD). The absorption is measured at 546 nm as a negative peak with a linear calibration curve up to 1000 pmol. In a modification of this method using a Mini-Q PC (3.2/3) column (the same anion exchanger as Mono-Q but a bead size of $3\ \mu\text{m}$) Guse *et al.* [410] described the detection limit of 1–3 pmol InsP_6 .

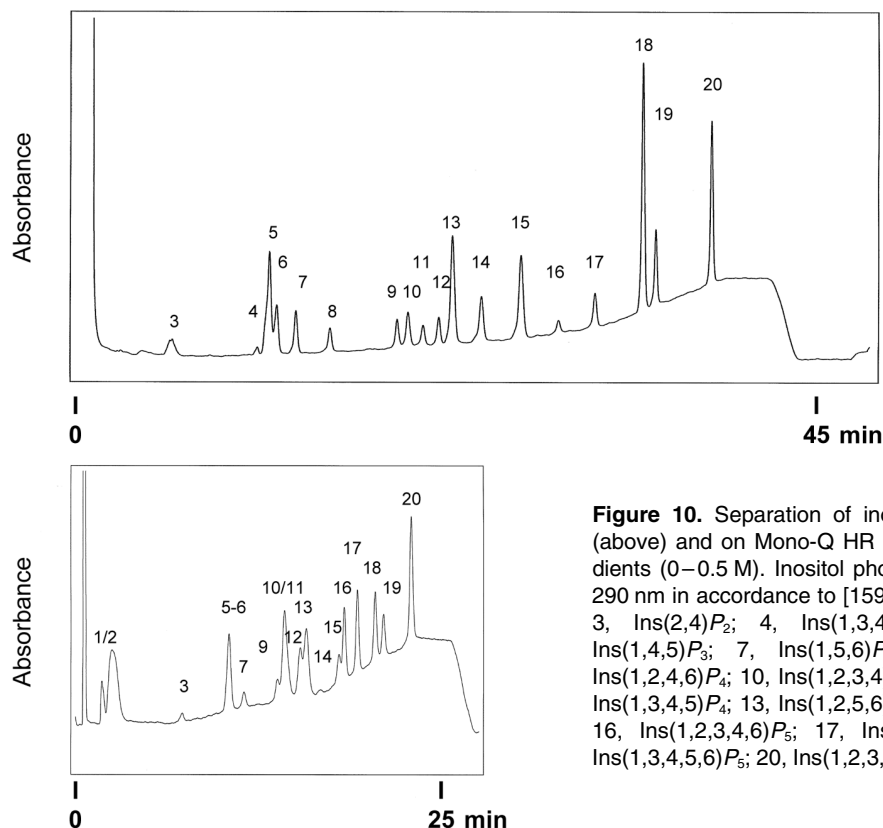


Figure 10. Separation of inositol phosphates on CarboPac PA-100 (above) and on Mono-Q HR 5/5 (0.5/5 cm) (below) by using HCl gradients (0–0.5 M). Inositol phosphates were detected with Fe^{3+} -ions at 290 nm in accordance to [159]. 1, $\text{Pi} + \text{InsP}_1$; 2, $\text{Ins}(1,2)\text{P}_2 + \text{Ins}(1,6)\text{P}_2$; 3, $\text{Ins}(2,4)\text{P}_2$; 4, $\text{Ins}(1,3,4)\text{P}_3$; 5, $\text{Ins}(1,2,6)\text{P}_3 + \text{Ins}(1,2,3)\text{P}_3$; 6, $\text{Ins}(1,4,5)\text{P}_3$; 7, $\text{Ins}(1,5,6)\text{P}_3$; 8, $\text{Ins}(4,5,6)\text{P}_3$; 9, $\text{Ins}(1,2,3,5)\text{P}_4 / \text{Ins}(1,2,4,6)\text{P}_4$; 10, $\text{Ins}(1,2,3,4)\text{P}_4 / \text{Ins}(1,3,4,6)\text{P}_4$; 11, $\text{Ins}(1,2,4,5)\text{P}_4$; 12, $\text{Ins}(1,3,4,5)\text{P}_4$; 13, $\text{Ins}(1,2,5,6)\text{P}_4$; 14, $\text{Ins}(2,4,5,6)\text{P}_4$; 15, $\text{Ins}(1,4,5,6)\text{P}_4$; 16, $\text{Ins}(1,2,3,4,6)\text{P}_5$; 17, $\text{Ins}(1,2,3,4,5)\text{P}_5$; 18, $\text{Ins}(1,2,4,5,6)\text{P}_5$; 19, $\text{Ins}(1,3,4,5,6)\text{P}_5$; 20, $\text{Ins}(1,2,3,4,5,6)\text{P}_6$.

