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Impact of Antinutritional Factors in Food Proteins on the Digestibility of Protein and the Bioavailability of Amino Acids and on Protein Quality

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Abstract

Dietary antinutritional factors have been reported to adversely affect the digestibility of protein, bioavailability of amino acids and protein quality of foods. Published data on these negative effects of major dietary antinutritional factors are summarized in this manuscript. Digestibility and the quality of mixed diets in developing countries are considerably lower than of those in developed regions. For example, the digestibility of protein in traditional diets from developing countries such as India, Guatemala and Brazil is considerably lower compared to that of protein in typical North American diets (54–78 versus 88–94 %). Poor digestibility of protein in the diets of developing countries, which are based on less refined cereals and grain legumes as major sources of protein, is due to the presence of less digestible protein fractions, high levels of insoluble fibre, and/or high concentrations of antinutritional factors present endogenously or formed during processing. Examples of naturally occurring antinutritional factors include glucosinolates in mustard and canola protein products, trypsin inhibitors and haemagglutinins in legumes, tannins in legumes and cereals, gossypol in cottonseed protein products, and uricogenic nucleobases in yeast protein products. Heat/alkaline treatments of protein products may yield Maillard reaction compounds, oxidized forms of sulphur amino acids, D-amino acids and lysinoalanine (LAL, an unnatural nephrotoxic amino acid derivative). Among common food and feed protein products, soyabeans are the most concentrated source of trypsin inhibitors. The presence of high levels of dietary trypsin inhibitors from soyabeans, kidney beans or other grain legumes have been reported to cause substantial reductions in protein and amino acid digestibility (up to 50 %) and protein quality (up to 100 %) in rats and/or pigs. Similarly, the presence of high levels of tannins in sorghum and other cereals, fababean and other grain legumes can cause significant reductions (up to 23 %) in protein and amino acid digestibility in rats, poultry, and pigs. Normally encountered levels of phytates in cereals and legumes can reduce protein and amino acid digestibility by up to 10 %. D-amino acids and LAL formed during alkaline/heat treatment of lactalbumin, casein, soya protein or wheat protein are poorly digestible (less than 40 %), and their presence can reduce protein digestibility by up to 28 % in rats and pigs, and can cause a drastic reduction (100 %) in protein quality, as measured by rat growth methods. The adverse effects of antinutritional factors on protein digestibility and protein quality have been reported to be more pronounced in elderly rats (20-months old) compared to young (5-weeks old) rats, suggesting the use of old rats as a model for assessing the protein digestibility of products intended for the elderly.

Key words: Antinutritional factors: protein digestibility & quality

Introduction

Protein digestibility and bioavailability of amino acids, amounts and proportions of dietary indispensable amino acids (IAA) and non-essential nitrogen are basic measures in determining the quality of a protein source. Based on regional and country balance sheets, dietary surveys and protein and amino acid composition data, there are very significant differences in protein and dietary essential amino acid availabilities between the developed regions and the developing economies[1]. Protein digestibility and quality of mixed diets in developing countries are also considerably lower than of those in developed regions[1,2]. For example, digestibility values of protein in traditional diets of India, Guatemala and Brazil were 54–78 % compared to those of typical North American diets (88–94 %)[3–5].

Diets in developed economies are mostly based on highly digestible proteins of animal and vegetable origin, while those in developing economies are predominantly based on poorly digestible proteins from less refined cereals and grain legumes, which contain high levels of less-digestible protein fractions, high levels of insoluble fibre and high concentrations of antinutritional factors[3–5]. Antinutritional factors may occur endogenously or may be formed during heat/alkaline processing of proteins. Examples of major naturally occurring antinutritional factors include trypsin

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inhibitors and haemagglutinins in legumes; tannins in legumes and cereals; phytates in cereals and oilseeds; glucosinolates in mustard and canola protein products; gossypol in cottonseed protein products; and uricogenic nucleic acid bases in yeast protein products[5,6]. Examples of important antinutritional factors formed during the heat/alkaline treatments of protein products include Maillard reaction products (MRP), oxidized forms of sulphur amino acids, D-amino acids and lysinoalanine (LAL, an unnatural nephrotoxic amino acid derivative)[5]. The negative impact of antinutritional factors on protein digestibility and amino acid availability in foods has been reviewed[5]. The purpose of this manuscript is to update and extend a previously published review[5] and to include a discussion of the negative effects of antinutritional factors on protein quality of foods in addition to effects on protein digestibility and amino acid bioavailability.

Important Naturally Occurring Antinutritional Factors

Trypsin Inhibitors

Many food products including legumes, cereals, potatoes and tomatoes contain inhibitors of enzymes such as trypsin, chymotrypsin, carboxypeptidases, elastase, and α-amylase[7]. Protease inhibitors isolated from soyabean (the richest source of dietary trypsin inhibitors) fall into two main categories including the Kunitz inhibitor and the Bowman-Birk inhibitor[8]. The Kunitz inhibitors have a molecular weight of about 21·5 kDa with 2 disulphide bridges and possess a specificity directed mainly against trypsin. The Bowman-Birk inhibitors have a molecular weight of about 8 kDa with a high proportion of disulphide bonds and the capability of inhibiting chymotrypsin and trypsin at independent binding sites. Other minor variants of these 2 main types of inhibitors have been isolated and characterized which differ in the length of amino acid sequence, electrophoretic mobility, specificity and sensitivity to thermal inactivation. Inhibition by soyabean extracts of trypsin and chymotrypsin in rats and humans has been reported to be similar[8].

Levels of trypsin inhibitors in food and feed products

Data on trypsin inhibitor contents of some commonly used food and feed products are reviewed in Table 1. Soyabeans are the most concentrated source of trypsin inhibitors among common food and feed products. Contents of tryspin inhibitors (mostly the Kunitz inhibitor) in soyabeans have been reported to vary from 8·6–48·2 mg/g sample or from 20·3–122·6 mg/g protein (Table 1). This large variation in contents of trypsin inhibitors could be due to differences in cultivars and, perhaps, to use of various methods of determination. Compared to raw soyabeans, peas, and other grain legumes,

Table 1. Trypsin inhibitor content of some common soya protein and other legume protein products

Product	Trypsin inhibitor activity (mg/g sample)	Trypsin inhibitor activity (mg/g sample)	Reference
Soyabeans, raw	16·7–27·2	34·7–122·6	Anderson & Wolf[9]
Soyabeans, raw	48·2	–	Miyagi *et al.*[10]
Soyabeans, raw	8·62–18·21	20·3–51·1	Giami[11]
Soya flour, raw	28·0–32·0	57·8	Anderson & Wolf[9]
Soya flour, raw	52·1	104·2	Liener[8]
Soya flour, raw	65·8	131·6*	Radha *et al.*[12]
Soyabeans, autoclaved	3·7–8·1	15·9–21·5	Giami[11]
Soyabeans, boiled	0·9–4·0	2·2–11·7	Giami[11]
Soya flour, roasted	62·6	–	Radha *et al.*[12]
Soya flour, autoclaved	4·2	–	Radha *et al.*[12]
Soya flour toasted	3·2–7·9	–	Liener[8]
Soya flour toasted	7·9–9·4	–	Anderson & Wolf[9]
Soya protein concentrate	5·4–7·3	8·4–11·2	Anderson & Wolf[9]
Soya protein concentrate	6·3–13·7	–	Liener[8]
Soya protein concentrate	4·4–7·3	6·8–11·2	Peace *et al.*[13]
Soya protein isolate	1·2–30·0	1·4–29·4	Anderson & Wolf[9]
Soya-based infant formula	0·2–2·7	1·3–15·4	Peace *et al.*[13]
Soya tofu	0·6	9·2	Anderson & Wolf[9]
Soya tofu	1·2–3·8	–	Miyagi *et al.*[10]
Soya milk	6·3	–	Miyagi *et al.*[10]
Soya sauce	0·3	3·3	Anderson & Wolf[9]
Soya miso	4·1	22·9	Anderson & Wolf[9]
Pea (various cultivars)	2·0–12·5	–	Gatel[14]
Pea, raw	–	11·9	El-adaway[15]
Pea, boiled	–	2·1	El-adaway[15]
Beans, Indian indigenous	–	13·5–62·3	Vedivel & Janardhanan[16]
Kidney bean	4·6	–	Shimelis & Rakshit[17]
Jack bean, flour	–	2·3	Betancur-Ancona *et al.*[18]
Jack bean, protein isolate	–	0·1	Betancur-Ancona *et al.*[18]
Velvet bean, flour	–	52·8	Betancur-Ancona *et al.*[18]
Velvet bean, protein isolate	–	6·2	Betancur-Ancona *et al.*[18]

* Assuming a protein content of 50 % in soya flour.

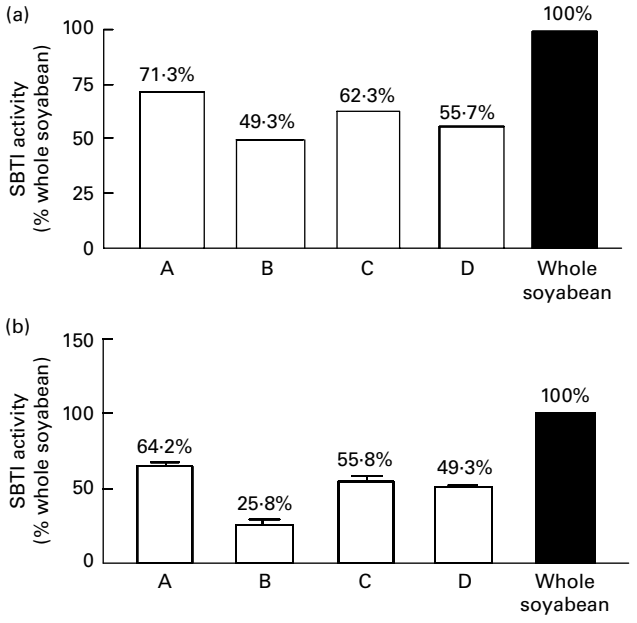

Fig. 1. Inhibition of bovine (a) and human (b) trypsin by SBTI (soyabean trypsin inhibitors) in some commercial soyabean beverages (A, B, C, and D) sold in Canada (Xiao CW, Wood CM, Robertson P& Gilani GS, unpublished data).

and properly processed soyabean products contain considerably lower levels of trypsin inhibitors.

