Hydrolyzed Vegetable Proteins (HVP) – Quality and Safety

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Introduction

The consumption of various food additives has been growing steadily around the world since the mid-20th century. The purpose of their use is always to increase the sensorial, nutritional or functional properties of foodstuffs of vegetable or animal origin and meals prepared from them. Seasonings containing hydrolyzed vegetable proteins (GVP) hold a very important place among widely used good additives. In the food processing industry they are primarily used to enhance the taste and aroma of soups, sauces, salads, meat and vegetable meals and finished meals.

Probably all kinds of foodstuffs and materials for food processing used for human consumption contain substances that pose a certain health risk. Likewise, all food processing operations can result in the production of substances unacceptable from a health point of view. The task of the food processing industry is to develop technology that would minimize such health risks.

In case of HVP the raw materials as such present a certain health risk as they contain a share of allergenic proteins. It has to be noted, however, that people consume such proteins to a far greater extent in other types of food, in particular in bread, rolls and other bakery products. Nevertheless, allergies to wheat and soy proteins are rare; the occurrence of the celiac disease (intolerance to wheat gluten) is more frequent. A comparison of the consumption of HVP, bakery products and other soy products points at only a marginal negative effect of the raw materials used for the preparation of HVP. The presence of allergens in the hydrolyzed protein as such is virtually impossible, as mentioned below.

The acid hydrolysis technology can result in the production of the so-called toxic glycerol chlorohydrins (MCPD and DCP). The action of their production, occurrence and hygienic-toxicological aspects are discussed below.

Materials for the production of acid hydrolysates

The production of acid hydrolyzed proteins employs the following raw materials: defatted soy soybean meal, wheat gluten and corn flour. All materials contain at least a 50% protein share as well as ballast substances (cellulose etc.), a smaller portion of saccharides (particularly starch) and residual fat. The protein share of wheat gluten and soybean meal contains protein fractions that can cause allergic reactions and, in case of gluten fractions, also the celiac disease.

Characteristics of the most important allergens of soy and wheat

Soy allergens

- Beta-conglycinin: glycoprotein with a molecular weight of 180 kDa; it comprises 3 subunits – α-, α´-, β-; the biggest allergenic effect is provided by α-subunit marked

also as (Gly m Bd 60K)
- Glycinin: comes from the group of legumins – combined proteins with a molecular weight of up to 360 kDa; 11 S protein fraction; constitutes 35 – 40% of soy proteins
- Papain protease: (P34/Gly m Bd 30 K) it is probably the most important allergen in soy causing constitutional dermatitis; however, it can also evoke other types of allergic response; it is part of vacuole proteins
- PR-10 Bet v 1 allergens: there is an allergen (SAM22/Gly m 4) in soy responsible for allergies in the upper respiratory tract, featuring swelling tongue and larynx (oral allergy syndrome – OAS)
- Kunitz-type protease inhibitors: they include allergens with a low molecular weight of up to 21 kDa with two disulfide bridges; they are especially contained in soy, but also in other legumes; they can induce anaphylactic response

Wheat allergens

- PR-9 peroxidase: peroxidases containing hemo-bound iron; glycoprotein with a molecular weight of 36 kDa is a significant wheat allergen (Tri a Bd 36 K); it easily disintegrated by hydrolysis
- Grain prolamins: grain allergens, e.g. in wheat; typical amino acid sequence Gln-Gln-Gln-Pro-Pro; they are rather thermo-stable and resistant to enzyme proteolysis; amino acid amides are easy to hydrolyze in acids
- Omega-5-gliadin: significant wheat allergen; invokes respiratory anaphylactic response, called baker's asthma
- Polysaccharides: the liquid fraction of wheat flour contains the polysaccharide mannoglucan (comprising glucose and mannose in the molar ratio of 4.4:1) with a molecular weight of about 50 kDa, which causes long-term allergic response
- Gluten intolerance (celiac disease - coeliac sprue) is a rather frequent occurrence in Europe. People with this disposition experience a reaction with an antigen into gliadin and glutelin (prolamin) fraction of wheat proteins (gluten) or glutelin fraction of barley, rye and oats proteins. The celiac disease is caused by two amino acid sequences in those proteins: Pro-Ser-Gln-Gln and Gln-Gln-Gln-Pro.

Technology

Hydrolysis is conducted using a rather concentrated solution of hydrochloric acid (about 20% solution) under high temperature (100 – 120°C) and usually a slightly increased pressure. The hydrolysis time depends on the type of the raw material used, temperature and pressure and usually ranges from 6 to 20 hours. The hydrolyzed product is then neutralized with sodium hydroxide and/or bicarbonate to a pH of about 5.5.