This method still is the most sensitive inline detection of nonradiolabelled inositol phosphate isomers after HPLC separation. However, other multivalent cations and especially Fe^{3+} -ions interfere strongly in the detection by affecting the Y^{3+} –PAR complex. Thus for reliable determination, all iron containing materials in contact with the eluent have to be eliminated and all chemicals and standards used need highest possible purity. The long running time of more than an hour for the long Mono-Q columns (250 mm), however, is disadvantageous for routine analysis and gives preference for inositol phosphate separation on the shorter Mono-Q column (HR 5/5) or the Mini-Q column.

In 1997 Skoglund *et al.* [411, 412] reported excellent separation of inositol phosphates including most of their stereoisomers by anion-exchange chromatography on Omni Pac PAX-100 (Dionex) and especially on CarboPac PA-100 (Dionex) by using HCl gradients (0–0.5 and 0–1.0 mol/L, respectively) along with the ferric ion detection [15]. This determination was proposed for mainly InsP_3 – InsP_6 . For lower phosphorylated inositol phosphates, such as InsP_1 – InsP_2 and InsP_3 , good separation on Omni Pac PAX-100 (Dionex) by using alkaline gradients with NaOH and 2-propanol in conjunction with anion micromembrane suppressor and conductivity detection in the picomole range of InsP_1 – InsP_3 was achieved [411, 413]. In 1988 and 1989 Smith and MacQuarrie [414, 415] already reported on the usefulness of the chemically suppressed conductivity detection, not only for lower phosphorylated (InsP_1 – InsP_3) but also for higher

phosphorylated inositol phosphates (InsP_4 , InsP_6) and Talamond *et al.* [416, 417] described it as an adequate detection also for the determination of phytic acid in food.

Very recently Letcher *et al.* [418] reported on an enzymatic method in which phytate can be determined after ^{32}P -labelling and HPLC separation as InsP_7 . However, even if the method is highly sensitive and might allow phytate detection below the nanomolar range (<1 nM InsP_6), it is unfortunately restricted to the determination of InsP_6 only. Other inositol phosphates, also significant in cells and tissues, are not detectable.

9.3 Problems of detection

Due to missing characteristic absorption spectra and of specific colorimetric reagents for inositol phosphates, further properties of the inositol phosphates had to be applied for analysis. In contrast to food samples, the concentration of phytate and other inositol phosphates in physiological samples such as tissues and cells is extremely low. Therefore, highly sensitive methods and adequate procedures for the sample preparations from different matrices were required.

9.4 Absorption

The high affinity of the phosphate groups of inositol phosphates to polyvalent cations, such as Fe^{3+} -, Y^{3+} - and Cu^{2+} -ions, is the basis most commonly used for the detection of

phytate and inositol phosphate in foods. Absorption can be measured either by direct interaction between cations, such as Fe^{3+} -ions and inositol phosphates [15], or by means of ligand exchange reactions, *e.g.* by removing Y^{3+} from the intensively coloured Y^{3+} –PAR complex by inositol phosphates, resulting in a bleaching of this complex [409]. Both methods, especially the latter one, provide sensitive detection of inositol phosphates. Nevertheless, other organic phosphates, such as nucleotides, interfere with this inositol phosphate determination as they coelute during chromatographic separation from the column with certain InsP_1 – InsP_3 isomers and can also be determined by this detection method (Fig. 9) [409]. Due to the low content of nucleotides in food, their effect on the determination of inositol phosphates in food is also low. When cells or physiological samples such as blood plasma, urine, tissues and organs are analysed, nucleotides and phosphorylated proteins need to be separated by adequate sample preparation, such as additional anion-exchange chromatography, charcoal treatment, *etc.* [409, 419].

