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REVIEW

Safety of protein hydrolysates, fractions thereof and bioactive peptides in human nutrition

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This paper evaluates the safety for humans with regard to consumption of protein hydrolysates and fractions thereof, including bioactive peptides. The available literature on the safety of protein, protein hydrolysates, fractions thereof and free amino acids on relevant food legislation is reviewed and evaluated. A new concept for the safety assessment of protein hydrolysates and fractions thereof is developed. Benchmarks for the evaluation are safety of total protein intake, safety of free amino acid intake, documented history of safe use, outcome of questionnaires in efficacy studies and safety studies. Similar to the intake of intact proteins with a history of safe use, the intake of hydrolysates made from them, does not raise concern about safety, provided the applied proteolytic enzymes are food grade and thus of suitable quality. The safety of hydrolysates and of fractions thereof, including the so-called bioactive peptides, should always be assessed by the company before market introduction (company safety assessment). Only when a novel protein source is used or a novel production process is applied, which results in significant changes in nutritional value, metabolic effect or increased level of undesirable substances, that products might fall under novel food regulations. This means that company safety assessment should be reviewed and approved by external independent experts (external safety evaluation) and the novel protein hydrolysate (fraction) is authorized by competent authorities before market introduction. It is argued that good judgement on the safety of hydrolysates and the fractions thereof can be obtained by comparing the anticipated intake of amino acids by these products with those levels to be reasonably expected to be ingested under normal conditions of consumption of a balanced and varied diet. The paper shows a decision tree that can be used for safety assessment.
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Introduction

Technological developments in food processing and advancements in separation technology have resulted in the widespread use of hydrolysates of dietary proteins and/or fractions thereof in feed and food. In the scope of this paper we define hydrolysates as mixtures of polypeptides, oligopeptides and amino acids that are manufactured from protein sources, using partial hydrolysis. Hydrolysis is achieved by using food grade proteolytic enzymes, heat or suitable acids and alkalies.

The 'crude' hydrolysates may undergo further processing, such as heat inactivation, (ultra)filtration, centrifugation, precipitation, concentration and drying. For the dairy sector, the world annual market for hydrolysates derived from whey protein, milk protein and casein has been estimated at 8000, 4000 and 3000 MT, respectively (Affertsholt, 2007). In human diets these products, including bioactive peptides, are often used as ingredients in specific nutritional products, such as hypoallergenic infant formulas and functional foods, such as sports beverages. Various traditional protein sources, including milk, fish, meat, collagen, egg, pea, soy, rice and potato are being used for the preparation of protein hydrolysates.

This paper reviews and evaluates current knowledge about this issue in order to assess conditions that should be fulfilled to ensure safety. A legal issue is whether or not (and if so when) protein hydrolysates and fractions thereof belong to a category of foods for which specific legislation exist, similar to the European Novel foods legislation and comparable legislation in the United States of America, Canada, Australia, China and Japan. This legislation requires an

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external evaluation (and approval) of the safety assessment by independent experts before the product is authorized by competent authorities to enter the market (Commission of the European Communities, 1997, 2008; Rodrigues, 2008).

Any food business operator is responsible for the safety of food that he introduces on the market. The General Food Law (Commission of the European Communities, 2002) lays down definitions, principles and obligations covering all stages of food/feed production and distribution. For the safety assessment of any newly developed food, it is generally useful to focus this evaluation on the differences with comparable traditional foods that have a documented history of safe use. For the safety evaluation of protein hydrolysates and fractions thereof, it appears appropriate to use the following benchmarks:

- safety of protein intake
- safety of free amino-acid intake
- documented history of safe consumption
- safety-related data obtained from efficacy studies
- outcome of safety studies
- safety aspects of production processes, such as processing aids, source materials and process conditions (Madden, 1995).

Safety of protein intake

During history of mankind different protein consumption patterns have been practiced. Most human diets contain animal-, vegetable- and microbial proteins. Wide variations in protein intake above the level of the average minimum requirement of good-quality protein (about 0.6 g/kg body weight per day for normal healthy adults (Institute of Medicine, 2002)) are compatible with good health. It is possible to remain healthy while living exclusively on a meat diet when the meat contains sufficient fat so that protein intake is less than 40% of energy. Consumption of more than 40% of energy as protein may be toxic and cause a condition known as 'rabbit starvation', referring to the very low-fat content of rabbit meat (Garlick, 2001). According to the Institute of Medicine in the US (2002), there is insufficient evidence to support the hypothesis that high protein diets are associated with chronic diseases, including osteoporosis, renal insufficiency and cardiovascular disease. The Institute defines an acceptable range of protein intake for adults as 10–35% of energy intake. Others (Eisenstein *et al*., 2002) are more reluctant by stating that consumption of protein greater than two to three times the US Recommended Daily Allowance (more than 20–30% of energy intake) contributes to urinary calcium loss and may in the long term predispose to bone loss. Caution with such diets is also recommended in those individuals who may predispose to nephrolithiasis or kidney disease.