In addition to water and sodium chloride (produced during the neutralization of hydrochloric acid), the hydrolyzed product contains free amino acids, as the main products of hydrolysis, water soluble brown pigments and aromatic substances. Colorful and aromatic compounds (volatile and non-volatile) are the products of side non-enzymatic browning reactions (especially Maillard reactions). The content of free amino acids usually corresponds to 75 – 90% of the original protein content.

The process waste includes a mixture of substances insoluble in water called humins. Humins contain a non-hydrolyzable share of the original materials (especially cellulose, possibly also lignin and other substances), color melanoidins insoluble in water (again produced as a product of non-enzymatic browning reactions), sodium chloride, and possibly also non-reacted proteins that precipitated during neutralization. The waste humins can be reprocessed and used as fertilizers or for feeding.

Reactions of raw material components in the production of acid hydrolyzed products

Effect of pH on the physical properties of proteins

The raw materials used for the production of hydrolysates contain the original proteins and the present peptides and free amino acids, if any, in a non-ionized form. In that form their water solubility is very difficult. In an acid environment the free amino group of the N-terminal amino acid is ionized and the proteins, peptides and amino acids are transferred to the form of hydrochlorides, which are easily soluble in water and prone to further reactions.

This reaction is reversible and does not result in the loss of activity of the protein molecule. Following the end of hydrolysis, the hydrolyzed product is neutralized to a pH close to the protein's isoelectric point. Under these conditions the protein returns back to the non-ionized form and has a very limited solubility. A small quantity of proteins that would otherwise remain in a non-hydrolyzed state precipitate during neutralization and are converted into waste humins.

Protein denaturation

Protein denaturation refers to a set of physical reactions leading to the change of conformation of the protein molecule and (in case of active proteins) to the loss of their activity.

The production of acid hydrolyzed products includes denaturation by way of high temperature, higher pressure and very low pH in the reaction mixture. The combination of all three physical phenomena results in an extensive denaturation of the present proteins at the beginning of the hydrolysis.

The denaturation as such would not be probably enough to remove allergens from the product. Some protein structures with allergenic activity are rather thermo-stable and could resist denaturation for some time. In addition, allergenic activity can be also observed in some polysaccharides, which are not obviously involved in denaturation.

Splitting of peptide bonds and other hydrolytic reactions

There is a very massive, rapid and efficient hydrolysis of proteins and peptides, hydrolyzable polysaccharides and fats during the production of hydrolyzed products due to the rather concentrated hydrochloric acid, high temperature and higher pressure, if applicable.

Hydrolysis occurs in proteins and peptides on the peptide bond, leading to the production of other peptides and subsequently amino acids. Because of the excessive hydrochloric acid in the reaction mixture and the high temperature, the differences in the stability of the miscellaneous peptide bonds are not important. The differences in the rate of hydrolysis of the individual proteins therefore only depend on the mechanical barriers to the smooth contact between the protein material and the acid, created due to the imperfect blending of the reaction mixture. The time interval of the hydrolysis is, however, very long, which ensures that each protein molecule will be fully hydrolyzed down to the respective amino acids or at least partially hydrolyzed to a mixture of peptides and amino acids. That is why the presence of any, even minimum, amount of allergenic proteins in the product is virtually impossible.

In case of polysaccharides, the glycosidic bond is hydrolyzed to produce simple oligosaccharides and monosaccharides. Polysaccharides that can be digested and used by humans, including saccharides exhibiting allergenic activity, have easily hydrolyzable glycosidic bonds. A massive hydrolysis during the production process once again ensures that there will be virtually no allergenic polysaccharides in the product. Saccharides that can be hydrolyzed with difficulty, such as cellulose, do not exhibit any allergenic activity.

The hydrolysis of saccharides results in the production of many reactive compounds, which then easily engage in non-enzymatic browning reactions.

The production process also includes a rather rapid hydrolysis of the present fats to glycerol and fatty acids. After dehydration to acrolein, most of the produced glycerol is involved in non-enzymatic browning reactions. In some of the glycerol, the hydroxy group can be subject to substitution with chlorine, subject to producing toxic glycerol chlorohydrins (in particular MCPD – monochloropropanediol and DCP – dichloropropanol). The MCPD limit in commercial hydrolyzed products is set by legislation; at present the limit value is 20 micrograms per kilogram of product. The MCPD content in hydrolyzed products in case of proper technology, with the inclusion of decontamination, is usually way below the hygienic limit. This issue will be discussed separately.