Meek and Nicoletti [420] proposed the determination of InsP_2 and InsP_3 after HPLC separation by cleaving the phosphate groups using immobilised alkaline phosphatase (calf intestine) in an on-line bioreactor, determining phosphate by means of ammonium molybdate at 340 nm.

9.5 Fluorescence detection

A similar ligand exchange reaction as described for the Y^{3+} –PAR complex [409] was also applied for the fluorescence detection of inositol phosphates. Irth *et al.* [404] removed Fe^{3+} -ions from the Fe^{3+} –methylcalcein blue complex by inositol phosphates, showing higher affinity than the methylcalcein blue complex to Fe^{3+} -ions. By this ligand exchange reaction the quenching of the methylcalcein blue by means of the Fe^{3+} -ions was attenuated and depending on the inositol phosphate concentration the fluorescence intensity increased.

March *et al.* [421] used the activation of phytic acid on the oxidation of 2,2'-dipyridyl keton hydrazone catalysed by Cu^{2+} -ions resulting in highly fluorescent reaction products to determine phytate in urine. The calibration curve for this determination is linear over the range of 76–909 nmol/L with a detection limit of 45 nmol/L.

Chen *et al.* [422] used the replacement of the Cu^{2+} -ions from the Cu–gelatine complex by phytic acid. Thus the quenching of the Cu–gelatine complex was eliminated and the fluorescence intensity increased. The calibration curve for this determination is linear from 606 to 3.600 nmol/L with a detection limit of 348 nmol/L. In urine phytate was detected in the range of 0.49–0.75 mg/L with a recovery of 96.2–108.8%.

9.6 Light-scattering detection

Light-scattering detection as in-line determination of phytate after HPLC separation on a strong anion exchange AS7 column (Dionex) was tested by Phillippy *et al.* [15] and compared to the Fe^{3+} -ion detection. The authors reported a detection limit of 1.5 nmol InsP_6 for the light-scattering detection which, however, is higher than that of the Fe^{3+} -ion detection with 758 pmol InsP_6 and so far it does not offer a real advantage over the Fe^{3+} -ion detection.

9.7 Conductivity

Smith and MacQuarrie [414, 415] showed that more than 20 different biologically important anions can be separated by anion-exchange chromatography on AS 4A columns (Dionex) and can be detected with high sensitivity by using chemically suppressed conductivity. They reported a range of ~20 pmol to 400 nmol InsP_1 for the calibration line with a good separation not only between different phosphorylated organic compounds, such as nucleotides (ATP, ADP, GTP, *etc.*), glucose 6-phosphate, fructose 6-phosphate and various low phosphorylated inositol phosphates (InsP_1 – InsP_3), but also of InsP_4 and InsP_6 . Skoglund *et al.* [411, 413] and Talamond *et al.* [416, 417] later used this anion micromembrane suppressed conductivity detection either for low phosphorylated inositol phosphates or for phytic acid, in physiological samples and in food, respectively.

9.8 NMR spectroscopy

NMR spectroscopy is an adequate technique for analysing InsP_6 and its various hydrolysis products (InsP_1 – InsP_5), including the different stereoisomers. It has been successfully applied in both food and biomedical research [302, 423, 424]. The ^{31}P -NMR spectroscopy has potential application as an analytical technique to determine phytate content in plants and as well as human tissue and is appropriate to detect its form and binding to other components. Moreover, it is also adequate to study phytate metabolism and phytate degradation during food processing, offering the advantage of high accuracy and specificity. High resolution ^{31}P -NMR has been used as a noninvasive method for the study of P-containing compounds in intact tissues and cell suspensions. In comparison with other techniques, such as HPLC, NMR allows direct detection and qualification of all phosphate compounds in the same experiment. Use of inositol phosphate standards for NMR analysis is not required, unlike with other analytical methods such as HPLC [425].