Adverse reactions to dietary proteins are allergy and celiac disease (gluten intolerance). Food allergy occurs in probably less than 2% of the adult population and in 3–7% of the children, and the prevalence of gluten intolerance is estimated at 1% of the population (Lessof, 1994; European Food Information Council, 2006). In case of allergy, failure of the digestion and/or barrier function of the gut, absorption of food protein fractions results in hypersensitivity reactions. Typical proteins involved are present in eggs, milk, shellfish, nuts, peanuts, soybean and rice. Celiac disease, the adverse reaction to the gliadin fraction of gluten from wheat, is associated with atrophy of intestinal villi, leading to malabsorption of nutrients and adverse reactions of the immune system. Before proteins, that do not have a history of safe use in human nutrition (novel food proteins), are allowed on the market, attention should be given to potential allergenicity in the safety assessment (Meredith, 2005).

The use of (partial) protein hydrolysates instead of intact proteins, in the so-called hypoallergenic infant formulas, has been shown to effectively reduce or even prevent allergic reactions (see below). Risk management measures for allergic patients are included in the food legislation of many countries. Information on the product label enables allergy sufferers to make adequate choices.

Safety of amino acids

The increasing use of free amino acids in dietary supplements and foods led to questions being raised by health authorities with regard to their safety. This was particularly the case in the United States after the occurrence of the eosinophilia myalgia syndrome in individuals who had used L-tryptophan supplements (which appeared later to result from impurities). Therefore, two different groups of experts, one in the United States (FASEB, Federation of American Societies for Experimental Biology, 1992) and one in the Netherlands (Health Council in the Netherlands, 1999) evaluated the safety of free amino acids. The safety of free amino acids was also evaluated in the United States by the Institute of Medicine (2002). The Federation of American Societies for Experimental Biology committee concluded that insufficient data are available to judge the safety of (single free) amino acids in food supplements. For none of the 22 amino acids, a safe upper limit could be established. Also the Institute of Medicine and the Dutch expert committee arrived at this conclusion. The latter committee recommended to comply to maximum acceptable levels of free amino acids in supplements and foods. These maximum levels correspond to amounts of amino acids in the Recommended Daily Allowance of protein for adult women. As a reference, a protein mixture was taken of casein and soy protein in a ratio of 2:1, simulating the ratio between animal and vegetable protein in Dutch diets. A special case was made for methionine. According to the Dutch expert committee, this amino acid should not be ingested in free form, because it may contribute to undesirably increased plasma levels of homocysteine. However, there is no safety

Table 1 Recommended maximum quantities (g/day) for extra amino-acid consumption by supplements or food enrichment

Indispensible	*Maximum*	*Dispensible*	*Maximun*
Histidine	1.2	Alanine	2.1
Isoleucine	2.9	Arginine	2.3
Leucine	5	Asparagine + aspartic acid	4.4
Lysine	3.7	Cysteine	0.5
Methionine	Not permitted	Glutamine	5.5
Phenylalanine	2.6	Glutamic acid	5.1
Threonine	2.3	Glycine	2
Tryptophan	0.6	Proline	4.5
Valine	3.1	Serine	3.3
		Tyrosine	2.5

Health Council in the Netherlands recommends that isoleucine, leucine and valine (branched chain amino acids) should only be permitted in combination, using proportions indicated in this table, and should not be consumed separately.

reason to forbid the presence of methionine in peptides and protein fractions when its content does not exceed the normal level in intact proteins. For this, an 'average' content of 2.9% (Sarwar and Peace, 1994) could be used, as in a 2:1 mixture of casein and soy. Table 1 shows the proposed maximally allowed quantities for amino acid consumption by supplements or food enrichment.

An important difference in the metabolism between amino acids from intact dietary proteins and free amino acids may result from their rate of entry into the circulation, which will be faster for free amino acids (Bilsborough and Mann, 2006). This rate of entry of free amino acids is also dependent on the composition of the mixture of free amino acids as a consequence of the different amino acids that compete for the same transporters. Free amino acids as such cannot be stored in the body. Therefore in the postprandial phase, body protein degradation is decreased and protein synthesis is increased (mediated by increased insulin levels) to create a temporally positive N-balance. If the net protein synthetic capacity in the postprandial phase is insufficient to compensate for the increased entry of free amino acids, amino-acid oxidative pathways will be stimulated to keep the plasma amino-acid levels within an acceptable safe range (Erlandsen *et al*., 2003). Thus, an optimal efficiency of amino-acid utilization requires that the postprandial amino-acid appearance rate does not exceed the net protein synthesis capacity of the human body (Nolles, 2006). Protein synthesis requires the availability of all needed essential amino acids in sufficient quantities within protein synthetic time limits. Therefore, it is clear that the postprandial synthetic capacity will be decreased in case the content of one or more of the indispensable amino acids in the meal is inadequate or when a time discrepancy exists in amino-acid absorption rates, creating an imbalanced and possibly even toxic postprandial plasma amino-acid pattern. Such an imbalance is not expected when supplementary free amino-acid intake does not exceed amounts shown in Table 1.