Since the predominant trypsin inhibitors in soyabeans are located mostly with the main storage proteins in the cotyledons, they tend to fractionate with the storage proteins when soyabeans are processed into food ingredients[9]. Therefore, when soyabeans are defatted to produce raw flour, trypsin inhibitors become concentrated to levels of 28·0–65·8 mg/g sample or 57·8–131·6 mg/g protein.

Due to their proteic nature, trypsin inhibitors can be inactivated by heat processing including extrusion, infrared radiation, micronizing, boiling, autoclaving, steam processing or flaking[14] or they can be removed by fractionation[7]. The extent of heat inactivation of trypsin inhibitors depends upon a number of factors including the initial endogenous level, temperature, heating time, particle size, moisture and perhaps crop species and cultivar[7,8,14].

Most properly processed commercial soyabean products for human consumption including concentrated soyabean protein products such as concentrates (70% protein) and protein isolates (90% protein), soya-based infant formulas, soya milk and miso, have been subjected to sufficient heat treatment to inactivate up to 80% of the trypsin inhibitor activity in raw soya flour[8]. Application of prolonged heating required to destroy all inhibitor activity would adversely affect protein digestibility and quality of the soyabean products. For example, heating at 100°C for 10 min reduced trypsin inhibitor activity by about 80% and resulted in an optimal PER (Protein Efficiency Ratio) of about 3·1. However, prolonged heating to further reduce trypsin inhibitor activity resulted in a lower PER of about 2·9[19].

In the absence of regulatory upper safe limits of dietary trypsin inhibitors, there is no guarantee that each and every commercial product would be properly processed and, consequently, would contain minimal residual levels of trypsin inhibitors, which may vary greatly with the extent of heat and other processing conditions used in the preparation of soyabean products. For example, several commercial soya beverages have been reported to contain significant levels of residual trypsin inhibitor activity (up to 71 and 64% of that of whole soybeans) against bovine and human trypsin, respectively (Fig. 1a and 1b; Xiao CW, Wood CM, Robertson P, Gilani GS, unpublished data). Similarly, some soya-based infant formulas have been reported to retain up to 28% of the trypsin inhibitor activity[13]. An outbreak of gastrointestinal illness in individuals who had consumed an unprocessed soya protein extender in tuna fish salad documented the fact that inadequately processed soya products can find their way into the human food chain[8].

Antinutritional effects of trypsin inhibitors

The feeding of raw soyabean protein preparations or extracted inhibitors from soyabeans caused an enlargement of the pancreas in susceptible animals[7]. Similarly, feeding of a soya protein isolate has been reported to significantly increase pancreatic weight of male and female rats but to significantly reduce their spleen weight (Fig. 2). Exposure to soyabean trypsin inhibitors results in increased synthesis and secretion of proteases (such as trypsin, chymotrypsin and elastase)

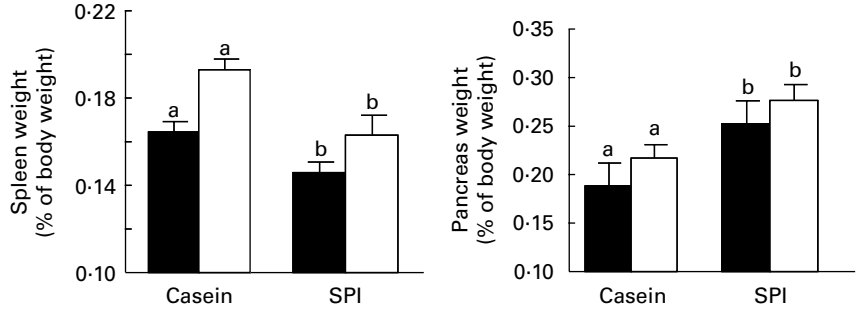

Fig. 2. Effects of feeding casein (control) and SPI (soya protein isolate) for 90 days on spleen and pancreas weights of female and male rats (Data were abstracted from Huang *et al.*[20]). [a,b] Means for spleen or pancreas weights between the two dietary treatments (casein and SPI) in the same gender of rats with unlike superscripts differ significantly ($P<0·01$).

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Table 2. Effects of feeding raw soyabean flour (Nutrisoy) and autoclaved Nutrisoy on the ileal digestibility (%) of protein and selected amino acids in growing pigs*

Product	Nutrisoy	Autoclaved Nutrisoy
Protein	37[a]	77[b]
Arginine	45[a]	90[b]
Histidine	44[a]	83[b]
Isoleucine	40[a]	86[b]
Leucine	37[a]	86[b]
Lysine	41[a]	80[b]
Methionine†	59[a]	86[b]
Cysteine	35[a]	68[b]
Phenylalanine	39[a]	88[b]
Tyrosine	34[a]	85[b]
Threonine	36[a]	73[b]
Valine	38[a]	84[b]

[a,b] Means in the same row between the two diets with unlike superscripts differ significantly ($P<0{\cdot}01$).
* Data were abstracted from Li *et al.*[21]. Two maize starch-based diets were fed which contained 200 g/kg diet from either Nutrisoy (a food-grade defatted soya flour containing active trypsin inhibitors) or autoclaved Nutrisoy (containing reduced amounts of trypsin inhibitors).
† Digestibility after correction for dietary supplementation of methionine.

and pancreatic hypertrophy and hyperplasia in animal models[7]. The increased secretion of proteases supported the suggestion that the growth depression caused by trypsin inhibitors was the consequence of an endogenous loss of amino acids in the form of enzymes being secreted by a hyperactive pancreas. Trypsin and chymotrypsin are particularly rich in sulphur-containing amino acids. Therefore, the effect of a hyperactive pancreas would be to divert these amino acids from the synthesis of body tissue proteins to the synthesis of enzymes, which are subsequently lost in the faeces[7].

Protein and amino acid digestibility and protein quality have been reported to be negatively affected in animal models by the presence of high levels of dietary trypsin inhibitors and other antinutritional factors from soyabeans, kidney beans and other grain legumes[5]. The effects of feeding a food-grade defatted soya flour (Nutrisoy) and autoclaved Nutrisoy on protein and amino acid digestibility were studied in growing pigs[21]. The trypsin inhibitor activities in the Nutrisoy and autoclaved Nutrisoy diets were 13·4 and 3·0 g/kg, respectively. The ileal protein and amino acid digestibility in the diet based on unheated Nutrisoy were about 50 % lower compared to those in the diet based on autoclaved Nutrisoy (Table 2).

The influence of thermal processing (raw; home processing, boiling in hot water at 100°C for 10 min; and commercial canning) on amino acid digestibility in red kidney beans (*Phaseolus vulgaris* L.) has been investigated[22]. Digestibility was determined as true faecal digestibility in rats fed red kidney beans as the sole source of dietary protein in diets containing 10 % protein. The raw bean diet containing the highest amounts of antinutritional factors had the lowest amino acid digestibility values. The digestibility values for the first limiting amino acid (methionine + cystine) and valine in the raw bean diet were negative, whereas the values for other IAA, arginine and histidine ranged from 4–32 % (Table 3). Home processing and commercial canning substantially improved the digestibility of amino acids. The beneficial effect of home processing was more pronounced than that of commercial canning (Table 3). The lower amino acid digestibility values for the canned beans compared to those for the home processed beans would suggest an adverse effect of severe heat treatment used during canning on amino acid digestibility, especially that of the first limiting amino acid, methionine + cystine.

In lentils and beans, the true faecal digestibility of limiting amino acids such as methionine (41–60 %), cystine (0–75 %), tryptophan (47–76 %), and threonine (62–77 %) were substantially lower than the digestibility of protein (72–86 %) (Table 4). This would suggest that protein digestibility may not be a good approximation of the bioavailability of amino acids in grain legumes.

The presence of trypsin inhibitors in legumes such as soyabean not only has adverse effects on protein and amino acid digestibility, it also negatively impacts on protein quality. For example, three breeding lines of soyabean developed in Nigeria in raw form that contained 20·3–51·1 mg/g trypsin

Table 3. Effects of processing on true rat faecal digestibility (%) of selected amino acids in red kidney beans*†

Amino Acid	Raw Kidney beans	Home-cooked Kidney beans	Canned Kidney beans
Arginine	28[a]	88[c]	78[b]
Histidine	32[a]	86[c]	80[b]
Isoleucine	12[a]	83[c]	76[b]
Leucine	4[a]	86[c]	74[b]
Lysine	27[a]	85[c]	75[b]
Methionine + cystine	−19[a]	68[c]	40[b]
Phenylalanine + tyrosine	8[a]	85[c]	79[b]
Threonine	11[a]	78[c]	73[b]
Tryptophan	13[a]	84[c]	63[b]
Valine	−8[a]	82[c]	68[b]

[a,b,c] Digestibility values within a row with unlike superscript letters among the three diets were significantly different ($P< 0{\cdot}05$).
* Data were abstracted from Wu *et al.*[22]. Diets were formulated to contain 10 % protein. A protein-free diet was fed to estimate metabolic faecal amino acids; used in the calculations of true digestibility.
† Treatments: raw, uncooked dry beans; home-cooked beans (boiled in water, 100°C for 120 min); canned, commercially canned beans, Progresso; Casein, ANRC casein.