The low sensitivity of NMR methods might cause difficulties in detecting low inositol phosphate contents in biological and physiological samples, such as cells or tissues. However, the inositol phosphate concentration is high

enough for a reliable ^{31}P -NMR determination in foods, chyme and faeces. Comparing to other analytical methods, good agreement of phytate determination in foods by ^{31}P NMR have been reported [426, 427]. NMR has also been successfully applied in routine analysis to differentiate inorganic phosphate from different inositol phosphates in plant extracts and in whole diet samples [428].

When analysing InsP_6 by ^{31}P NMR, four resonance peaks are observed since the molecule has plane symmetry through C-2 and C-5. Accordingly, the P signals from C-1 and C-3, as well as C-4 and C-6 are identical. In the adopted system, proton decoupling is used to eliminate interactions between protons and ^{31}P [425, 426]. When phytate is hydrolysed, *e.g.* by phytases, new signals appear due to the formation of phytate hydrolysis products (InsP_1 – InsP_5). Inositol pentaphosphate, the first hydrolysis product of the stepwise phytate degradation, exhibits five equally strong peaks and shows an asymmetrical molecule with phosphorylated carbon at positions 2 and 5 [423, 428]. The assignment of peaks arising from tetra- and tris-phosphates becomes increasingly difficult when different isomers are generated. Inositol mono- and diphosphates eventually formed can also be identified. Phillippy applied 2-D ^1H – ^1H -NMR to differentiate the two inositol trisphosphate isomers, $\text{Ins}(1,2,3)\text{P}_3$ and $\text{D-Ins}(1,2,6)\text{P}_3$, formed by wheat phytase hydrolysis of InsP_6 . Both inositol phosphate isomers cannot be distinguished by HPLC [429], as they coelute during chromatographic separation from the column. In this respect MNR offers a distinct advantage over HPLC analysis. Another advantage is that NMR is an excellent noninvasive analytical method with low effort for sample preparation.

It should be stressed, however, that NMR analyses are expensive and require skilled expertise to correctly interpret the complex spectra from heterogeneous biological systems such as foods.

9.9 Mass spectrometric detection

Studying inositol phosphates in cells, tissues and body fluids, concentrations <200 pmol inositol phosphates/mL or g may occur. For this purpose, the mass spectrometric determination of inositol phosphates offers excellent sensitivity.

March *et al.* reported a GC-MS method was reported for the determination of phytate [430]. It is based on the purification by anion-exchange chromatography, enzymatic hydrolysis of phytate to *myo*-inositol and consecutive derivatisation to trimethylsilyl derivative. *Scyllo*-inositol was applied as internal standard [431]. The method offers a linear calibration line of 15–500 μg phytate/L with a CV of 1.9% and a detection limit of 10 nmol phytate/L. It has been successfully applied to a variety of biological samples, such as various rat organs (kidney, liver, brain and bone), human plasma, urine and kidney stones [430]. This method is tedious and requires highly active phytases with sufficient stability.

HPLC-MS was described as another method for the determination of phytate in human urine by [432]. The method is based on hydrolysing phytate and determining the *myo*-inositol [433]. Urine was purified and InsP_6 separated by anion-exchange chromatography and acid hydrolysis of phytate performed at 120°C for 11 h. Chromatographic separation was performed on an Aminex HPX-87C column with ultra pure, deionised H_2O (18 M Ω ; Milli-Q system) as mobile phase and 5 mM ammonium acetate was added consecutively (postcolumn). The detector counted positive ions by monitoring $m/z = 198$, which corresponds to the adduct of *myo*-inositol with the ammonium cation. The RSDs obtained for standards containing 0.5, 1 and 1.5 mg phytate/L were 4.1, 3.0 and 2.7% respectively ($n = 5$). The LOD was 60 $\mu\text{g/L}$ of phytate.

Different urine samples were analysed both by this method and by the GC-MS method, described above [430]. The results of both methods were comparable, however, the HPLC-MS method is more suitable than the GC-MS method because derivatisation is avoided. Hydrolysis of phytate to inositol can be accomplished by extended acid heating but this process has to be carefully governed as *myo*-inositol easily degrades in acidic media.