Safety of protein hydrolysates

Technological aspects, enzymes

Protein hydrolysates can be produced by acid and alkaline hydrolysis, by heat treatment and by incubation with enzymes. As compared with the chemical process, proteolysis by enzymes has several advantages. These include mild process circumstances, specificity, high reaction velocity and a lot of choices. The evaluation of the safety of protein hydrolysates in this paper is restricted to hydrolysates, which are made from sources of proteins with a history of safe use in human nutrition. The processes used are common processes used in the food industry using food grade source materials, processing aids and appropriate equipments. The enzymes used are digestive proteolytic enzymes (such as pepsin, chymotrypsin, trypsin and so on) obtained from animals, or food grade enzymes obtained from edible parts of plants and from microorganisms with an accepted safe use in human nutrition. A detailed evaluation of the safety of proteolytic enzymes is beyond the scope of this paper. For more information, the reader is referred to an EU report on this issue (Commission of the European Community, 1992). In brief, from this report the following information is obtained.

Proteolytic activity from a large variety of microorganisms used in traditional food fermentations has not been demonstrated to imply any health risk. Enzymes from edible parts of plants and animals are generally considered as posing no health problems. Regarding enzymes from microorganisms, the source organism should not be pathogenic and should not produce toxic compounds that remain in the final product. Toxicological test programs should be performed on all individual strains. Proteolytic enzymes belong to class 3 of enzymes (hydrolases). Important microorganism sources are strains of *Aspergillus niger*, *Saccharomyces cervisiae*, *Bacillus* and *Escherichia coli* (Kluifhooft, 2005). Food grade enzymes do not require labeling when they are considered as processing aids (Codex Alimantarius Commission, 1992). Important safety issues of enzyme preparations are toxicological properties (absences of enzyme contaminants, such as mycotoxins and antibiotics), the possibility of occupational health problems, such as skin irritations (caused by skin exposure to the enzyme), unintended reactions (such as transformation of histidine in histamine) and the safety of the source organism. There are no confirmed cases of allergic reactions in consumers, caused by the intake of enzyme-treated food.

Transport rate of amino acids

The rate of entry into the circulation of amino acids from protein hydrolysates will be faster than that from intact dietary proteins and may even be faster than that from free amino acids (Bilsborough and Mann, 2006). Amino acids from di- and tripeptides are reported to be absorbed more quickly than free amino acids and intact proteins, because peptide transport over the brush border membrane has a

greater capacity than the amino-acid transporters (Di Pasquale, 1997). Theoretically, this high rate of transport may have consequences for the metabolic effects and utilization of protein hydrolysates. However, so far to the knowledge of the author, no significant adverse effects of ingestion of protein hydrolysates have been reported.

Applications, history of safe use

A large body of evidence, substantiating the safety of protein hydrolysates, originates from their application in infant-feeding practice. For more than 60 years, extensively hydrolyzed proteins have been successfully used in the treatment of many children with food protein allergies; more recently also products based on amino-acid mixtures are used for this purpose (Host and Halken, 2004). Research in preterm infants and in term infants with cow's milk protein allergy has learned that the use of hypoallergenic hydrolyzed good-quality proteins is an effective and safe way to feed these children. In general, growth of infants on formulas with hydrolyzed good-quality protein is not different from that of breast-fed or standard formula-fed infants (Committee on Nutrition of the American Academy of Pediatrics, 1989). However, frequently, differences in plasma levels of urea and several amino acids were reported (Rigo and Senterre, 1994; Decsi *et al*., 1996; Szajewska *et al*., 2001; Hernell and Lönnerdal, 2003), but the significance of these differences for human safety is difficult to evaluate. At least a part of the differences in plasma amino-acid concentrations is attributable to the higher levels of amino acids in the hydrolyzed formulas compared with those in breast milk, standard formulas and amino-acid requirements (Hernell and Lönnerdal, 2003). In one study (Vanden plas *et al*., 1993), infants fed on hydrolyzed whey formula exhibited higher plasma urea levels than infants on a whey predominant standard formula with similar protein content. This may reflect a slightly lower postprandial amino acid utilization of the hydrolyzed formula.