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Table 4. Values (%) for the true digestibility of protein and limiting amino acids in lentils and beans, as determined by the rat balance method*

Product	Protein	Met	Cys	Thr	Trp
Lentils (canned)	84	41	40	77	73
Kidney bean (canned)	81	44	0	74	76
Pinto bean (canned)	79	45	56	72	70
Pinto bean (autoclaved)	80	61	59	68	58
Seafarer bean (autoclaved)	84	60	72	77	74
Black bean (autoclaved)	72	51	46	62	47
Fababean (autoclaved)	86	59	75	76	53

* Data were abstracted from Sarwar & Peace[23].

inhibitors had true faecal protein digestibilities of 47–58% and had negative PER values (−0·46–0·88) compared to a PER value of 2·47 for a casein control (Table 5). Boiling (for 30 min) was more beneficial than autoclaving (for 20 min) in reducing levels of trypsin inhibitors (2·2–12·7 vs. 15·9–22·5 mg/g protein), and in improving true faecal protein digestibility (75–93 vs. 60–70%) and PER values (1·35–2·30 vs. 0·91–1·33) (Table 5). An early study in humans showed that intake of raw soyabean meal supported 20% lower nitrogen retention compared to that of heated soyabean meal[24].

Tannins

Tannins are naturally occurring water-soluble polyphenolic compounds with the ability to complex and precipitate proteins in aqueous solutions[25]. Their molecular weights range from 0·5 to 3 kDa, and they are present in various plant species including cereal grains and legume seeds. The chemistry, occurrence in food and feed products, and antinutritional properties of tannins in monogastric animals have been reviewed[26,27].

Tannins are generally classified into hydrolysable and condensed tannins[25]. The hydrolysable forms are readily hydrolyzed by acids, alkalis, and some enzymes, while the condensed tannins, mainly polymerized products of flavan-3-ol (catechin) and flavan-3,4-diol or mixture of these, are resistant to hydrolysis. The condensed tannins which are also referred to as flavolans or procyanidins, are the main polyphenols present in commonly consumed food products while the hydrolysable forms are present only in small amounts[26].

Amounts of tannins in foods and feeds

The limited published data on tannin contents of various foods and feedstuffs are summarized in Table 6. Among important food and feed products, sorghum, millet, various types of beans and peas may contain considerable amounts of tannins (up to 72 g/kg) (Table 6). Some forage crops such as browse legumes may contain even higher amounts of tannins (up to 111 g/kg). Sorghum is an important food crop in many parts of Africa, Asia and the semi-arid tropics worldwide due to its drought resistance[30]. Similarly, other cereal crops and beans and peas, which are high in tannins, form the main sources of nutrients in diets of people living in the economically disadvantaged countries of the world. Therefore, the total dietary intake of tannins in these countries could be considerable. The prevalence of protein malnutrition in these countries would further aggravate the antinutritional effects of dietary tannins. In general, tannins are resistant to heat[27]. Several technological treatments have been studied to reduce tannin contents of food and feed products including sorghum and fababean[27]. These included dehulling, soaking in water or alkaline solutions, addition of chemicals with a high affinity for tannins such as polyvinylpyrrolidone and polyethylene glycol or gelatin and germination. However, most of these processing treatments appeared to be rather laborious, expensive or ineffective[27]. More recently, genetic manipulation has been successful in producing fababean cultivars containing highly reduced levels of tannins[28].

Antinutritional effects of tannins

While tannins protect the grains against insects, birds and fungal attacks, this agronomic character is accompanied with reduced nutritional quality[30]. It is well known that tannins are potential protein precipitants and they reduce protein and amino acid digestibility in animals fed tannin-containing cereals such as sorghum, and grain legumes such as field beans and fababean[31]. It is believed that under optimal

Table 5. Effects of processing on contents of trypsin inhibitors, protein digestibility and protein quality of three soyabean breeding lines developed in Nigeria*

Soyabean line	Trypsin inhibitors (mg/g protein)	True faecal protein digestibility (%)	Protein Efficiency Ratio (PER)
TG-923-2, raw	20·3^{c}	58^{a}	−0·88^{a}
TG-923-2, autoclaved	15·9^{b}	70^{b}	1·33^{b}
TG-923-2, boiled	2·2^{a}	93^{c}	2·30^{c}
TG-1019-2, raw	51·1^{c}	48^{a}	−0·46^{a}
TG-1019-2, autoclaved	22·5^{b}	60^{b}	0·91^{b}
TG-1019-2, boiled	9·8^{a}	75^{c}	1·38^{c}
TG-1497-1	46·6^{c}	47^{a}	−0·46^{a}
TG-1497-1	21·5^{b}	61^{b}	0·92^{b}
TG-1497-1	12·7^{a}	75^{c}	1·35^{c}

^{a,b,c} Means for trypsin inhibitor contents, true protein digestibility or PER within each soyabean line bearing unlike superscript letters were significantly ($P<0·05$) different.

* Data were abstracted from Giami[11]. The trypsin inhibitor contents, true protein digestibility and PER of the casein control diet were 0·00, 95 and 2·47, respectively.

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Table 6. Contents of Tannins in some food and feeds products

Product	Tannin content, (g/kg)	Source of data
Chick pea (*Cicer ariterium*)	0·6–2·7	Jansman & Longstaff[27]
Cowpea (*Vigna sinensis*)	1·4–10·2	Jansman & Longstaff[27]
Pea (*Pisum sativum* L.)	0·6–10·5	Jansman & Longstaff[27]
Pigeonpea (*Cajanus cajan*)	3·8–17·1	Jansman & Longstaff[27]
Winged bean (*Psophocarpus tetragonolobus*)	0·3–7·5	Jansman & Longstaff[27]
Dry beans (*Phaseolus vulgaris* L.)	0·3–12·6	Jansman & Longstaff[27]
Kidney beans (*Phaseolus vulgaris* L.)	5·3–17·55	Shimelis & Rakshit[17]
Faba bean (*Vicia faba* L.)	0·1–6·6	Krepon *et al.*[28]
Faba bean (*Vicia faba* L)	0·5–24·1	Jansman & Longstaff[27]
Sorghum (*Sorghum vulgare*)	0·5–72·0	Jansman & Longstaff[27]
Barley (*Hordeum sativum*)	5·5–12·3	Jansman & Longstaff[27]
Finger millet (white seed)	0·3–0·8	Salunkhe *et al.*[26]
Finger millet (brown and dark brown seed)	5·7–20·0	Salunkhe *et al.*[26]
Browse legumes	0·0–110·7	Jansman & Longstaff[27]
Sal (*Shoree robusta*) seed meal	26·0	Ahmed *et al.*[29]

conditions, sorghum tannin is capable of binding and precipitating at least 12 times its own weight of protein[31].

The influence of feeding 20 sorghum cultivars varying in tannin (catechin equivalent) content of 0 to 3·88 to caecectomized cockerels on amino acid digestibility has been studied[32]. There were significant overall inverse relationships between tannin content and the mean digestibility of IAA or dispensable amino acids. It was also noted that sorghum varieties with similar tannin contents may vary greatly in their amino acid digestibility. The quantity of α-Kafirin (low digestibility principal storage protein) in various sorghum varieties was also inversely related to the amino acid digestibility, and may explain the somewhat inconsistent relationship between tannin content and amino acid digestibility in sorghum[32].

Addition of increasing amounts of tannin-rich fababean hulls to a casein diet resulted in a linear decrease in the apparent rat faecal digestibility of total ($r^2 = 0{\cdot}97$) and individual ($r^2 = 0{\cdot}27$ to $0{\cdot}99$) amino acids[25]. The digestibility of most IAA was affected to a lesser degree than that of some of the dispensable amino acids, particularly proline, glycine and glutamic acid (Table 7). It was hypothesized that the marked reduction in digestibility of these dietary dispensable amino acids was primarily due to the interactions of tannins with the proline-rich proteins that were secreted by the parotid gland, as these three amino acids make up 73% of the weight of isolated proline-rich proteins from parotid-glands of tannin-fed rats[25].

Table 7. Influence of the addition of varying amounts of tannins extracted from fababean on apparent rat faecal digestibility values for amino acids in casein*

Dietary Tannins	0·0%	1·32%	1·99%
Total amino acids	91[c]	74[b]	60[a]
Lysine	93[c]	87[b]	71[a]
Threonine	89[c]	77[b]	54[a]
Arginine	90[c]	62[b]	43[a]
Histidine	94[c]	82[b]	60[a]
Glutamic acid	93[b]	74[a]	71[a]
Glycine	79[c]	−29[a]	−20[b]
Proline	96[b]	50[a]	52[a]
Serine	87[c]	73[b]	57[a]

[a,b,c] Means for amino acid digestibility among the three diets bearing unlike superscript letters were significantly ($P < 0{\cdot}05$) different.
* Data were abstracted from Jansman *et al.*[25].

