Mixed β-glucanase, xylanase from *Humicola insolens*

Chemical and Technical Assessment (CTA)

First draft prepared by Jim Smith

1. Summary

The mixed β-glucanase and xylanase enzyme produced by *Humicola insolens* is used to hydrolyse β-glucans, pentosans and other gums during the mashing process of brewing. This improves filtration rates better than β-glucanase alone due to the hydrolysis of the pentosans other than β-glucans.

2. Description

The enzyme preparation described is a mixed β-glucanase and xylanase produced by submerged fermentation of a non-pathogenic and non-toxigenic strain of *Humicola insolens.*

The enzyme preparation contains the two main activities: β-glucanase, Xylanase, and several side activities, e.g.: Cellulase, Hemicellulase, Pentosanase. The enzyme preparation is standardized on the main activity: β-glucanase.

According to the Nomenclature Committee of the International Union of Biochemistry and Molecular Biology (1992) and the Chemical Abstracts Service, the main activities are classified as:

Generic name:	Carbohydrases	
IUB nomenclature:	Endo-1,4-β-glucanase	Endo-1,4-β-xylanase
IUB No.:	3.2.1.6	3.2.1.8
CAS number:	62213-14-3	9025-57-4
EINECS No.:	263-462-4	231-800-2
Trade name:	traflo™ L Novozymes A/S	

Microbial sources:

The production strain for the enzyme is a non-pathogenic and non-toxigenic strain of *Humicola insolens.*

Classification of the strain:

The description of the strain used by Novozymes for the manufacture of the mixed β-glucanase and xylanase enzyme preparation is as follows:

Name:	*Humicola insolens*
Subdivision:	Deuteromycotina
Class:	Hyphomycetes
Order:	Bactridiales
Family:	Sepedoniaceae
Genus:	*Humicola*
Species:	*insolens*

This strain of *Humicola insolens* is non-pathogenic and non-toxic in man or other animals.

The description of *Humicola insolens* characteristics has been first published by Cooney & Emerson (1986).

The production strain has been obtained through a selection process leading to isolates with improved enzyme production.

The production strain produces no spores (normally the *Humicola insolens* strain produces very few spores). Growth rate on agar is slower. Pigmentation is less. After 5 days on YPPS at 37 C the diameter is approx. 2.5 cm. The colonies never become dark-greyish.

The identification has been confirmed by the German Collection of Microorganisms (DSM, Deutsche Sammlung von Mikroorganismen).

Safety of the production organism:

The *Humicola insolens* strain was first used at Novozymes A/S as a producer of alkaline cellulase. In the research phase the product was called SP227. The alkaline cellulase is still used in the detergent and textile industries.

Later on the other carbohydrases were exploited as processing aids in food industries. The β-glucanase is very valuable in the brewing industry, and the commercial product is called Ultraflo™ L . The xylanase was used in the baking industry. The commercial product was called Pentopan. Pentopan has now been replaced with mono-component xylanase (GMO).

The mixed β-glucanase and xylanase preparation is also marketed within the EU as a feed-additive under the name Bio-Feed Plus. Before a provisional permission to market a feed-additive within EU the product in question must be evaluated by the Scientific Committee on Animal Nutrition (SCAN).

The SCAN concluded in its Opinion of the Scientific Committee on Animal Nutrition on the use of certain enzymes in animal feeding stuffs (updated 16 October 2002) Annex, Part A that:

> *The Scientific Committee on Animal Nutrition concludes on the basis of the information provided and knowing of no adverse reports elsewhere that the following enzyme preparations are safe for use as feed additives when used according to the manufacturers instructions and with the animal categories specified. The Committee also concludes that these enzyme preparations pose no risk to the wider environment, to those handling the preparations, or to individuals of any age consuming products derived from animals fed the enzyme-treated feed.*

Greenough, Everett and Stavnsbjerg (1991) conclude that *Humicola insolens* can be regarded as a non-toxigenic and a non-pathogenic strain.

3. Manufacturing

3.1 Manufacturing principle

The manufacturing process is composed of a fermentation process, a purification process, a formulation process and finally a quality control of the finished product, as outlined by Aunstrup et al., 1979.

Ultraflo™ L is manufactured in accordance with current Good Manufacturing Practices, Food. The quality management system used in manufacturing process complies with ISO 9001.