The adequate intake of high-quality protein for formula-fed infants in the age between 0–6 months is in the range between 1.4 and 1.8 g/kg body weight per day or per 100 kcal (Health Council in the Netherlands, 2001; Institute of Medicine, 2002). The safe replacement of intact proteins by protein hydrolysates in infants and extrapolation of the 1.8 g/kg/day or 1.8 g/100 kcal to adults with a body weight of 70 kg, consuming a 2500 kcal diet, indicates that protein hydrolysates are most probably safe up to adult intake levels of $1.8 \times 25 = 45$ g/day or, if extrapolated on a body weight basis, even up to intake levels of about $1.8 \times 70 = 126$ g/day.

Other applications of protein hydrolysates are found in clinical nutrition and sports nutrition. In patients with digestive disturbances, partial hydrolysis of dietary protein will facilitate the uptake of amino acids and peptides. In sports nutrition enhanced absorption of amino acids is known to be associated with insulinotrophic effects, leading to the stimulation of post-exercise protein anabolism and glycogen repletion (Manninen, 2004).

The overall conclusion is that partially or extensively hydrolyzed good-quality proteins (such as milk, soy and egg) have a long history of safe use and that, so far, no unacceptable side effects of this practice have been reported.

Safety of protein hydrolysate fractions and (bioactive) peptides

Hydrolysis of proteins with specific proteolytic enzymes and subsequent fractionation may result in the isolation of fractions with particular nutritional characteristics. Two types of characteristics can be differentiated: (1) protein fractions with a relatively high content of specific amino acids and (2) bioactive peptides with particular amino-acid sequences, which are inactive in the intact protein molecule and become bioactive after their release from the intact molecule through the action of digestive enzymes in the body or through the action of proteolytic enzymes in food processing.

Protein fractions with relatively high contents of specific amino acids

Examples of peptides with relatively high contents of specific amino acids are glutamine peptides, derived from wheat, and cysteine/glycine and tryptophan peptides derived from whey protein (US Patent, 2003, 2004; World Intellectual Property Organization, 2004). Such peptides may find their application in clinical nutrition, sports nutrition and weight management. In principle, it is possible to prepare specific peptides by selecting a suitable combination of protein source, proteolytic enzyme(s) and fractionation procedure. Regarding safety, there is no argument to differentiate peptides from free amino acids. As upper safe limits for amino acids have not been assessed, one should be careful with high levels of intake of these nutrients not just in free form, but also by hydrolysate fractions. Although the safety of free glutamine was addressed in several studies and doses up to about 40 g/day did not seem to cause side effects in short-term experiments, long-term safety is uncertain (Garlick, 2001). A prudent attitude is that in the situation that the anticipated intake of amino acids either in free form or by protein hydrolysate fractions exceeds amounts of amino acids that are reasonably expected to be ingested under normal conditions of consumption of a balanced and varied diet, there should be safety concern. Table 1 could serve as a guideline for acceptable upper levels of amino acid intake by hydrolysate fractions. As argued before, extra intake of methionine is acceptable when its content in the hydrolysate fraction or peptides does not exceed 2.9%.

Bioactive peptides

These peptides (mostly hydrophobic) generally contain 3–20 amino acids. The C- or N-terminal fragments are crucial for their activities. Activities are shown on the digestive system,

the immune system, the cardiovascular system, the nervous system and body defense. Many of the bioactive peptides are derived from milk proteins (Korhonen and Pihlanto, 2006). Examples are β-casomorphin (opiate activity), casein macro peptide (stimulation of release of CCK), β-casein fragments (angiotensin-I-converting enzyme inhibition), casein phosphopeptides (enhancement of mineral absorption), α-lactalbumin fragments (immune stimulation) and a wide range of antimicrobial peptides derived from caseins and whey proteins. For an extensive overview of food proteins and their bioactive amino-acid sequences, the reader is referred to Dziuba and Darewicz (2007).