In a dose-response study, increasing the amount of dietary tannin-rich fababean hulls (providing 0·0 to 1·99% tannins as catechin equivalents) caused a linear increase in both the relative size of the parotid glands in the rat (multiple regression correlation coefficient, $r^2 = 0{\cdot}90$) and the quantity of proline-rich proteins in the glands ($r^2 = 0{\cdot}89$)[25]. Similarly, the consumption of high tannin sorghum or purified condensed tannins was shown to increase specifically the size of the parotid gland and the synthesis and secretion of proline-rich proteins in rats[33]. It was suggested that proline-rich proteins are secreted in the saliva and are bound to dietary tannins in the oral cavity to protect dietary protein. Binding of tannins to both dietary and endogenous proteins (such as digestive enzymes and proteins located at the luminal side of the intestinal tract) has been used to explain reduced protein and amino acid digestibility in tannin-containing diets[25,33].

Phytic acid

Phytic acid (*myo*-inositol hexaphosphoric acid) is a naturally occurring substance in the plant kingdom[34]. This is mainly found in seeds, grains and nuts, where it functions as a source of mineral nutrients and inositol to be used during germination[35]. Collectively known as phytate, phytic acid is typically present in plant tissues as salts of mono- and divalent cations such as Mg, Ca, Na and K. Phytate accumulates primarily in the protein-rich aleuronic layers of monocotyledonous seeds, while it is distributed uniformly throughout the kernels in dicotyledonous seeds, including oilseeds and grain legumes[36]. The chemistry, occurrence and antinutritional effects of phytate have been reviewed in detail[37].

Phytate, with its abundance of negatively charged phosphate groups, is best known to chelate several nutritionally essential nutrients in the gastrointestinal tract of humans and animals, making them less bioavailable[38]. Phytate interferes with zinc homeostasis and also negatively impacts on the bioavailability of other nutrients including proteins. Phytate can negatively influence the activity of digestive enzymes,

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such as carboxypeptidases and aminopeptidases, by the chelation of mineral cofactors or interaction with the protein (either enzyme or substrate). Binding of phytate to proteins may be direct (phytate: protein) or indirect (through a cation bridge). These complex interactions vary with pH, time, and relative concentrations. At low pH (for example in the stomach), phytic acid forms electrostatic linkages with the basic arginine, lysine and histidine residues resulting in insoluble complexes. Above its isoelectric point, a protein carries a negative charge, and multivalent cation bridging (typically involving calcium) appears to be involved in the formation of a complex between phytate and proteins[36]. Phytate:cation: protein interaction would be expected to dominate at the higher pH found in the small intestine[39]. Another indirect mechanism of phytate inhibition of digestive enzyme activity measured *in vitro* was suggested to involve complex interactions among phytate, digestive enzymes and other proteins present in the solution[40].

Phytic acid contents of food and feed products

Phytate contents of a large number of foods have been previously summarized[34,38]. The data on phytate contents of some commonly consumed food and feed products are summarized in Table 8. Phytate is present in seeds, grains and nuts at concentrations of up to several percent on a dry weight basis. Due to its uneven distribution within grains, most of the phytate can be found in the germ of corn, the bran of wheat and the pericarp of rice[38]. Processing treatments that remove or concentrate these portions can, therefore, result in dramatic changes in the phytate content of the finished product. For example, rice polishings, wheat bran and middlings and defatted meals of oilseeds (soyabean, cottonseed, sunflower and canola) contain substantial amounts of phytates (up to 5·5 per cent (Table 8). In pulses (peas, lentils and beans), the greatest proportion of the phytate is found in edible cotyledons. Therefore, mechanical processing does not result in much reduction in phytate content. Moreover, phytate is relatively heat-stable, with only a small fraction being destroyed during heat processing, while the phytase enzymes that might break down the phytate are heat labile[44]. However, extrusion (with a central temperature and moisture content of 150°C and 20 g/100 dry weight, respectively) of hard-to-cook common beans was reported to reduce phytate contents by about 20–30 % (Table 8).

Antinutritional effects of phytate on protein digestibility and quality

Phytic acid has been reported to interfere with the proteolytic action of pepsin in a number of vegetable and animal proteins as determined *in vitro*, possibly through the formation of phytate:protein interaction complexes at low pH[45]. Phytate has also been reported to inhibit trypsin activity in some studies but not in all investigations. Addition of phytate to multienzyme proteolytic assay systems was shown to significantly (up to 25 %) inhibit *in vitro* digestion of casein[46]. Fermentation treatment of finger millet to decrease phytate content by 23–26 % caused a 14 to 26 % improvement in *in vitro* digestibility of the finger millet protein using pepsin and pancreatin[47].

The effects of phytate on protein and amino acid digestibility and protein quality have involved studies of phytase supplementation to production rations for animals such as poultry and swine. In such studies, microbial phytase is added to animal rations to degrade phytate, improve phosphorous bioavailability and reduce the environmental impact

Table 8. Phytic acid contents of some common food and feed products

Product	Phytic acid (g/kg)	Phytic acid (g/kg protein)	Reference
Rice, white, polished	3	–	Harland & Oberleas[34]
Rice, wild	22	–	Harland & Oberleas[34]
Rice polishing	55	377	Ravindran *et al.*[36]
Wheat	6	53	Ravindran *et al.*[36]
Wheat middlings	28	170	Ravindran *et al.*[36]
Wheat, bran	34	–	Harland & Oberleas[34]
Sorghum	7	101	Ravindran *et al.*[36]
Sunflower meal	27	89	Ravindran *et al.*[36]
Corn	7	88	Ravindran *et al.*[36]
Cottonseed meal	33	78	Ravindran *et al.*[36]
Canola meal	26	72	Ravindran *et al.*[36]
Soyabeans	26	–	Harland & Oberleas[34]
Soyabean meal	32–41	62–78	Chitra *et al.*[41]
Soyabean flours	10–20	20–37	Anderson & Wolf[9]
Soya protein isolates	10–20	11–22	Anderson & Wolf[9]
Soya milk	17	–	Anderson & Wolf[9]
Tofu	15–29	–	Anderson & Wolf[9]
Common bean (*P. vulgarus*), unextruded	8–11	459–578	Batista *et al.*[42]
Common bean (*P. vulgarus*), extruded	7–8	351–423	Batista *et al.*[42]
Chick pea	5–12	29–47	Chitra *et al.*[41]
Pigeonpea	7–17	29–72	Chitra *et al.*[41]
Mung bean	10–15	45–57	Chitra *et al.*[41]
Urd bean	13–15	46–54	Chitra *et al.*[41]
Lentils	7	27	Porres *et al.*[43]
Cashew nuts	20	–	Harland & Oberleas[34]

of high phosphate effluent from intensive pig and poultry units[39]. This environmental problem prompted the development and acceptance of microbial phytase feed enzymes for monogastric animals.

While the primary function of phytase added to animal feeds was to improve the availability of phytate-bound phosphorous, it has provided new insights into the antinutritional properties of phytates. The interaction of phytate with proteins may have substantial practical and economical significance in animal nutrition[39].

Studies on the effects of phytase supplementation in improving protein and amino acid digestibility and protein utilization in pigs and poultry have been reviewed[39]. Only a few studies have been reported on the influence of phytase supplementation on the ileal digestibility of amino acids in pigs. Differences in methodology make comparisons among these studies rather difficult, but an increase of 3–10 % in the ileal digestibility of at least some amino acids has been reported which appears to correlate with the magnitude of improved growth rates and protein retention reported by others[39].

Phytase supplementation has been reported to significantly improve the digestibility of protein and amino acids in 5-wk old broilers fed a number of feedstuffs including three cereals (corn, sorghum, and wheat) and four oilseed meals (soybean meal, cottonseed meal, canola meal and sunflower meal) and two cereal by-products (wheat middlings and rice polishings)[36]. In the latter study, apparent ileal protein digestibility increased by 2–6 % in different diets due to phytase supplementation of 1200 FTU/g kg diet[36]. One FTU (unit of phytase) is defined as the quantity of enzyme that releases 1 μmol of inorganic phosphorus/min from 0·00 015 mol/L sodium phytate at pH 5·5 at 37°C. The mean apparent ileal digestibility of 15 amino acids increased by 2–9 % with phytase addition to various diets. For individual IAA, average improvements across various feedstuffs tested were 4–8 % with the greatest improvement being noted for threonine and valine[36]. Dietary phytate concentration was negatively correlated with the inherent protein digestibility ($r = -0{\cdot}81$, $P<0{\cdot}001$) and amino acid digestibilities ($r = -0{\cdot}85$, $P<0{\cdot}001$) of the feedstuffs tested.

Supplementation of a marginally (91 % of the recommended level) lysine-deficient diet (wheat-soyabean meal-sorghum) for broiler chicks with increasing levels of microbial phytase significantly improved (3–5 %) the apparent ileal digestibility of protein and amino acids[48]. Statistically significant linear dose responses were noted for the digestibility of protein and all amino acids using phytase supplementation levels up to 1000 FTU/kg diet. Phytase supplementation also increased apparent metabolizable energy in that study, attaining a plateau effect at 750 FTU/kg diet[48].

The beneficial effects of phytase supplementation on the protein and amino acid digestibility of various feedstuffs for poultry and swine could be due to the release of protein from protein-phytate complexes occurring naturally in feedstuffs, the prevention of formation of binary and tertiary protein-phytate complexes within the gut, the alleviation of the negative impact of phytate on digestive enzymes, and the reduction in endogenous amino acid losses[39].