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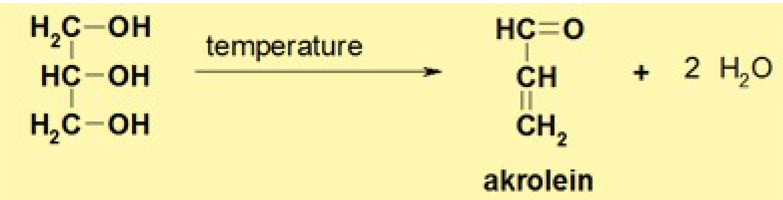


Some fatty acids oxidize during hydrolysis, producing mostly aldehydes, which also engage very easily in non-enzymatic browning reactions.

Non-enzymatic browning reactions

This process primarily involves a reaction of the amino group with the carbonyl group, resulting in the production of the so-called Schiff bases, which then react and produce color and aromatic products.

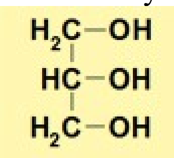
The production of hydrolyzed proteins includes a reaction of the amino group of free amino acids or the N-terminal amino acid of peptides and proteins with the free aldehyde group of saccharides present in the input material. This one type of a series of non-enzymatic browning reactions is called the "Maillard reactions". The aldehyde group of the products of oxidation of fats or fatty acids can react with the free amino acid similarly.

The activation energy of these reactions for protein and polysaccharide is very high. Under the conditions of production of hydrolyzed proteins, however, proteins and polysaccharides are able to engage in the sequence of Maillard reactions (especially thanks to the high temperature). That is why a protein or polysaccharide with allergenic activity will lose this activity through the chemical reaction.

<u>Chlorinated propanol derivates</u>

In acid hydrolyzed products as well as in other foodstuffs, chlorinated derivates are created by substituting one or two hydroxy group of glycerol with chlorine. The source of chlorine in HVP is hydrochloric acid and in other foodstuffs the commonly present chlorides. Free

(R,S)-3-chloro-propane-1,2-diol (3-MCPD) occurs most often, while the production of other derivates (2-chloro-propane-1,3-diol; 1,3-dichloro-propane-2-ol; 2,3-dichloro-propane-1-ol; 3-chloro-propane-1-ol) is highly suppressed and they occur in quantities bordering on detectability.



(S)-(+)-3-chlor-propan-1,2-diol

The legislative limit for the content of 3-MCPD in hydrolyzed products is 20 µg/kg (ppb). For most other foodstuffs there is no set limit. Its content in common foodstuffs is, however, usually close to this value. A higher content can be found e.g. in fermented salamis (up to 50 ppb) or processed cheese (up to 30 ppb). A very high content has been detected in Parmesan (82 ppb). There is an extreme increase in the content of 3-MCPD in bread during toasting or grilling (up to 300 ppb).

<u>General composition of dried hydrolyzed proteins from Rieber & Son, ASA</u>

Solids	min. 95 %
Amino acid content (Nx6.25)	26 ± 4 %
Fat	2 ± 1 %
NaCl content	50 ± 4 %
Glutamic acid content (natural)	7 ± 1 %
pH (10% solution)	5,6 ± 0,2
3-MCPD	< 10 µg/kg (ppb)

<u>Conclusion</u>

The production of hydrolyzed proteins is a process during which the input materials containing especially proteins and a smaller portion of saccharides are hydrolyzed by a rather concentrated hydrochloric acid at high temperature and, if applicable, increased pressure for a relatively long period of time. This process involves virtually a complete destruction of all proteins and saccharides, resulting in the production of free amino acids and products of non-enzymatic browning reactions. In addition, the neutralization of the hydrolyzed product after the end of hydrolysis leads to the precipitation of other potentially non-hydrolyzable proteins. The consequence of this process is practically a complete absence of the original proteins and polysaccharides in the final product. Likewise, all proteins and saccharides with allergenic activity occurring in the original product are inactivated. Acid hydrolyzed proteins can be therefore considered absolutely safe in terms of occurrence of allergic reactions in the organism.

In case of proper technology with the inclusion of a decontamination stage, the content of 3-MCPD is below the hygienic limit and is comparable to, and usually lower than, its content in common foodstuffs.

Hydrolyzed vegetable proteins can be therefore considered fully conforming in terms of food safety.

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