Opinion on The use of urease prepared from *Lactobacillus fermentum* in wine production (expressed on 10 december 1998)

Terms of Reference

To give an opinion on the safety in use of urease prepared from *Lactobacillus fermentum* in the production of wine and in particular to advise on any conditions that should be placed on its use to protect public health.

Background

The use of urease in oenology is authorised in the EU by Council Regulation (EEC) No 822/87 as amended by Regulation EC No 536/97, Article 4(c). This Regulation also requires that the precise conditions of use are to be determined. The purpose of urease treatment is to reduce the urea content in wine after ageing in order to minimise the eventual formation of ethyl carbamate (urethane). This specific use was also approved by the Conseil Supérieur d'Hygiène Publique de France (3).

Introduction

The objective of adding urease at the end of the fermentation process for making wine is the reduction of the urea content by conversion via hydrolysis to CO_2 and NH_3. If excess urea is formed in wine it will combine with the ethanol in wine during the storage and ageing period to form ethyl carbamate, a known carcinogen, the presence of which in wine is undesirable.

The safety of the urease preparation submitted was assessed according to the guidelines on the evaluation of an enzyme preparation to be used in foodstuffs published by the SCF (1).

Specification of the commercial preparation

a) General specification

- Loss on drying: not more than 10%
- Heavy metals: not more than 30 ppm
- Pb: not more than 10 ppm
- As: not more than 2 ppm
- Total coliforms: none detectable
- *Salmonella spp:* absent in 25 g sample
- Aerobic count: not more than $5x10^4$ cells/g

b) Substance specific specification

- Urease activity: not less than 5 U/mg
- *Lactobacillus fermentum* viable cells absent

Technical information

The principal enzyme activity of the commercial preparation is characterized as urease EC 3.5.1.5, CAS No: 9002-13-5, EINECS No: 232-656-0. It is optimally active at acidic pH and has an activity of not less than 5 U/mg. It is claimed that no other secondary enzymatic activities are present.

One unit of urease enzyme activity is defined as the amount that produces 1 µmol of NH_3 per minute at 37 C from 5000 mg urea/L at pH 4.

The enzyme is produced by a culture fermentation process from *Lactobacillus fermentum*, a non-pathogenic, non-toxigenic organism, commonly present in many kinds of fermented European foods and in the Indian fermented food "dosa" The organism has been consumed by man traditionally as part of his daily food. It is also a normal inhabitant of the gastrointestinal tract of humans and of the rat.

For production of the urease preparation a pure culture of *L. fermentum* is aseptically grown in fermentors in a medium containing only dextrose, casein digest, meat extract, yeast extract, sodium chloride, sodium acetate and manganese sulphate. The biomass is homogenised in 50% ethyl alcohol for several hours and the final suspension is converted into a powder by drying. This procedure kills all viable cells of the producer organism. The activity of the final enzyme preparation is adjusted by dilution with cellulose powder or dextrin

The enzyme preparation is mixed carefully into those wines destined for ageing for more than 1 year at doses ranging from 25 mg/L to 75 mg/L and treatment is continued for at least 4 weeks at temperatures above 15 C. When the concentration of urea has fallen to at least <1mg/L any residual enzyme preparation is removed by filtration using a filter of pore size <1 µm and filtering aids.

Toxicological information

In a 28-day gavage study doses of 0, 200, 600 or 2000 mg/kg b.w./day of a preparation of urease with an urease activity of 6.0 U/mg were administered to rats. Based on the increased red cell count and haemoglobin at the top dose, the NOEL in this study was 600 mg/kg b.w. No other abnormal clinical signs or toxicologically relevant adverse findings were noted (2).

No evidence for a mutagenic potential was found in a modified rec-assay using *B.subtilis H17* (rec $^+$) and *M 45* (rec $^-$). A gene mutation test using *E.coli* WP2uvrA did not show any increase in mutant colonies and a modified Ames test, using *Salmonella typhimurium* TA100 and TA98 with and without S9 mix also produced no increase in the number of revertant colonies. A sex-linked recessive lethal test in *Drosophila melanogaster* was negative (2).

No antimicrobial activity was demonstrable in the culture medium in which *Lactobacillus fermentum* IFO 14511 was cultivated statically for 2 days at 37 C and at pH 7.0, when tested against 6 known common pathogenic organisms (2).

Conclusion

In this submission (2) for a urease to be used in wine production the enzyme is retained in the spray-dried biomass preparation of *Lactobacillus fermentum*, a non-pathogenic, non-toxigenic organism, that is a common constituent of many foods and a commensal of the human gut flora. The toxicological tests on the enzyme preparation do not give any cause for concern. Neither are there any concerns about the constituents of the fermentation medium. Furthermore, the enzyme preparation solids are eventually removed from the treated wine.

Although the studies submitted did not fully correspond to the requirements of the SCF guidelines (1), the abovementioned considerations enabled the Committee to conclude the following.

There is no reason to object to the use of a urease preparation, complying with the technical specification mentioned earlier, for reducing the urea content of a wine destined for ageing, when used under GMP conditions. The Committee is informed, that at present a dose up to 100 mg/L is sufficient for this purpose.

References

1. SCF (1992). Opinion adopted on 11 April 1991. Twentyseventh Series of Reports of the SCF, pg 13-22. Cat. N° EUR 14181. Luxembourg
2. Takeda Chemical Industries Ltd, (1997) Petition submitted to the European Commission
3. Conseil Supérieur d'Hygiène Publique de France (1994) Avis issued 21.6.1994