Uricogenic nucleobases

Foods rich in nucleic acids, which elevate serum uric acid levels are restricted in the diets of hyperuricaemic individuals. This restriction has been based on the amount of total dietary nucleic acids or all purines, assuming no differences in uricogenic effects of individual purines (adenine, guanine, hypoxanthine and xanthine). However, in spite of their biochemical similarity, purines are metabolized differently and produce different uricogenic effects in animals and humans[49]. Adenine and hypoxanthine were reported to be more uricogenic than guanine and xanthine. Moreover, free adenine was shown to exert a greater uricogenic effect than its nucleoside or nucleotide when fed to animals. Addition of adenine to a casein control diet also exerted a negative effect on its protein quality as demonstrated by reduced rat growth and increased serum urea nitrogen concentrations, and resulted in a nephrotoxic condition including the deposition of crystals in the renal tubules causing damage to the epithelial tissues[6]. The differences in metabolic effects of individual purines, and modification of the amount and form of purines caused by processing would suggest that the uricogenic potential of processed foods should be based on the nature and quantity of dietary proteins.

The main food sources of nucleic acids are meats, leguminous seeds, some types of seafood, some vegetables and food yeasts[6,50]. Among animal foods, organ meats are considered to be a rich source of nucleic acids. The use of new non-traditional protein sources such as inactive food yeasts may lead to increased consumption of nucleic acids and uricogenic bases. Inactive dried food yeasts, their autolysates and co-precipitates are increasingly used as functional ingredients in various foods and are also offered for sale as food supplements. Due to their low cost, these protein-rich products have been commercially promoted for various nutritional applications. Various food yeasts may contain 8 to 12 % nucleic acid and 0·7 to 1·0 % adenine, depending upon the organism and the growth conditions used. The Protein-Calorie-Advisory Group (PAG) of the United Nations System has recommended that a safe limit of nucleic acid from single cell protein products is 2 g/d and that the total nucleic acids from all dietary sources should not exceed 4 g/d for adults[51]. The use of inactive dried food yeasts at low levels (3–5 %) for functional purposes would not pose any problems if the yeasts contained not more than 8 % nucleic acids[52]. However, their use as a major protein source without significant reduction in their contents of nucleic acids, would have negative health effects including hyperuricaemia and inferior protein quality[6].

Important Antinutritional Factors Formed During the Processing of Foods

Maillard reaction products (MRP)

During thermal processing and the storage of foods, proteins undergo chemical changes leading to a reduction of their nutritional value. This may happen in the case of Maillard reactions which occur between reducing sugars and lysine

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in various products including dairy foods, eggs and cereals[53]. During the "early" Maillard reaction, lysine is modified into fructose or lactuloselysine (in dairy products), which are both biologically unavailable. Under severe heat treatments, "blocked lysine" generates reactive carbonyls which may react with other amino acids or polymerize into brown melanoidins, adversely affecting nutritional parameters other than lysine. Furosine {*e*-*N*-(furosylmethyl)-L-lysine) is related to the early stage of MRP such as fructoselysine, lactuloselysine, and lysinoalanine[54]. However, hydroxymethylfurfural and fluorescence are indices of the presence of intermediate and advanced Maillard reaction[55].

The metabolism and antinutritional effects of MRP have been reviewed mainly in rats[56]. Limited human studies relate to the effects of MRP on protein digestibility and utilization[57]. The effects of diets with different MRP contents on dietary protein utilization were investigated in adolescent males aged 11–14 y, as they are nutritionally at risk because of their high physiologic needs and dietary habits[57]. MRP are widely consumed by this population, mostly as a result of their high intake of fast foods and snacks. In a 2-period crossover trial, healthy adolescent males were randomly assigned to two diets (the brown diet and the white diet). The brown diet was rich in MRP and the white diet was low in MRP contents (hydroxymethylfurfural: 3·87 and 0·94 mg/kg; fluorescence intensity: 20·04% and 7·31%, respectively; the two indicators of Maillard reaction). Subjects collected urine and faeces on the last three days. Fasting blood samples were collected after both periods. Compared with consumption of the white diet, consumption of the brown diet resulted in a 47% higher faecal nitrogen excretion, 12% lower apparent nitrogen absorption, and a 6% lower nitrogen digestibility (Table 9). The apparent nitrogen retention and utilization of the ingested nitrogen did not differ significantly between the two diets, although values supported by the white diet tended to decrease. It was concluded that the consumption of a diet rich in MRP negatively affects protein digestibility and that the possible long term effects of an excessive intake of MRP during adolescence warrant further investigation[57].

Table 9. Comparative dietary nitrogen utilization in adolescent males aged 11–14 y during crossover dietary treatments with white and brown diets*

Parameter	White diet	Brown diet
Nitrogen intake (mg kg^{-1} d^{-1})	245 ± 13[b]	227 ± 9[a]
Faecal nitrogen excretion (mg kg^{-1} d^{-1})	23 ± 3[a]	33 ± 3[b]
Urinary nitrogen excretion (mg kg^{-1} d^{-1})	145 ± 10	130 ± 6
Nitrogen absorption (mg kg^{-1} d^{-1})	222 ± 11	194 ± 8
Apparent nitrogen digestibility (%)	91[b]	85[a]
Nitrogen retention (mg kg^{-1} d^{-1})	64 ± 11	52 ± 8

[a,b] Means between the two diets bearing unlike superscript letters were significantly ($P < 0.05$) different.

* Data were abstracted from Seiquer *et al.*[57]. All values are means ± SE, n = 18. The subjects consumed the white diet (low in MRP) and the brown diet (rich in MRP) for 14-d periods with a 40-d washout period. The white diet was essentially free of foods in which the Maillard reaction develops during cooking practices (i.e. frying, toasting, or roasting) or foods that naturally contain MRP (e.g. bread crust, chocolate or coffee). The brown diet was rich in processed foods with an evident development of browning, which therefore were high in MRP. This diet contained corn flakes, baked potatoes, chocolates, fried foods, toasted foods and breaded foods. Both the diets were balanced and adjusted to meet the nutritional requirements of this age group and to maintain the eating patterns of the subjects as much as possible.

Different processing conditions used in the preparation of infant formulas such as sterilization, spray-drying, and treatment at ultra-high temperatures were reported to cause strong protein-protein and protein-lipid interactions in processed milk[58]. These interactions were more pronounced in conventionally (in-can) sterilized than in spray-dried (powdered) and UHT (ultra heat treated) products demonstrating their temperature dependency. Analysis of raw materials, intermediate and end products revealed that in-can sterilization caused irreversible denaturation of proteins. *In vitro* analysis demonstrated significantly lower protein digestibility of in-can sterilized infant formulas compared to their spray-dried and UHT counterparts[58]. The amount of "available lysine" was lower in sterilized than in powdered infant formulas demonstrating the higher occurrence of MRP in the sterilized form. Similarly, values for true digestibility of protein, lysine, and protein quality (measure as relative PER) in liquid concentrates were up to 21% lower than those in powders (Table 10). Lower levels of digestible lysine in liquid concentrates compared with powders prepared by the same manufacturer would suggest that inferior protein quality of liquid concentrates may be due to more heat treatment involved in their preparation[59]. Compared with the preparation of powders, the preparation of liquid concentrates involves more heat treatment including sterilization at ~122–132°C for 5–8 minutes (higher temperature being associated with shorter time) in the presence of high levels of moisture, lysine-containing proteins and lactose (reducing sugar). Therefore, lower protein and lysine digestibility and inferior protein quality of liquid concentrates compared with powders prepared by the same manufacturer would suggest more protein damage by Maillard reactions in liquid concentrates compared to powders[59]. This observation was supported by the lower values for plasma lysine in rats fed liquid concentrates compared with those fed powders. The plasma amino acid data suggested that the liquid concentrates were first limiting in lysine for rat growth[59].

The nutritional consequences of feeding milk containing a high level of lactulose-lysine to pigs has been studied[53]. Pigs fed an equal amount of dried skim milk (SM) either lyophilized or heat treated to obtain an intense Maillard reaction (M-SM) resulting in a 50% lysine blockage. Pigs were fitted, under anaesthesia, with permanent catheters in the portal vein, carotid artery and urethra. In M-SM containing 50% blocked lysine, no other amino acid was chemically modified. Fructoselysine appeared very late in blood compared to amino acids, resulting from a very slow release and corresponded to 8·2 and 18·6% of the ingested amount after 12 and 72 h, respectively. Significant differences in the appearance of amino acids in the portal blood were observed only for lysine (−60%), alanine (−17%) and cystine (+37%). A small decrease in the faecal digestibility of most amino acids during the same period was observed, which was significant after 48 h for lysine, phenylalanine, cystine, aspartic acid, glycine and total amino acids (−6%). The loss in protein

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Table 10. True faecal protein digestibility (rat) of protein and lysine and protein quality of selected milk-based infant formulas fed to rats*

Formula	Protein digestibility (%)	Lysine digestibility (%)	Relative PER (%)†	Digestibility (%)
Casein + methionine (control)	98[d]	98[f]	100[e]	80
Powder-1	94[c]	96[e]	76[c]	76
Liquid Concentrate-1	88[a]	87[b]	52[a]	52
Powder-2	93[c]	93[d]	98[e]	76
Liquid Concentrate-2	88[a]	85[a]	85[d]	61
Powder-3	97[d]	98[f]	88[d]	79
Liquid Concentrate-3	90[b]	86[a,b]	83[d]	63
Liquid Concentrate-4	90[b]	89[c]	64[b]	60

[a-f] Means (n = 8) in each column bearing unlike superscript letters differ significantly ($P<0{\cdot}05$).
* Data were abstracted from Sarwar *et al.*[59].
† PER (Protein Efficiency Ratio) for the casein diet was 4·73.

nutritive value was mostly due and proportional to the deterioration of lysine and, to a lesser degree, to the decrease in the digestibility of other IAA. Taking into account the very high level of lactuloselysine in the M-SM sample, it may be concluded that in common foods such as milk, biscuits, bread and pasta containing lower levels of blocked lysine, the nutritional loss is mainly associated with the loss of digestible lysine and to a lesser extent to the decrease in digestibility of other IAA[53].