Hsu *et al.* [434] applied a thermospray liquid chromatographic/mass spectrometric combination for the determination of inositol phosphates. They separated inositol phosphates (InsP_1 , InsP_3 , InsP_6) by anion-exchange chromatography on Mono-Q with ammonium formate, heated at 260°C (pH 4) and determined mass spectrometrically the dephosphorylated moiety as m/z ion 198 [NH_4^+ · inositol] $^+$. The method is sensitive with a detection limit of 100 pmol/ μL for $\text{Ins}(1,2,6)\text{P}_3$.

An inductively coupled plasma-MS (ICP-MS) by ^{31}P method [435] for phytate determination in human urine based on total phosphorus determination of separated phytate was also described. Separation of accompanying inorganic phosphates, pyrophosphates or any other phosphorus compounds from phytate is required by using anion-exchange SPE. Separation of phytate and recovery were verified in artificial urine. The linear range of the phytate determination is 20–600 μg phytate/L with a detection limit of 5 $\mu\text{g/L}$. The lack of selectivity of phosphorous compounds when determining phosphorous *via* ICP-MS can be satisfactorily overcome by selective anion-exchange separation and purification of phytate in urine.

Alternatively, an ICP atomic emission spectrometry (ICP-AES) for routine phytate analysis, also based on the determination of phosphorous, was developed [435]. This procedure also requires chromatographic separation of phytate from other phosphorous containing compounds and shows a linear working range of 0–7 mg phytate/L with a detection limit of 64 μg phytate/L and a limit of quantification of 213 μg phytate/L. This method is less sensitive than the ICP-MS [436] described above but shows sufficient sensitivity and is more suitable for routine phytate analysis in

urine. Comparison studies of both methods show consistent results ($p < 0.05$) [436].

For the discussion of the recent development in mass spectrometric determination of phytic acid the reader is referred to the interesting contribution of Cooper *et al.* [438].

9.10 Discussion and conclusions

Surprisingly, some principles of the early phytate determination by Heubner and Stadler [389] survived the last 100 years and are still being applied successfully. This is the acidic extraction of phytic acid by hydrochloric acid from dry and ground food powder to liberate phytic acid from the food matrix on the one hand, and the use of Fe^{3+} -ions for phytate detection on the other. While Heubner and Stadler applied ferric ions to precipitate phytate and by doing so determined the phytate content, Phillippy *et al.* used Fe^{3+} -ions to form soluble Fe^{3+} -inositol phosphate complexes, which could be applied for quantitative determination of inositol phosphates [15].

Graf and Dintzis [74] introduced the concentration and purification of the sample HCl extract on AG 1X8 resin. This is not only essential for the separation of inositol phosphates by RP chromatography but is also helpful in maintaining good HPLC separation of the different stereoisomers of inositol phosphates on strong anion exchangers such as Mono-Q- or CarboPac PA-100 columns. Carlsson *et al.* [438], however, showed good separation of inositol phosphates of mainly food samples on CarboPac PA-100 without purification on AG 1X8 resin but by direct injection of the ultrafiltrated sample HCl-extracts. Whether or not, this sample preparation is sufficient to prevent poisoning and blockage of HPLC columns, such as CarboPac PA-100, with high backpressure (>300 bar at ~ 1.3 ml/min), when long series of samples of complex biological matrices are analysed, remains to be clarified. Phillippy *et al.* [15] used purification by means of RP chromatography which might be an alternative for purification on AG 1 resins, and Mayr [409] applied charcoal to the purification. This charcoal treatment does not only separate cations such as iron but also phosphopeptides and nucleotides, which all strongly interfere with the determination of inositol phosphates by using the Y-PAR reaction.

Depending on the matrix, reliable determination of inositol phosphates in the long run and by using automated analysis by means of autosamplers make it desirable to purify the sample HCl extract before HPLC analysis. This is relevant for complex biological matrices, such as gastro-intestinal content, blood plasma, urine and tissues. Whether this is also true for the analysis of raw and unprocessed food, containing mainly phytate and inositol pentaphosphates, remains to be clarified. Oberleas and Harland [407, 408] recently conducted an interlaboratory comparison trial for the specific determination of phytic acid in food by separating phytic acid on a short PL-SAX column (50×4.6 mm)

and detecting it by means of the Fe^{3+} -sulfosalicylate complex (Wade reagent). They found good correlation between the results of the samples (predominantly cereal samples) among the participating laboratories [439]. This is remarkable, as after filtration ($0.45 \mu\text{m}$) the phytic acid containing HCl sample extracts after filtration were directly applied for HPLC analysis on the unguarded anion exchange column without any further purification. Unfortunately, it was not reported how many injections can be successfully applied by this method in a row, as poisoning and clogging of the column seems to be inevitable.