The raw materials are Food Grade Quality and have been subjected to appropriate analysis to ensure their conformity with the specifications.

Fermentation:

The enzymes in Ultraflo™ L are a β-glucanase and a xylanase produced by submerged fed-batch pure culture fermentation of the production strain of *Humicola insolens*, described in section 2.

3.2 Detailed description (confidential)

[Removed for reasons of confidentiality]

4. Chemical characterization

4.1 Composition of the food additive

Formulation B Ingredients and additives

The liquid concentrate is standardized to the declared enzyme activity 45 FBG/g by addition of water, sorbitol, glycerol and potassium sorbate.

Immobilized enzyme preparation:

Not relevant.

TOS and composition:

As described in section 4.1 above, Ultraflo™ L is obtained from the liquid enzyme concentrate.

Ultraflo™ L is typically composed as follows:

Enzyme solids (TOS)	approx. 5%
Water	approx. 40%
Glycerol	approx. 28%
Sorbitol	approx. 27%
Potassium sorbate	approx. 0.2%

4.2 Possible impurities (including degradation products)

Good Manufacturing Practice (GMP):

Ultraflo™ L is produced according to good manufacturing practice for manufacturing, packing or holding human food, in order to prevent serious food hazards.

The quality management system used in manufacturing process for Ultraflo™ L complies with the requirements in ISO 9001.

Influence on total microbial count in final foodstuff:

Ultraflo™ L complies with JECFA and FCC recommended purity specifications for food-grade enzymes, as detailed in section 8. Therefore, its application in food products will not cause an increase in the total microbial count of the concerned foodstuff.

Purity specifications/absence of contaminants:

The mixed β-glucanase and xylanase enzyme preparation comples with the purity criteria recommended for enzyme preparations in Food Chemicals Codex 3rd supplement to 4th edition, 2001.

In addition to this, the mixed β-glucanase and xylanase enzyme preparation also conforms to the General Specifications and Considerations for Enzyme Preparations Used in Food Processing as proposed by the Joint FAO/WHO Expert Committee on Food Additives in Compendium of Food Additive Specifications, Addendum 9, FAO 2001.

In total, the mixed β-glucanase and xylanase enzyme preparation conforms at least to the following complete specification:

Enzyme activity	According to declaration
Lead	Not more that 5 ppm
Arsenic	Not more that 3 ppm
Cadmium	Not more that 0.5 ppm
Mercury	Not more that 0.5 ppm
Total viable count/g	Not more that 1 x 10^4
Total coliforms/g	Not more that 30
Enteropathogenic E. coli/25 g	Negative by test
Salmonella/25 g	Negative by test
Antibiotic activity	Negative by test
Production organism	Negative by test
Mycotoxins	Negative by test

Heavy metals:

Ultraflo™ L does not contain toxicologically significant amounts of heavy metals as outlined in the complete specification above.

Microbiological contaminants:

Absence of the microbiological contaminants is part of the complete specification as outlined above.

Production organism:

The manufacturing process includes a final germ filtration step to ensure that no production organism is present. Absence of the production organism is part of the complete specification as outlined above.

Antibiotic Activity:

Absence of antibiotic activity is part of the complete specification as outlined above.

Toxins:

Absence of mycotoxins is part of the complete specification as outline above.

4.3 Analytical methods

Ultraflo™ L is standardised to have a typical activity of 45 FBG/g. In addition the enzyme preparation will contain

approximately 470 FXU/g.

The definition of FBG (Fungal β-glucanase) is a follows:

1 β-glucanase unit (BG) is the amount of enzyme, which under standard conditions (pH 5.0 and 30.0 C) liberates glucose or other reducing carbohydrates with a reduction power corresponding to 1 μmol glucose per minute.

Principle:

Beta-glucanase converts β-glucans to glucose and other reducing carbohydrates which are determined according to the Somogyi-Nelson method (J. Biol. Chem. 153, p.375 (1944).

The definition of FXU (Farbe Xylanase Unit) is as follows:

The FXU activity is determined relative to the activity of a reference enzyme standard. The activity of the reference standard, *Humicola insolens* xylanase batch no. 17-1194, is defined to have an enzymatic activity of 3550 FXU/g at pH 6.0 and 50.0 C in 30 min reaction time of colour release from remazol-xylan substrate.