Protein-bound D-amino acids and lysinoalanine (LAL)

Exposure of food proteins to heat and/or alkaline treatments results in two major chemical changes including racemization of amino acids to D-enantiomers and concurrent formation of LAL[60]. Formation of D-amino acids in proteins is pH-, time- and temperature dependent. The degree of racemization caused by the processing conditions varies from protein to protein but the relative order of the degree of racemization of the individual amino acids within different proteins shows a high level of similarity (Table 11).

The chemistry, nutritional quality and safety of D-amino acids have been reviewed[61,62]. The presence of D-amino acids in proteins leads to impaired protein and amino acid digestibility and nutritional quality. The nutritional utilization of different D-amino acids varies widely in animals and humans[61].

D-amino acid contents of some common food products

Data on D-amino contents of foods have been recently reviewed[62]. Although some insects, worms and marine invertebrates contain substantial quantities of D-amino acids, such organisms are not major components of the human diet. However, in communities where marine shellfish is an important source of food, the antinutritional implications of D-amino acids must be taken into account[62]. Milk, meat and various grains do not contain substantial quantities of D-amino acids. However, during the course of preparation for consumption, processing treatments are applied which may give rise to racemization. The influence of processing treatments on racemization of amino acids was investigated by determining D-aspartic acid concentrations[63]. In this study, raw milk contained the lowest level of D-aspartic acid (1·48 %) but quantities of D-aspartic acid increased with the extent of processing. For example, fat-free milk powder, kefir, evaporated milk, yoghurt and milk-based infant formulas contained 2·15, 2·44, 2·49, 3·12 and 4·95 % of D-aspartic acid, respectively[63]. A D-aspartic acid content of 31 % was found in casein heated at 230°C for 20 min[62]. Similarly, high ratios of D-aspartic acid have been reported in textured soya protein and bacon (up to 13 %), alkaline/heat treated soya protein (up to 27·7 %), and alkaline/heat treated zein (up to 40·2 %)[62,64]. Among common food products, substantial quantities of D-aspartic acid were reported in crackers

Table 11. D-amino acid composition (%) of eight alkali/heat treated protein sources*

Amino Acid	Casein	Lactalbumin	Wheat gluten	Zein	Fish	Soyabean	Bovine albumin	Haemoglobin
Serine	41	47	42	44	42	44	43	44
Cystine	–	32	32	44	23	21	23	30
Methionine	25	32	33	30	29	24	30	26
Threonine	29	29	30	36	33	28	28	31
Phenylalanine	24	24	24	32	28	25	28	30
Aspartic acid	29	23	26	42	25	31	27	19
Glutamic acid	20	19	32	35	19	21	18	20
Tyrosine	15	19	19	35	16	14	15	23
Alanine	15	14	19	22	19	16	22	17
Lysine	8	7	9	8	11	11	13	10
Leucine	7	5	7	8	7	6	8	7
Isoleucine	3	3	4	5	4	4	6	5
LAL†	4·4	5·4	0·9	0·3	2·8	3·2	8·5	4·4

* Data were abstracted from Friedman[60]; Conditions: 0·1 N NaOH; 75°C; 3 h.
† Mixture of (LD + LL) lysinoalanine isomers in grams per 16 g of N.

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made from wheat flour (9·5 %), Mexican pancake (11·6 %) and corn cake (15·4 %)[65]. In mature cheeses, D-aspartic acid, D-alanine and D-glutamic acid were reported to be up to 68 %, suggesting that foods which have undergone microbial fermentation would contain substantial quantities of D-amino acids[66]. In general, the IAA do not racemize rapidly unless exposed to high temperatures[62].

LAL-amino acid contents of some common food and feed products

LAL (an unnatural amino acid derivative) is formed when proteins are subjected to an alkaline treatment. It is formed mainly by the addition of an ϵ-amino group of lysine residue to the double bond of a dehydroalanine residue that has been generated by the β-elimination reaction of cystine, phosphoserine, or glycoserine residue[64]. The amount of LAL formed in processed protein products is dependent upon temperature, concentration of alkali, time of exposure to alkali, type of protein, and type of cations in the solution[67]. Heat treatment under non-alkaline conditions was also reported to form LAL in a variety of proteins[67].

Although protein-containing food and feed products are commonly subjected to alkaline/heat treatments, information on the amounts of LAL found in food products that are part of the every day diet are limited. The published data vary widely according to the treatments applied. The most representative results are summarized in Table 12. Domestic cooking may produce up to 1820 ppm LAL in foods (such as sausage, chicken meat and eggs) initially free of LAL. Commercial food products that have undergone processing treatments may contain LAL in variable amounts according to the type of product and certainly the conditions of preparation including alkaline/heat treatment. Sterilized milk and milk powders have been reported to contain up to 1160 ppm LAL. Special attention has been paid to the high LAL contents of sterilized liquid concentrate infant formulas which may contain as much as 2120 ppm LAL. Since such formulas are often the sole source of protein for infants over a long time period, it has been recommended that the LAL content of infant formulas be kept under <200 ppm[69]. Protein-rich ingredients such as dried egg white, soya protein isolate and sodium caseinates have been reported to contain significant amounts (up to 6900 ppm LAL) (Table 12). Among the common food products, whipping agents are known to contain the highest levels of LAL, i.e. up to 53 150 ppm LAL.

Table 12. Levels of lysinoalanine (LAL) in some common food products

Food product	LAL, ppm	Reference
Frankfurter	0–170	Finot[68]
Chicken meat	370	Finot[68]
Eggs	160–1820	Friedman[69]
Corn chips	390	Friedman[69]
Pretzels	220–500	Friedman[69]
Polished rice	1000	Finot[68]
Infant formula, powdered	150–920	Finot[68]
Infant formula, liquid concentrate	160–2120	Friedman[69]
Evaporated milk	150–860	Finot[68]
Milk powders	150–620	Finot[68]
Sterilized milk	200–1160	Finot[68]
Soya protein isolate	370–1300	Friedman[69]
Sodium caseinate	430–6900	Friedman[69]
Whipping agents	6500–53150	Finot[68]; Friedman[69]

Antinutritional effects of protein-bound D-amino acids and LAL

Protein-bound D-amino acids formed during processing, especially at alkaline pH, may have adverse effects on protein digestibility and the quality and safety of processed foods. When absorbed, D-amino acids may be made utilizable by the action of racemases or epimerases or D-amino acid oxidases[60]. The amino acid oxidase system (which varies in amount and specificity of oxidases in different animal species) may become saturated when foods containing high concentrations of D-amino acids are consumed. Proteins containing D-amino acids can be hydrolyzed at peptide bonds containing L-amino acids. However, the hydrolysis rates may be slower than those for corresponding unprocessed proteins. These changes adversely affect the nutritional quality and safety of foods by generating biologically nonutilizable forms of amino acids, creating D-D, D-L, and L-D peptide bonds partly or fully inaccessible to proteolytic enzymes. Moreover, these racemized proteins may compete with proteins that do not have racemized amino acids for the active site of digestive proteinases in the gut and thus adversely affect the biological utilization of the unracemized proteins. The slower absorption of free and peptide-bound D- compared with L-amino acids may result in decreased protein digestibility[70]. It is not known whether D-amino acid containing oligopeptides can change the microflora of the digestive tract. Wide variations in the biological utilization of free D-methionine by various animal species have been reported[60]. Free D-methionine was well utilized by mice and rats but was poorly utilized by humans when consumed either orally or during total parenteral nutrition.

The formation of D-amino acids and LAL and their influence on protein digestibility has also been studied[71]. Three protein sources (casein, β-Lactoglobulin and wheat protein) were subjected to heat and alkaline treatments (heating for 6 or 24 h at 65°C, pH 10·5–11·5). Treatment of these proteins for 24 h increased levels of D-amino acid residues. For example, about 11–15 % of L-asparagine and aspartic acid, the most susceptible amino acids, were racemized in the three protein sources[71]. Moreover, the alkaline/heat treatment resulted in increased amounts of LAL, and about 10–12 % of the total lysine was converted to LAL in these protein sources. True ileal protein digestibility of the treated casein, β-lactoglobulin and wheat protein, decreased by 13, 14 and 17 %, respectively, when fed to minipigs[71]. Similarly, the heat/alkaline treatment of casein caused significant reductions (up to 17 %) in digestibility of aspartate, serine and glycine. However, digestibility of other amino acids was not affected. This study provides evidence that even small amounts of D-amino acids and LAL within a protein can impair protein digestibility. This significant impairment was already present at a level of 8 %

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D-asparagine + aspartic acid as found in treated β-lactoglobulin (24 h at 65°C, pH 10·5)[71].