Excellent separation of inositol phosphates can be achieved by HPLC on strong anion exchangers such as CarboPac PA-100 (250×4 mm) (Fig. 10, above) and Omni Pac PAX-100 (250×4 mm) [411, 412] and Mono-Q (Fig. 9) [409] and Mini-Q [410]. Mono-Q with a 250 mm column (250×5 mm; $10 \mu\text{m}$ beads) shows optimum separation of the inositol phosphates at a long run of ~ 60 – 90 min, while only half of that time is required for excellent separation of inositol phosphates on CarboPac PA-100 (250×4 mm; $10 \mu\text{m}$ beads) and Mini-Q (50×4.6 mm; $3 \mu\text{m}$ beads). Currently separation on CarboPac PA-100 by anion-exchange chromatography offers the best discrimination of the different inositol phosphate isomers. However, running analysis on the CarboPac PA-100 column requires adequate equipment due to the high back-pressure of ~ 350 bar at the requested elution velocity of 1.5 mL/min. Good separation of inositol phosphates also was achieved by Schlemmer *et al.* [159] on a short Mono-Q column HR 5/5 (50×5 mm) in less than half an hour, showing good separation of InsP_6 and the four InsP_5 -isomers and sufficient differentiation of the main InsP_4 - and InsP_3 -isomers (Fig. 10, below). If more information on the inositol tetrakis- and inositol-trisphosphate isomers is needed, further gradients have to be applied and detailed analysis of the inositol mono- and inositol diphosphates requires extra separation anyway (*e.g.* by alkaline gradients). As the back-pressure on the Mono-Q column HR 5/5 does not exceed 30 bar during the run at a flow of 1.3 mL/min, many practical problems connected with high pressure analysis at $> \sim 300$ bar as frequent leaking and blockage of the column, typical for long HPLC columns (250 mm) are absent. The analysis of inositol phosphates on Mono-Q column HR 5/5 (50×5 mm) is suitable for food samples [72] as well as for most studies in human and animal nutrition and has been successfully applied in various pig studies [159, 177].

The most sensitive detection of the different inositol phosphate isomers can be achieved by the Y-PAR determination, with a detection limit of ~ 1 – 3 pmol InsP_6 [409, 410]. Although mass spectrometric detection of inositol phosphates shows comparably high sensitivity [430, 432, 436], the separation of inositol phosphate on AG 1X8 resin, with consecutive mass spectrometric detection of either the phosphorous or the inositol proportion, does not reach the excellent separation of the different inositol phosphate iso-

mers that can be achieved with Mono-Q or CarboPac 100 and subsequent Y–PAR detection.

The method of detecting inositol phosphates by using Fe^{3+} -ions [15] offers good sensitivity, with a detection limit of $\sim 0.68\text{--}2\text{ nmol InsP}_6$, along with high stability and reproducibility [159]. It is very suitable and recommendable for any food analysis [15] and many physiological samples with complex matrices such as the gastro-intestinal chyme [159] and is more sensitive than the ferric sulfosalicylate-complex detection [440].

If low phosphorylated inositol phosphates such as $\text{InsP}_1\text{--InsP}_2$ are the focus of the analysis, alkaline gradients along with conductivity detection in conjunction with anion micromembrane suppression seem to be the suitable detection. Alkaline gradients offer good separation of lower phosphorylated inositol phosphates in combination with sensitive detection in the picomole range [411, 413].

In food and nutrition research, HPLC methods for the determination of phytate and other inositol phosphates are most commonly used. Other methods such as CZE and capillary isotachopheresis [441–443] are also used but have not been widely applied. This is probably due to the lower sensitivity and discrimination of the inositol phosphate isomers compared to HPLC methods.