Most of the research on bioactive peptides has focused on efficacy and not on safety and it should be stressed that the efficacy data are largely based on *in vitro* data and animal model studies. Efficacy studies in humans are scarce with the exception of clinical studies on angiotensin-I-converting enzyme-inhibiting peptides. As far as these studies have included safety parameters, no adverse effects, which could be related to these bioactive peptides, have been reported. So in the clinical studies peformed in Japan on the blood pressure lowering effects of angiotensin-I-converting enzyme inhibition by the peptides valine–proline–proline, and IPP (isoleucine–proline–proline), side effects, that occasionally appear during treatment with angiotensin-I-converting enzyme-inhibiting drugs, were not detected, neither in studies with dairy products containing casein-derived valine–praline–proline and IPP, nor in a study with tablets, which provided much higher amounts of these bioactive peptides (Kajimoto *et al*., 2001). Moreover, it appeared that the natural concentration of both these peptides in some hard cheeses was such that the normal intake of valine–roline–proline and IPP by cheese may even exceed the dose of valine–praline–proline and IPP, given with functional foods and dietary supplements in clinical studies (Bütikofer *et al*., 2007). In a recent study by Ponstein-Simarro Doorten *et al*. (2009), the safety of an IPP tripeptide-containing milk protein hydrolysate, designed as a functional food ingredient or dietary supplement to manage blood pressure, was evaluated. The product was examined in three *in vitro* genotoxicity tests and in a repeated dose 90-day oral toxicity study with rats. The product was not mutagenic or clastogenic and the no observed adverse effect level (NOAEL) in the rat study was 4% in the diet, corresponding to an IPP intake of 40 mg/kg body weight. This is more than 100 times higher than the anticipated intake in humans.

One can argue about the necessity of toxicity testing of biologically active peptides made from a well-known food protein. In any case, it can be reassuring to do a limited set of toxicity tests. When, as also indicated above for protein fractions with relatively high contents of specific amino acids, an anticipated intake of amino acids by bioactive peptides largely exceeds levels that are reasonably expected to be ingested under normal conditions of a balanced diet, the company safety assessment should be evaluated and approved by an external independent expert committee and market introduction should be approved by the competent authorities (see also the legal aspects below).

Legal aspects

In all current food law systems, the safety of products is the responsibility of the food business operator. Companies should adequately document this safety assessment. With respect to the safety of hydrolysates, fractions thereof, and enzymes used, the following safety considerations are applicable.

Enzymes

In the United States, two series of enzymes are generally recognized as safe (GRAS): those with a GRAS status approved by the Food and Drug Administration (FDA, 2001) and those with a GRAS status assessed independently by qualified experts. In Europe, there exist no harmonized rules on the use of enzymes. France and Denmark have their national legislation. The European Commission has proposed new legislation that would harmonize EU rules on food enzymes (Commission of the European Communities, 2006a). This proposal aims at creating, maintaining and updating a general positive list of enzymes and a common authorization procedure, 'insofar as the existence of different national authorization procedures could potentially lead to different results and, in consequence hinder-free movement of substances in concern and distort-free competition'.

Hydrolysates and fractions thereof

Dietary proteins and protein hydrolysates have the GRAS status in the United States and are allowed in most countries (FDA, 2003). No specific legislation exists about the use of amino acids and bioactive peptides as dietary supplements or as food ingredients in most countries. Officially, in the Netherlands the addition of single amino acids to foods is not allowed, but the Health Council in the Netherlands (1999) advised positively about their use, although under defined restrictions. For foods for particular nutritional uses amino acids are on an EU positive list (Commission of the European Communities, 2001). There are no positive lists of food ingredients for common foods, or food supplements (except vitamins and minerals).

An important question regarding the regulatory position of protein hydrolysates and fractions thereof is whether these products fall under a category of foods, food ingredients or dietary supplements for which specific legislation exists. One realistic possibility in this regard is the group of novel foods. If the novel food regulation is applicable for a newly developed or imported food or food ingredient, then such a product will not be allowed on the market without a review of the company safety evaluation by external independent experts and approval by the competent authorities.

Novel foods are defined in Europe as foods and food ingredients that were not used for human consumption to a significant degree within the European Community before 15 May 1997 (Commission of the European Communities, 1997, 2008). In addition, they should fall in one of the four categories. Of these categories, the last two categories could be of interest for the safety assessment of protein hydrolysates and fractions thereof, because these categories include the following:

'foods and food ingredients consisting of or isolated from plants and food ingredients isolated from animals, except for foods and food ingredients obtained by traditional propagating of breeding practices and having a history of safe use' (e.g. hydrolysates with a documented history of safe use) and 'foods and food ingredients to which a production process has been applied, where that process gives rise to significant changes in the composition or structure