The health concerns of LAL are twofold: LAL formation in foods results in a loss of the IAA, lysine, cysteine and threonine, and reduced protein digestibility; and secondly in kidney damage[72,73]. Nephrocytomegaly (i.e. enlarged kidney cells) and nephrokaryomegaly (enlarged nuclei) are unique lesions induced in rats by LAL. The amount of dietary LAL needed to produce nephrocytomegaly depends on whether LAL is fed as the free amino acid or bound in a protein. Enlarged nuclei have been reported in rats by feeding as little as 1200–1400 ppm protein- bound LAL[74,75]. In comparison, feeding of free LAL was found to produce nephrocytomegaly at much lower levels (100–250 ppm)[76,77]. L-lysyl-D-alanyl-LAL, the most potent isomer of the four optical isomers of LAL, induced nephrocytomegaly when fed at levels as low as 30 ppm, whereas D-lysyl-D-alanyl-lysinoalanine did not produce lesions at less than 1000 ppm[78]. LAL, which is a strong chelator of mineral nutrients such as calcium, iron, copper and zinc, may exert its toxic effect by metal binding in renal tubule cells. The human kidney is more susceptible to damage by LAL than that of several animal species[69]. The altered mineral (iron and copper) status due to consumption of LAL from processed foods was demonstrated in one of the first *in vivo* studies in rats[79]. Liver and kidney iron levels were greatly reduced which is of particular concern, as this is the mineral in which status is often already reduced in the elderly and infants, persons likely to consume sole-source foods. The susceptibility of humans to the nephrotoxic effect of LAL is unknown. Because nephrotoxic effects were not observed after long-term feeding of alkali-treated soya protein to baboons, consumption of low amounts of dietary LAL is probably safe for human consumption[69] However, further analytical data are needed for assessment of the actual quantity of LAL ingested[72]. In addition, the influence of chronic consumption of alkaline-treated foods high in LAL on the balance of copper and other minerals should be examined.

It has been recommended that the LAL content of infant formulas, which are often the sole source of protein for some infants, should be kept at <200 ppm[69]. Significant amounts of LAL were found in infant formulas and formulated liquid diets sold in Canada. The liquid concentrate forms of milk-based infant formulas and commercially available formulated liquid diets were found to contain up to 1200 and 2400 ppm of LAL in the formula/diet protein, respectively (Gilani GS, unpublished data). It is not known whether growing children and infants are more sensitive to the adverse effects of LAL than are adult humans.

To establish an NOAEL (no observed adverse effect level) for dietary LAL, a 16-wk rat study investigating the effect of alkaline-treated soya protein isolate (to provide 0·05, 0·10, 0·20 and 0.30 % of dietary LAL) on growth, protein and mineral status were investigated (Gilani GS, L'Abbé MR, unpublished data). Body weights of rats fed a casein control, and 0·05, 0·10, and 0·20 % LAL diets were 522 ± 25^{a}, 500 ± 22^{a}, 370 ± 20^{b} and 280 ± 16^{c} g, respectively. Growth data suggested that the NOAEL for protein-bound LAL would probably be between 0·05 and 0·10 % of diet. Alkali-treated proteins containing LAL have lower digestibility compared to untreated proteins. For example, an inverse relationship between the LAL content of casein and the extent of *in vitro* proteolysis by trypsin has been demonstrated[80]. The biological utilization of LAL as a source of lysine in a mouse growth assay was only 3·8 % that of crystalline lysine[81]. Similarly, LAL was completely unavailable as a source of lysine to the rat but it was 37 % bioavailable to the chick[82]. The alkaline treatment of soya protein reduced protein digestibility from 97 to 83 % in rats, and lowered body weight gain in baboons[69]. Similarly, alkaline/heat treatment had significant negative effects on the true faecal protein digestibility of lactalbumin (99 vs. 73 %) and soya protein isolate (96 vs. 68 %) in rats[79]. The protein quality of these protein sources as predicted by rat growth was also significantly reduced (Table 13). The treated proteins contained considerably higher levels of LAL compared to the untreated proteins. The amount of LAL in the treated lactalbumin was more than two-fold higher than in the treated SPI (soya protein isolate). As expected, the formation of LAL in the treated proteins was associated with a loss of lysine (19–20 %), cystine (73–77 %), and serine (18–30 %). There was also a loss of threonine (35–45 %) in the treated proteins (Table 13). Most other amino acids were not greatly affected by the alkaline treatment of the two proteins. Since the methodology used did not distinguish between D- and L-isomers of amino acids, the influence of the processing conditions on the formation of D-amino acids could not be determined in this study[79]. Exposing soyabean protein isolate to alkaline conditions (pH 8–14) for various time periods (10–480 min),

Table 13. Selected amino acids, true faecal protein digestibility (rat) and protein quality of untreated and alkaline/heat-treated lactalbumin and soya protein isolate (SPI)*

Amino acid, (g/100 g protein)	Lactalbumin, untreated	Lactalbumin, treated†	SPI, untreated	SPI, treated†
LAL	0·1	4·42	0·03	1·94
Lysine	10·5	8·35	6·08	4·94
Cyst(e)ine	2·81	0·76	1·16	0·27
Threonine	5·73	3·13	3·98	2·57
Serine	4·93	3·46	5·12	4·16
True Protein digestibility§	99	73	96	68
RPER (Casein + Met = 100)§	89	0	56	0

* Data were abstracted from Sarwar *et al.*[79].
† Protein sources were subjected to alkaline treatment with 0·1 N NaOH at room temperature for 1 h, followed by heat treatment at 75°C for 3 h, neutralization with 10 N HCl to pH 7·5, with ultrafiltration to remove salts and spray drying of the ultrafiltrate retentate.
§ Determined in rats; RPER (relative protein efficiency ratio) values were calculated using the following equations: RPER = [PER of test diet/PER of control diet] × 100; where PER (protein efficiency ratio) = weight gain of test rat/protein consumed by test rat.

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and temperatures (25–95 at 19°C intervals) has been reported to destroy all of the cystine and part of the arginine, lysine, serine and threonine residues[69]. These losses were accompanied by the appearance of LAL and unidentified ninhydrin-positive compounds, and the formation of D-amino acids. Therefore, possible causes for the reduction in protein digestibility and quality following heat/alkaline treatment may include destruction of lysine, threonine, cystine and arginine, isomerization of L-amino acids to less digestible D-isomers, formation of inter- and intra-molecular cross-links, and inhibition of proteolytic enzymes[69,79].

Protein digestibility and quality of some enteral products based on caseinates and SPI were inferior to casein[83]. For example, the true faecal protein digestibility of five commercial enteral products, as determined in rats, was significantly lower than that of casein (89–92 vs. 95%). The enteral products also contained higher levels of LAL than casein (998–2333 vs. 0 μg/g protein). Since the formation of LAL was also reported to occur in a variety of proteins under non-alkaline conditions[69], the lower protein digestibility of the enteral products could be explained by the presence of LAL in these products[83]. Similarly, heat treatment at pH 12·2 was reported to cause a significant reduction in protein digestibility and NPU (net protein utilization) of casein and soyabean[69].

In comparing the protein nutritional values of milk-based infant formulas sold in Europe, the formation of LAL was found to be one of the most sensitive predictors of protein damage in infant formulas caused by heat processing[84]. It was found that liquid forms of milk-based formulas contained up to ten times more LAL than powder forms; suggesting more severe heat treatment in the preparation of liquid forms compared to powders[84]. The question as to whether either D-amino acids and D-amino acid-containing peptides or cross-links such as LAL alone are responsible for lower protein digestibility, or whether these effects are additive remains unresolved. Both racemization and cross-links were shown to inhibit proteolysis and to decrease protein and peptide digestibility with *in situ* experiments using isolated loops of rat intestine[85]. The adverse effects of D-amino acids and LAL formed during heat processing of proteins may have considerable implications in animal nutrition. For example, feeding roller-dried milk powder to calves may impair their growth. Some NaOH and/or heat treated feedstuffs may contain considerable amounts of D-amino acids and LAL, for example, as a consequence of alkaline detoxification of aflotoxins. The diminished nutritive value for a racemized, heat and alkali-treated dietary protein may also have relevance for human nutrition. This reduction in protein nutritional value may not only be due to a reduction in L-amino acid content but also of a diminished overall digestibility. Even if many IAA amino acids are not racemized to D-isomers and would, after breakdown of the protein, be available for absorption, adjacent D-amino acids might nevertheless interfere with their absorption[86]. The effect of adjacent amino acids on the bioavailability of methionine in some tripeptides found in casein and soya protein has been examined[87]. The relative bioavailability of methionine (L-methionine = 100) in

Table 14. Net protein ratio (NPR) and methionine bioavailability values for some Synthetic tripeptides containing L- and D-methionine (Met)*

Diet	NPR	Methionine Bioavailability (%)
Basal	1·17	–
Basal plus L-Met (reference)	3·82[a]	100[a]
Basal plus D-Met	3·81[a]	100[a]
Basal plus tripeptides		
Ala-Met-Ala	3·05[b]	71[b]
Ala-D-Met-Ala	2·37[d]	45[d]
Val-Met-Phe	2·71[c]	58[c]
Val-D-Met-Phe	1·09	0
Thr-Met-Arg	2·64[c]	55[c]
Thr-D-Met-Arg	1·15	0
Thr-Met-Lys	1·13	0

† Means (n = 8); the NPR of the casein + Met diet was 5·60, suggesting that the experimental conditions of the feeding study were properly controlled.
[a-d] Means within the same column bearing unlike superscript letters differ significantly ($P<0·05$).
* Data were abstracted from Sarwar *et al.*(1985).

