

**35.07 - Enzymes; prepared enzymes not elsewhere specified or included.**

3507.10 - Rennet and concentrates thereof

3507.90 - Other

Enzymes are organic substances produced by living cells; they have the property of causing and regulating specific chemical reactions inside or outside living cells, without themselves undergoing any change in their chemical structure.

Enzymes may be referred to as follows :

**(I) According to their chemical constitution, e.g. :**

- (a) Enzymes in which the molecule consists solely of a protein (e.g., pepsin, trypsin, urease).
- (b) Enzymes in which the molecule consists of a protein combined with a non-protein compound of low molecular weight, acting as a cofactor. The cofactor may be either a metal ion (e.g., copper in ascorbate oxidase, zinc in human placental alkaline phosphatase) or a complex organic molecule called a coenzyme (e.g., thiamine diphosphate in pyruvate decarboxylase, pyridoxal phosphate in glutamine-oxo-acid aminotransferase). Sometimes both are required.

**(II) According to :**

- (a) **their chemical activity** as oxidoreductases, transferases, hydrolases, lyases, isomerases, ligases; or
- (b) **their biological activity** as amylases, lipases, proteases, etc.

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This heading includes :

**(A) “Pure” (isolated) enzymes.**

These are generally in crystalline form, and are mainly intended for use in medicine or in scientific research. They are not as important in international trade as enzymatic concentrates and prepared enzymes.

**(B) Enzymatic concentrates.**

These concentrates are generally obtained from either aqueous or solvent extracts of animal organs, of plants, of micro-organisms or of culture-broths (the latter derived from bacteria, moulds, etc.). These products, which may contain several enzymes in various proportions, can be standardised or stabilised.

It should be noted that certain standardising or stabilising agents may already exist in the concentrates in variable quantities, deriving either from the fermentation liquor or from the clarifying or precipitating processes.

The concentrates can be obtained, for example, in powder form by precipitation or freeze-drying or in granular form by using granulating agents or inert supports or carriers.

## 35.07

### (C) Prepared enzymes not elsewhere specified or included.

Prepared enzymes are obtained by further dilution of the concentrates mentioned in Part (B) above or by intermixing isolated enzymes or enzymatic concentrates. Preparations with substances added, which render them suitable for specific purposes, are also included in this heading, **provided** they are **not** covered by a more specific heading in the Nomenclature.

This group includes, *inter alia* :

- (i) Enzymatic preparations for tenderising meat, such as those consisting of a proteolytic enzyme (e.g., papain) with added dextrose or other foodstuffs.
- (ii) Enzymatic preparations for clarifying beer, wine or fruit juice (e.g., pectic enzymes containing added gelatin, bentonite, etc.).
- (iii) Enzymatic preparations for desizing textiles such as those with a basis of bacterial  $\alpha$ -amylases or proteases.

This heading **excludes**, *inter alia*, the following preparations :

- (a) Medicaments (**heading 30.03 or 30.04**).
- (b) Enzymatic preparations for pre-tanning (**heading 32.02**).
- (c) Enzymatic soaking or washing preparations and other products of **Chapter 34**.  
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The following are the most important among the enzymes found in trade :

#### (1) Rennet (lab-ferment, chymosin, rennin).

Rennet is obtained either from the fresh or dried fourth stomach of calves or by the cultivation of certain micro-organisms. It is a proteolytic enzyme which curdles milk by coagulating its casein. It is available in liquid, powder or tablet form. It may contain salts (e.g., sodium chloride, calcium chloride, sodium sulphate), remaining from the manufacturing process or added for standardisation, and preserving agents (e.g., glycerol).

Rennet is mainly used in the cheese industry.

#### (2) Pancreatic enzymes.

The most important enzymes produced by the pancreas are **trypsin** and **chymotrypsin** (which break down proteins),  $\alpha$ -**amylase** (which breaks down starches) and **lipase** (which breaks down fatty substances). They are mainly used in medicine and pharmacy for treating digestive disturbances.

Enzymatic concentrates of the pancreas are normally obtained from fresh or dried pancreas. They may contain highly absorbent salts (added to take up part of the water of crystallisation) and certain protective colloids (to facilitate storage or transport). They are used in the manufacture of preparations for desizing, washing, hair-removal or tanning.

The enzymatic preparations of the pancreas classified in this heading include those used for de-sizing textiles.