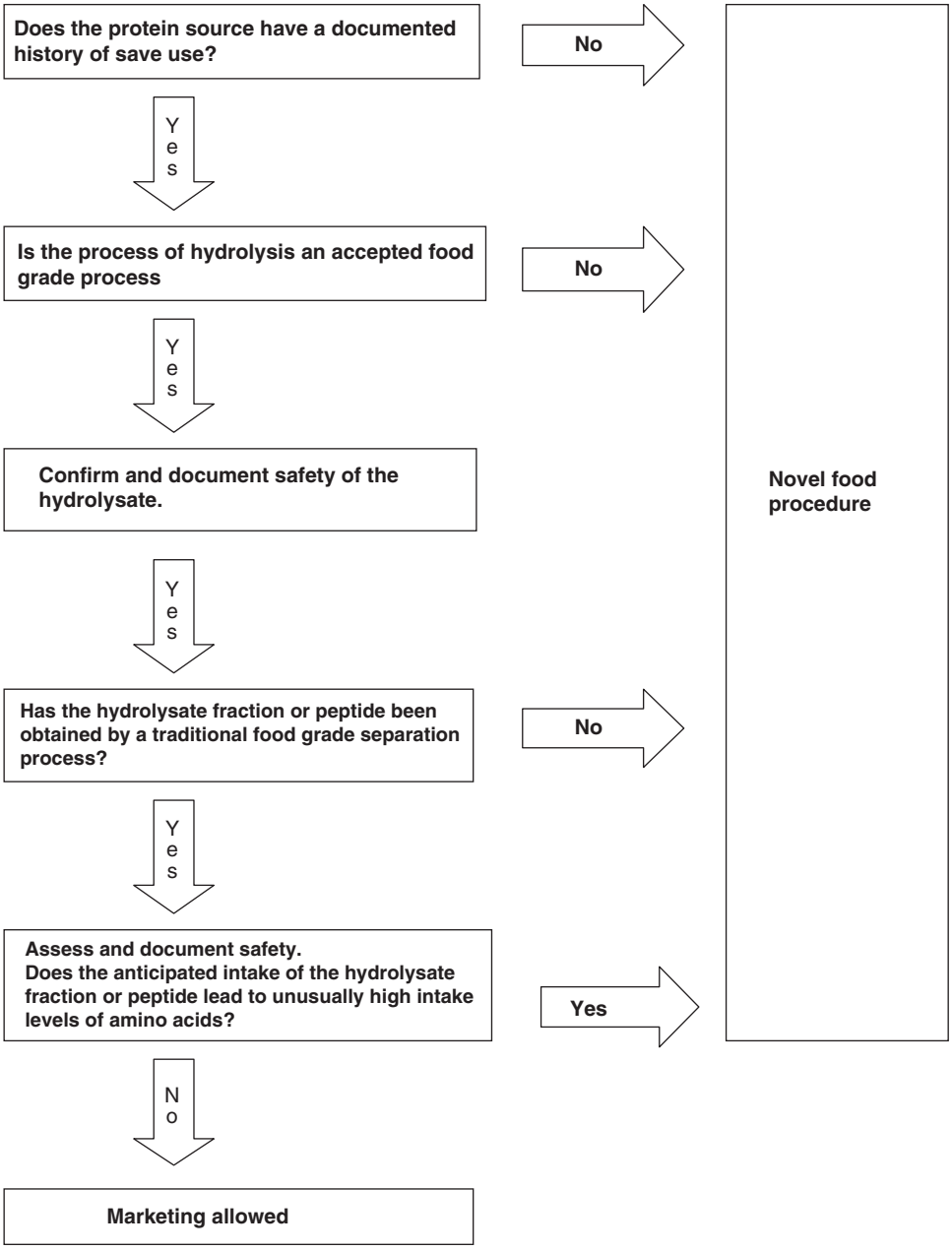

Figure 1 Decision tree on the safety assessment of protein hydrolysates and fractions thereof. A novel food procedure implicates a premarket safety evaluation by independent experts of a safety assessment by the company and subsequent authorization by competent authorities.

of the food or food ingredients, which affects its nutritional value, metabolic effect or level of undesirable substances'.

These categories refer to novel protein sources and novel processes (for example, new enzymes) used to produce peptides where this leads to significant changes in composition or structure that affects the nutritional value, metabolic effect and level of undesirable substances.

For hydrolysates derived from traditional protein sources and made with food grade enzymes, it can be argued that the process does not lead to undesirable changes in nutritional value and metabolic effects, when these hydrolysates are compared with the intact proteins from which they are obtained. This should be checked by the company.

For hydrolysate fractions and bioactive peptides, derived from these hydrolysates, the following consideration is applicable. Regulation (EC) No. 1925/2006 (Commission of the European Communities, 2006b) provides a handle to deal with the safety issue. Article 8 of this Regulation gives the possibility to put under scrutiny and, if necessary to restrict the use of substances added to foods or used in the manufacture of foods under conditions that would result in the ingestion of amounts largely exceeding those reasonably expected to be ingested under normal conditions of consumption of a balanced and varied diet and/or would otherwise represent a potential risk to consumers. Therefore, we suggest that, in situations where the anticipated intake of amino acids by bioactive peptides and protein fractions exceed the levels of free amino acids of Table 1 and/or when the methionine content is higher than 2.9%, the products must be viewed as novel foods, because of their potential significant influence on metabolism. Under these circumstances the safety assessment by the company should be evaluated and approved by independent experts (external evaluation and approval) before market introduction. Figure 1 shows a decision tree that summarizes the proposed safety assessment of protein hydrolysates and fractions thereof.