Principle:

Xylanase sample is incubated with remazol-xylan substrate. The background of non-degraded dyed substrate is precipitated by ethanol. The remaining blue colour in the supernatant is proportional to the endoxylanase activity. The activity is determined relatively to an enzyme standard.

Reaction conditions: pH = 6.0, temperature 50 C, and reaction time 30 min.

The Novozymes methods used to determine the enzyme activities, EB-SM-0338 for the FBG unit, and EB-SM-0352 for the FXU unit are available (Jensen, 2002).

5. Functional use

5.1 Technological function

The most important active principles in the enzyme preparation are β-glucanase and xylanase. The product is standardized according to the β-glucanase activity, characterized by IUB AEnzyme Nomenclature 1992" as EC 3.2.1.6, (systematic name 1,3-(1,3;1,4) -β-D-glucan 3 (4) -glucanohydrolase).

The preparation is mainly used in beer brewing but may also be used in the alcohol industry to hydrolyse β-glucans, pentosans, and other gums. This reduces the viscosity of the solution and thereby increases the filtration rate of both wort and beer, and haze is avoided.

The enzyme is normally inactivated and removed during the industrial production process.

Further information regarding the enzyme is given in the Ultraflo™ L Product Sheet (Novozymes, 2001).

Types of foodstuffs:

The preparation is mainly used in the brewing industry, but may also be used in the alcohol industry to hydrolyse β-glucans, pentosans, and other gums.

5.2 Food categories and use levels

Maximum dosage of the enzyme preparation:

The use levels of the enzyme are according to requirements for normal production (GMP).

The recommended dosage is 200 g of the enzyme preparation per ton of total raw materials, but is dependent on the raw material composition.

6. Reactions and fate in foods

Amount of enzyme in the final food preparation:

The enzyme preparation is added at mashing in, and the enzyme activities will be completely inactivated during wort boiling.

Main reaction products:

Enzymes are proteins and biological catalysts. Each enzyme is specific in character, acting on a particular substrate to produce a particular product.

The β-glucanase will hydrolyse the barley β-glucan to monomers or oligomers.

The xylanase enxyme hydrolyses xylosidic linkages in the arabinoxylan backbone resulting in a depolymerisation of the arabinoxylans into smaller oligosaccharides.

No reaction products, which could not be considered normal constituents of the diet, are formed during the production or storage of the enzyme treated food.

Possible effects on nutrients:

No effects on nutrients are known. Enzymes are proteins and are nutritionally not different from other proteins in the food.

7. References

Jensen, B.R. (2002) Mixed β-glucanase, xylanase enzyme preparation, produced by a strain of *Humicola insolens*. Ultraflo_ L. Unpublished report No. 2002-37749-01 from Novozymes A/S, Bagsvaerd, Denmark. Submitted to WHO by Novozymes A/S, Bagsvaerd, Denmark.

Novozymes (2001) Product Sheet Ultraflo™ L. Brewing/2001-00468-02. Submitted to WHO by Novozymes A/S, Bagsvaerd, Denmark.

Aunstrup, K. Production, Isolation and Economics of Extracellular Enzymes in Applied Biochemistry and Bioengineering, Volume 2, Enzyme Technology, Eds. Wingard, L.B., Katchalski-Katzir, E. and Goldstein, L., pp. 28-68, 1979.

Pariza, M.W. and Johnston, E.A. Evaluating the Safety of Microbial Enzyme Preparations Used in Food Processing: Update for a New Century. Regulatory Toxicology and Pharmacology 33: 173-186 (2001).

Enzyme Applications in Encyclopedia of Chemical Technology, 4th Ed. Kroschwitz, J.I., Volume 9, pp. 567-620, 1994.

Aunstrup, K., Andersen, Ol, Falch, E.A., and Nielsen, T.K. Production of Microbial Enzymes in Microbial Technology, 2nd ed., Vol. I, Eds. Peppler, H.J. and Perlman, D., Chapter 9, pp. 282-309, 1979.

Greenough, R.J., Everett, D.J., and Stavnsbjerg, M. Safety Evaluation of Alkaline Cellulase, Fd. Chem. Toxic. Vol. 29, No. 11, pp. 781-785, 1991.

International Union of Biochemistry & Molecular Biology (IUB) (1992) Enzyme Nomenclature Cooney and Emerson (1986) Thermophilic Fungi.