In order to get more information on the role of phytate in nutrition and for health protection, simple and evaluated methods for the determination of phytic acid and other inositol phosphates present in food and diets are required (see the respective discussion in Sections 2 and 3). Most probably, the HPLC separation of phytic acid and other inositol phosphates from accompanying compounds after acidic extraction of ground samples by applying anion-exchange chromatography along with a simple and evaluated detection seems to be the method of choice. Using long anion exchange columns ($250 \times 4\text{ mm}$), such as Omni Pac PAX-100 or CarboPac PA-100 [411, 412, 438], excellent separation of the inositol phosphates can be obtained (see Fig. 10, above). Using short columns (L. 50 mm), such as the PL-SAX column [439], a specific determination of phytic acid is also achievable. Although Oberleas and Harland [439] did not describe how other inositol phosphates also present in food can be discriminated from phytic acid by this method, it can be assumed that this should also be possible on this column. Using the Mono-Q column HR 5/5 ($50 \times 5\text{ mm}$) [159], another short anion exchange column, sufficient discrimination of the relevant inositol phosphates present in foods and biological samples was shown by Schlemmer *et al.* [159] (Fig. 10, below). It should be pointed out, however, that the columns need to be guarded by precolumns to protect them and to guarantee reliable analysis in the long run, independent of the fact that the samples will be just filtered or further purified by additional sample preparations prior to the HPLC separation. For quantitative detection different methods are available, preferably UV detection, as these detectors are wide spread

in laboratories. One method could be the detection by Rounds and Nielsen [408] using the Fe^{3+} –sulfosalicylate complex which was successfully applied by Oberleas and Harland in their interlaboratory comparison trial [439] or the highly sensitive determination of Mayr by means of the Yttrium–PAR complex detection [409]. Another opportunity for in-line detection of inositol phosphates is the iron detection (Fe^{3+}) of Phillippy *et al.* [405] which has shown to be sensitive along with high stability and good reproducibility [159, 405]. There might be other methods of specific, simple, fast and reliable determination of phytic acid and other inositol phosphates in foods and the future will show which method of determination and detection will be most suitable to determine phytic acid in foods and diets.

10 Final conclusions

Phytic acid is one of the most fascinating bioactive food compounds and is widely distributed in plant foods. It has different properties with varying effects for humans and animals. Due to its molecular structure, phytic acid shows a high affinity to polyvalent cations, such as minerals and trace elements, and interferes in their intestinal absorption. With unbalanced nutrition or undernourishment this may lead to serious deficiencies and is of particularly great significance for developing countries. However, with a well balanced nutrition this seems to be a less significant problem. In industrialised countries where various civilisation diseases are prevalent, the beneficial properties of phytic acid, such as its anticancer, antioxidative and anticalcification activities, are of great importance. Due to the enormous problems of civilisation diseases, any contribution to prevent these diseases is highly significant. If phytate really does show these beneficial properties in humans then phytate will be no longer considered an antinutrient. For developing countries, however, where iron and zinc deficiencies are widely spread, adequate strategies for preventing deficiencies of minerals and trace elements induced by phytate are of utmost significance. This can be done either by supplementation or by degrading food phytates or by improving the daily diet to obtain a better balanced supply of essential nutrients.

For optimum use of the beneficial phytate activities in the gut, phytate on the one hand has to be degraded to avoid inhibitory effects on the intestinal mineral absorption. On the other hand, if anticancer, antioxidative and anticalcification activities of phytate are to be used, any phytate hydrolysis would be counterproductive. This is the dilemma of phytate in human nutrition! Thus, the actual demand of a population to either improve mineral and trace element bioavailability or to help prevent cancer, kidney stone formation or other civilisation diseases, will decide whether or not phytate will be welcome in our daily diet. Terms for phytate such as ‘antinutrient’ or ‘bad food compound’ should belong to the past.

Evaluating the literature, some tasks seem to be in the focus of future research:

(i) the application of standardised and specific methods for the determination of phytic acid and other inositol phosphates in foods and other biological samples.

(ii) Additional information regarding phytic acid intake.

(iii) A better understanding of the mechanism of the gastro-intestinal absorption and cellular uptake of inositol phosphates and of the role of phytate in the metabolism and of its significance for human health.

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