Conclusions

(1) According to the general food law, the safety of products is the responsibility of the food business operator. Safety of any food should be documented.
(2) Protein hydrolysates, as defined in this paper, can be considered as safe when they are derived from proteins with a history of safe use and when they are made with food-grade proteolytic enzymes and commonly used food-processing methods.
(3) The safety of fractions and bioactive peptides, derived from safe hydrolysates, should be assessed by the company before market introduction.
(4) A review of the safety assessment of the company by an external independent committee and subsequent approval by the competent authorities according to novel food procedures is indicated in three cases: (1) novel protein source, (2) novel process and (3) unusual high intake of amino acids.

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References

Affertsholt T (2007). 3A Business Consulting, Aarhus, Denmark, personal communication.
Bilsborough S, Mann N (2006). A review of issues of dietary protein intake in humans. *Int J Sport Nutr Exerc Metab* **16**, 129–152.
Bütikofer U, Meyer J, Sieber R, Wechsler D (2007). Quantification of the angiotensin-converting enzyme-inhibiting tripeptides Val-Pro-Pro and Ile-Pro-Pro in hard, semi-hard and soft cheeses. *Int Dairy J* **17**, 968–975.
Codex Alimantarius Commission (1992). Inventory of processing aids. *Codex Alimantarius* **1A**, 1–39.
Commission of the European Community (1992). Scientific Committee for Food of the European Communities, twenty seventh series, Guidelines for the presentation of data on food enzymes, opinion expressed on 11 April 1991. Luxembourg, office for official publications of the European Communities pp 13–21.
Commission of the European Communities (1997). Regulation (EC) No 258/97 of the European Parliament and of the Council of 27 Januari 1997 concerning novel foods and novel food ingredients. Official Journal of the European Communities No L 43/1.
Commission of the European Communities (2001). Commission directive 2001/15/EC of 14 February 2001 on substances that may be added for specific nutritional purposes in foods for particular nutritional uses. Official Journal of the European Communities No. L 52/19.
Commission of the European Communities (2002). Regulation EC No 178/2002 of the European Parliament and of the Council of 28 January 2002, laying down the general principles and requirements of food law, establishing the European Food Safety Authority laying down procedures in matters of food safety. Official Journal of the European Communities No. L31/1.
Commission of the European Communities (2006a). Proposal for a regulation of the European Parliament and of the Council. Establishing a common authorisation procedure for food additives, food enzymes and food flavourings, Brussels 28-07-2006 COM (2006)423 final, 2006/0143 COD.
Commission of the European Communities (2006b). Regulation (EC) No 1925/2006 of the Eropean Parliament and of the Council of 20 December 2006 on the addition of vitamins and minerals and of certain other substances to foods. Official Journal of the European Union No. L 404/27.
Commission of the European Communities (2008). Proposal for a regulation of the European Parliament and of the Council on novel foods and amending Regulation (EC) No xxx/xxx. Brussels 14 01 2008, COM (2007) 872, final.
Committee on Nutrition of the American Academy of Pediatrics (1989). Hypoallergenic infant formulas. *Pediatrics* **83**, 1068–1069.

Decsi T, Veitl V, Szasz M, Pinter Z, Mehes K (1996). Plasma amino acid concentrations in health, full-term infants fed hydrolysate infant formula. *J Pediatr Gastroenterol Nutr* **22**, 62–67.

Di Pasquale MG (1997). *Amino acids and proteins for the athlete; The anabolic edge*. CRC Press: Boca Raton, Florida.

Dziuba M, Darewicz M (2007). Food proteins as precursors of bioactive peptides—classification into families. *Food Sci Technol Inter* **13**, 393–404.

Eisenstein J, Roberts SB, Dallal G, Salzman E (2002). High-protein weight loss diets: are they safe and do they work/ A review of the experimental and epidemiological data. *Nutr Rev* **60**, 189–200.

Erlandsen H, Patch MG, Gamez A, Straub M, Stevens RC (2003). Structural studies on phenylalanine hydroxylase and implications toward understanding and treating phenylketonuria. *Pediatrics* **112**, 1557–1565.

European Food Information Council (2006). Food allergy and food intolerance. The Basics nr. 06. http://www .eufic.org/article/en/page/BARCHIVE/expid/basics-food-allergy-intolerance.

FDA (2001). Partial list of enzyme preparations that are used in foods. US Food and Drug Administration. Centre of Food Safety and Applied Nutrition, Office of Food Additive Safety.

FDA (2003). Code of Federal Regulations: 21 CFR 184, Direct food substances affirmed as generally recognized as safe, 1551. http://www.cfsan.fda.gov/~lrd/FCF 184 html.