Ala-Met-Ala; Ala-D-Met-Ala; Valine-Met-Phe; Val-D-Met-Phe; Thr-Met-Arg; Thr-D-Met-Arg; Thr-Met-Lys was determined using a rat growth method (NPR, Net Protein Ratio) as the performance index. Crystalline D-met was completely bioavailable to the growing rat, but in the tripeptide form, D-Met (0–45%) was markedly less bioavailable than L-Met (38–71%) (Table 14). The bioavailability of Met was also influenced by the side chain of the adjacent amino acids. Met in the tripeptides with the bulky amino acids (Val-Met-Phe, Thr-Met-Arg, Thr-Met-Lys) was less bioavailable than in those with lighter amino acids (Ala-Met-Ala). D-Met in the tripeptides with bulky amino acids (Val-D-Met-Phe, Thr-D-Met-Arg) was completely unavailable for rat growth. The lower bioavailability of D-met when compared to L-Met in Ala-Met-Ala or Val-Met-Phe (Table 14) may be due to lower hydrolysis (by peptidases of the brush border membrane of the intestinal mucosa) of the respective peptide containing D-Met than that containing L-Met[86]. These findings suggested that protein-bound D-Met (formed during processing) may be considerably lower in bioavailability than protein-bound L-Met. Moreover, bioavailability of protein-bound Met may be influenced by the amino acids adjacent to Met in the polypeptide chain[87].

Effect of age on the digestibility of protein in products containing antinutritional factors

Protein Digestibility-corrected Amino Acid Score (PDCAAS) has been recommended to be the most suitable method for routine evaluation of protein quality of foods by FAO/WHO[88]. The PDCAAS method includes the use of young rats for predicting the protein digestibility of foods for all ages including the elderly. The possible age-related differences in protein digestibility were investigated by determining the true faecal protein digestibility of 15 products in 5-wk and 20-mo old rats[89]. Each protein product was fed as the sole source of 10% dietary protein. A protein-free diet was also included to obtain an estimate of metabolic faecal protein. True fecal protein digestibility values (corrected for metabolic faecal protein loss on a protein-free diet) in old

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rats were significantly ($P<0{\cdot}05$) lower than in young rats for most products. These differences were, however, small (up to 3%) for properly processed animal products (casein, whey protein concentrate, whey protein hydrolysate, lactalbumin and skim milk powder). Similarly, the differences due to age were not large (up to 5%) for properly processed vegetable protein products (soya protein isolate and autoclaved soyabean meal, black beans and fababean). However, digestibility values in old rats were considerably lower (7–17%) than in young rats when fed products containing antinutritional factors. These included mustard protein flour containing glucosinolates, alkaline/heat treated soya protein isolate and lactalbumin containing D-amino acids and LAL, raw soyabean meal and black beans containing trypsin inhibitors and heated skim milk powder containing MRP. Therefore, the inclusion of protein digestibility data obtained using young rats in the calculations of PDCAAS may overestimate protein digestibility and the quality of these products for the elderly. For products specifically designed for the elderly, protein digestibility should be determined using old rats, but the result needs to be confirmed in humans.

The digestibility of protein is considered a good approximation for the bioavailability of most amino acids in mixed diets and of properly processed products which contain minimal amounts of residual antinutritional factors[88]. However, there often are quite large differences between the digestibility of protein and the individual amino acids, especially in coarse cereals and grain legumes and in those products which contain antinutritional factors present naturally or formed during processing[2] or storage[90]. Therefore, there may be a need to include corrections for the bioavailability of individual amino acids and not just for the digestibility of protein in calculating PDCAAS values of such products. The effects of age on the bioavailability of individual amino acids have not been studied. It is, however, quite possible that differences due to age in the bioavailability of individual amino acids may be even larger than the digestibility of protein in products containing antinutritional factors. Trypsin and chymotrypsin are the major pancreatic serine proteases secreted into the duodenum during the digestion process. An elevation in their concentration at the ileum, caused by the presence of antinutritional factors in under-processed beans and peas, could suggest enhanced pancreatic secretion, reduced protein hydrolysis and amino acid absorption and/or enhanced protein secretion or loss from the gastrointestinal tract and leakage from the blood caused by the assault on the gut lining by the dietary constituents[91]. Although the effect of age on losses of endogenous proteins at the terminal ileum has not been studied, the lower protein digestibility in old rats compared to young rats fed diets containing antinutritional factors (Table 15) would perhaps suggest lower biological adaptability to the dietary nutritional insults in old rats than in young rats[92].

It is generally recognized that the abilities of rats and humans to digest a variety of foods are similar[88]. However, human studies are needed to validate the adverse effect of age on protein digestibility of products containing antinutritional factors, as reported in rats[89].

Table 15. Effect of age of rat on true faecal protein digestibility for some animal and vegetable protein products*

Product	Protein digestibility (%) (5-wk old rat)	Protein digestibility (%) (20-mo old rat)
Casein	99[a]	96[a]
Whey protein concentrate	100[a]	97[a]
Lactalbumin (ALBM)	98[a]	96[a]
ALBM, alkaline/heat treated	71[e]	64[f]
Skim milk powder (SMP)	93[b]	92[b]
SMP, heated	79[d]	70[e]
Soya protein isolate (SPI)	95[b]	93[b]
SPI, alkaline/heat treated	66[f]	49[g]
Soyabean meal, autoclaved	81[c,d]	78[d]
Soyabean meal, raw	80[c,d]	72[e]
Black beans, autoclaved	83[c]	78[d]
Black beans, raw	71[e]	60[g]
Fava beans, autoclaved	82[c,d]	77[f]
Mustard protein flour	92[b]	79[d]

[a-f] Within each column, digestibility values (attributed to source of protein) with unlike superscript letters differ significantly ($P<0{\cdot}05$).
* Data were abstracted from Gilani & Sepehr[89].

Conclusions

The presence of high levels of naturally occurring antinutritional factors such as trypsin inhibitors in legumes; tannins in legumes and cereals; and/or phytates in cereals have been reported to cause substantial reductions in protein and amino acid digestibility values (up to 50%) and protein quality (up to 100%) in animal models. Similarly, high levels of antinutritional factors formed during heat and/or alkaline processing of proteins such as Maillard reaction compounds, D-amino acids and/or LAL can reduce protein digestibility by up to 28% in rats and pigs, and can cause marked reduction (up to 100%) in protein quality as assessed by rat growth methods.

Due to their proteic nature, trypsin inhibitors in soyabeans (the most concentrated source of trypsin inhibitors; about 17–27 mg/g sample or 35–123 mg/g protein) can be readily reduced by heat processing, and the application of optimal heat processing can inactivate up to 80% of the trypsin inhibitor activity in commercial soya protein products used for human consumption such as protein concentrates and isolates, soya-based infant formulas and soya-based beverages. However, in the absence of regulatory upper safe limits for dietary trypsin inhibitors, the application of proper processing to obtain minimal residual levels in each and every product is not guaranteed. For instance, the residual levels of trypsin inhibitor activity in several commercial soya beverages were up to 71 and 64% of that of whole soyabeans against bovine and human trypsin, respectively. Moreover, some commercial soya-based infant formulas retained up to 28% of their trypsin inhibitor activity.

Some important food crops such as sorghum, millet, various types of beans and peas, which are staple sources of nutrients for populations in the economically disadvantaged countries, may contain substantial amounts of tannins (up to 72 g/kg). The prevalence of protein malnutrition in these regions

would further aggravate the negative effects of tannins on protein and amino acid digestibility. Although processing treatments such as dehulling, soaking in water or alkaline solutions, addition of chemicals with a high affinity for tannins and germination have been studied to reduce tannin contents of sorghum and fababean, these treatments were found to be cost ineffective. However, recent genetic improvements have enabled the production of fababean cultivars with significantly reduced contents of tannins.

The Maillard reaction commonly occurs during the thermal processing or storage of foods that are rich in protein and reducing sugars and it produces several compounds that contribute to the aroma, colour and flavour of cooked foods. Since controlled browning is used in roasting, baking, frying and other food technology processes, the MRP are widely consumed as a part of the human diet. The formation of MRP causes significant reductions in protein digestibility and bioavailable lysine and protein quality of the liquid concentrated forms (subjected to in can sterilization) of milk-based infant formulas in rats. Similarly, the consumption of a diet rich in MRP has been reported to negatively affect protein digestibility in adolescent males, aged 11–14 y.

Commercial food products may contain variable amounts of LAL based on the type of product and the extent of heat and/or alkaline heat processing. LAL formation in foods results not only in the loss of some IAA like lysine, cysteine and threonine but it also reduces protein digestibility and causes kidney damage. Dried egg white, soya protein isolate and sodium caseinates have been reported to contain up to 6900 ppm LAL. Sterilized milk-based liquid infant formulas have been reported to contain up to 2120 ppm of LAL, while the NOEL for dietary LAL has been suggested to be between 500 and 1000 ppm.

The negative effects of antinutritional factors on protein digestibility were more pronounced in elderly rats (20-months old) compared to young (5-weeks old) rats. For example, protein digestibility values in elderly rats for mustard flour (containing glucosinolates), alkaline/heat treated soya protein isolate and lactalbumin (containing D-amino acids and LAL), raw soyabean meal and black beans (containing trypsin inhibitors) and heated skim milk powder (containing MRP) were 7–17 % lower than in young rats. This would suggest the use of old rats as a model for assessing protein digestibility of products intended for the elderly.

In mixed diets and properly processed products which contain minimal amounts of residual antinutritional factors, the digestibility of protein is generally considered a good approximation for the bioavailability of most amino acids. However, in coarse cereals and grain legumes and in those products which contain antinutritional factors (either present naturally or formed during processing) there are quite large differences (up to 81 %) between the digestibility of protein and individual limiting IAA. Therefore, there may be a need to include corrections for the bioavailability of individual IAA and not just for the digestibility of protein in calculating PDCAAS values of such products. Although the effect of age on the bioavailability of individual amino acids has not been investigated, it is quite possible that the differences due to age in the bioavailability of individual amino acids may be even greater than the observed effect on the digestibility of protein in products containing antinutritional factors.

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