(3) **Pepsin.**

Pepsin is obtained from the stomach mucosa of hogs or cattle. For the purposes of stabilisation, it is sometimes preserved in a saturated solution of magnesium sulphate or is mixed with sucrose or lactose (powdered pepsin).

Pepsin is used mainly for medicinal purposes, combined with hydrochloric acid or betaine hydrochloride, or as pepsin wine.

(4) **Malt enzymes.**

This group covers **only malt amylases**.

Malt extracts are classified in **heading 19.01**.

(5) **Papain, bromelains, ficin.**

The term **papain** is used to describe both the dried latex of the papaya tree (*Carica papaya*) and the two fractions obtained from this product, viz., **papain** (in the more limited sense of this term) and **chymopapain**.

Papain is used, for example, for the manufacture of chillproof beer, in the preparation of meat tenderisers (see paragraph (C) (i) above) and in medicine.

Papain as the dried latex which is only partly water-soluble, falls in **heading 13.02**.

**Bromelains** are obtained from pineapple plants.

**Ficin** is obtained from the latex of certain varieties of fig trees.

(6) **Amylases and proteases obtained from micro-organisms.**

Certain micro-organisms, when grown in appropriate culture media, secrete a considerable quantity of amylases and proteases.

After removal of the cells and other impurities, the solutions are either concentrated by low temperature vacuum evaporation or the enzymes are precipitated by the addition of inorganic salts (e.g., sodium sulphate) or organic, water-miscible solvents (e.g., acetone).

Examples of microbial amylases and proteases are :

(a) **Bacterial  $\alpha$ -amylases.**

Bacterial  $\alpha$ -amylases (obtained, for example, by use of *Bacillus subtilis*) are starch-liquefying enzymes, used for the production of adhesives and of starch-based paper coatings, in bakeries and other food industries and for desizing textiles.

(b) **Fungal amylases.**

Fungal amylases are essentially  $\alpha$ -amylases derived from mould cultures, mainly of the genus *Rhizopus* or the genus *Aspergillus*.

Although their liquefying power is marked, it is much less than that of bacterial amylases.

Fungal amylases have many uses in the food industry.

It should be noted that fungal amylases sometimes contain proteases, glucose oxidase and invertase.

(c) **Amyloglucosidases.**

These enzymes, obtained, for example, from moulds of the genus *Rhizopus* or the genus *Aspergillus* are strong saccharifying agents but have no liquefying properties. They are used to obtain a high yield of dextrose from starchy materials.

Their main applications are in the production of glucose syrups and dextrose, and as saccharifying agents for grain alcohol fermentation mashes.

(d) **Proteases.**

Bacterial proteases (obtained by use of, for example, *Bacillus subtilis*) are proteolytic enzymes used to prepare textile desizing agents, as ingredients in certain washing preparations and in beer-making. Proteases produced from moulds are used for medicinal and pharmaceutical purposes.

(7)  **$\beta$ -Amylases.**

These enzymes are obtained from vegetable materials, such as malted barley, wheat and soya beans. They produce maltose from starch and dextrins.

(8) **Pectic enzymes.**

These enzymes are manufactured by cultivating various mould types, mainly of the genus *Rhizopus* or the genus *Aspergillus*. They are used in the manufacture (in order to facilitate the pressing operation and increase the juice recovery) and processing of fruit and vegetable juices.

(9) **Invertase ( $\beta$ -fructofuranosidase).**

Invertase is usually derived from low fermentation brewer's yeast.

This enzyme splits sucrose into glucose and fructose. It is used in the manufacture of golden syrup, chocolate and marzipan.

(10) **Glucose isomerase.**

This enzyme is manufactured by culture of certain micro-organisms, mainly of the genus *Streptomyces* or the genus *Bacillus*. It is used for the partial conversion of glucose to fructose in the production of syrups with a high degree of sweetness.

In addition to the other exclusions referred to above, this heading **does not cover** :

- (a) Yeasts (**heading 21.02**).
- (b) Coenzymes such as cocarboxylase (aneurine pyrophosphate) and cozymase (nicotinamide-adenine dinucleotide) (**Chapter 29**).
- (c) Dried glands and other products of **heading 30.01**.
- (d) Cultures of micro-organisms, blood enzymes (e.g., thrombin), blood fractions and truncated variants (parts) thereof with enzymatic properties/activity and other products of **heading 30.02**.