Federation of American Societies for Experimental Biology (1992) In: Anderson SA, Raiten DJ (eds). *Safety of Amino Acids Used as Dietary Supplements*. Federation of American Societies for Experimental Biology: Bethesda, USA.

Garlick PJ (2001). Assessment of the safety of glutamine and other amino acids. *J Nutr* **132**, 2556S–2561S.

Health Council in the Netherlands (1999). *Committee on amino acid supplementation Safety of amino acid supplementation*. Health Council of the Netherlands, publication No 1999/06: The Hague.

Health Council in the Netherlands (2001). *Dietary reference intakes; energy proteins, fats and digestible carbohydrates*. Health Council of the Netherlands, publication No 2001/19E: the Hague.

Hernell O, Lönnerdal B (2003). Nutritional evaluation of protein hydrolysate formulas in healthy term infants: plasma amino acids, haematology, and trace elements. *Am J Clin Nutr* **78**, 296–301.

Host H, Halken S (2004). Hypoallergenic formulas—when, to whom and how long: after more than 15 years we know the indication!. *Allergy* **59**, 45–52.

Institute of Medicine (2002). Institute of Medicine in the US. Dietary reference intakes for energy, carbohydrates, fiber, fat, fatty acids, cholesterol, protein, and amino acids (macronutrients). The National Academies Press: Washington, DC, 2005.

Kajimoto Y, Aihara K, Hirata H, Takahashi R, Nakamura J (2001). Safety evaluation of excessive intake of the tablets containing Lactotripeptides (VPP, IPP) on healthy volunteers. *J Nutritional Food* **4**, 37–46.

Kluifhooft JD (2005). An introduction to the unlimited world of enzymes VMT symposium additives and processing aids in foods. Ede, The Netherlands 13 April 2005.

Korhonen H, Pihlanto A (2006). Bioactive peptides: production and functionality. *Int Dairy J* **16**, 945–960.

Lessof MH (1994). *Food allergy and other adverse reactions to food International Life Sciences Institute Europe Concise Monograph Series*. ILSI Europe: Brussels, Belgium.

Madden D (1995). *Food biotechnology, an introduction International Life Sciences Institute Europe Concise Monograph Series*. ILSI Europe: Brussels, Belgium.

Manninen AH (2004). Protein hydrolysates in sports and exercise: a brief review. *J Sports Sci Med* **3**, 60–63.

Meredith C (2005). Allergenic potential of novel foods. *Proc Nutr Soc* **64**, 487–490.

Nolles JA (2006). *Postprandial fate of amino acids: adaptation to molecular forms Thesis*. Wageningen University and Research Center: Wageningen, The Netherlands.

Ponstein-Simarro Doorten AY, van der Wiel JAG, Jonker DD (2009). Safety evaluation of an IPP tripeptide-containing milk protein hydrolysate. *Food Chem Toxicol* **47**, 55–61.

Rigo J, Senterre J (1994). Metabolic balance studies and plasma amino acid concentrations in preterm infants fed experimental protein hydrolysate preterm formulas. *Acta Paediatrica Suppl* **405**, 98–104.

Sarwar G, Peace RW (1994). The protein quality of some enteral products is inferior to that of casein as assessed by rat growth methods and digestibility-corrected amino acid scores. *J Nutr* **124**, 2223–2232.

Rodrigues ICC (2008). *The European Novel Food Regulation, where do we stand after 10 years. An evaluation comprising genesis, present and future*. MSc thesis Law and Governance, Wageningen University and Research Center, Department of law and Governance: Wageningen, The Netherlands. pp 60–72.

Szajewska H, Albrecht P, Stoinska B, Prochowska A, Gawecka A, Laskowska-Klita T (2001). Extensive and partial protein hydrolysate perterm formulas: The effect on growth rate, protein metabolism indices, and plasma amino acid concentrations. *J Pediatr Gastroenterol Nutr* **32**, 303–309.

US Patent (2003). US patent 6620778-Cysteine/glycine rich peptides. http://www.patentstorm.us/patents/6620778/description html.

US Patent (2004). US patent 6692933-Method for producing a gluten-free peptide preparation and preparation this obtained. http://www.patentstorm.us/patents/6692933/description html.

World Intellectual Property Organization (2004). Use of tryptophane rich peptides. International Application No: PCT/NL 2003/000084.http://www.wipo.int/pctdb/en/wo.jsp?wo = 2004069265.

Vanden plas Y, Hauser B, Blecker U, Suys B, Peeters S, Keymolen K, Loeb H (1993). The nutritional value of a whey hydrolysate formula compared with a whey-predominant formula in healthy infants. *J Pediatr Gastroenterol Nutr* **17**, 92–96.