EUROPEAN COMMISSION
HEALTH & CONSUMER PROTECTION DIRECTORATE-GENERAL

Directorate B - Scientific Health Opinions
Unit B3 - Management of scientific committees II

SCIENTIFIC COMMITTEE ON FOOD

SCF/CS/ADD/EDUL/193 Final
6/12/99
Annex III to the minutes of
the 119th Pleanry meeting

OPINION ON MANNITOL MANUFACTURED BY FERMENTATION

(expressed on 2 December 1999)

Rue de la Loi 200, B-1049 Bruxelles/Wetstraat 200, B-1049 Brussel - Belgium - Office: BE232 - 6/37.
Telephone: direct line (+32-2) 295.81.10 / 296.48.70, exchange 299.11.11. Fax: (+32-2) 299.48.91
Telex: COMEU B 21877. Telegraphic address: COMEUR Brussels.
http://www.europa.eu.int/comm/dg24/health/sc/scf/index_en.html

Opinion on mannitol manufactured by fermentation

(expressed on 2 December 1999)

Terms of Reference

To evaluate the safety-in-use of mannitol manufactured by fermentation.

Background

Mannitol (E421) was evaluated by the Committee in 1984 and considered acceptable as a sweetener for general food use (1). According to the current EC Sweeteners Directive (2), it can be used "quantum satis" in desserts and similar products, in confectionery as a sweetener and, with some exceptions, in foodstuffs in general for purposes other than sweetening (3).

Currently, mannitol is produced by the catalytic hydrogenation of a mixture of glucose and fructose, made from invert sugar. A petitioner has proposed a new manufacturing process by discontinuous fermentation under aerobic conditions using a conventional strain of the yeast *Zygosaccharomyces rouxii* (4).

Specification aspects

The analytical data provided show that this form of mannitol manufactured by fermentation conforms to present specifications for mannitol. Analysis of six batches gave purity >99% (assayed by HPLC), compared to the EU specification of >96% purity.

A minor difference from the usual commercial mannitol is the absence of sorbitol and the presence of traces of arabitol (up to 0.3%). At present, there is no mention of the percentage of arabitol in the specification for mannitol.

The applicant has provided acceptable microbiological specifications for the final product:

Aerobic mesophilic bacteria	< 1000/g
Coliforms	Absence in 10 g
Salmonella	Absence in 10 g
E.coli	Absence in 10 g
Staphylococcus aureus	Absence in 10 g
Pseudomonas aeruginosa	Absence in 10 g
Moulds	< 100/g
Yeasts	< 100/g

Mannitol is the final product resulting from the fermentation and is unlikely to contain microorganisms of public health significance if produced by Good Hygienic Practice (GHP) and HACCP principles have been applied.

Microbiological aspects

The product (mannitol) is obtained by fermentation using a strain of the yeast *Zygosaccharomyces rouxii*. The organism has been characterised phenotypically and its identity confirmed by an internationally recognised culture collection (CBS, The Netherlands). The organism has not been derived by genetic manipulation.

The yeast *Zygosaccharomyces rouxii* is a spoilage organism of low water activity foodstuffs and has a long history of use in traditional fermented foods such as miso soy sauce. No evidence was found in the literature to suggest that the yeast is pathogenic to man. The end product of the process (mannitol) will not expose the consumer to *Zygosaccharomyces rouxii*.

Toxicological aspects

Studies on the acute toxicity of a *Zygosaccharomyces rouxii* culture containing yeast cells, growth media and fermentation products were performed by oral and intraperitoneal administration to mice. No mortality and no compound-related effects were observed at doses of about 10^9 cells/g body weight (4).

In a subchronic toxicity study, a material prepared by filtering the fermentation broth on a pre-coat covered with potato starch was fed to rats for 90 days in concentrations of 0, 1.25, 2.5 and 5% in the diet. The study, carried out according to OECD guidelines, did not reveal any treatment related effects (4).

Both the supernatant of a culture of *Zygosaccharomyces rouxii* and the dimethylsulfoxide extract from freeze-dried lysed cells of this yeast were tested for genotoxicity in *Salmonella typhimurium*, strains TA 1535, 1537, 1538, 98 and 100, and at the TK locus of L5178Y mouse lymphoma cells, with and without metabolic activation. In these studies, conducted according to OECD guidelines, using a microtiter cloning technique, the test materials did not induce gene mutations in either system (4).

The tested materials were complex mixtures of yeast cells, growth media and fermentation products. While these studies are relevant for the evaluation of mannitol produced by the fermentation process, these studies are not relevant for the toxicological evaluation of the mannitol component itself. There are, however, short- and long-term feeding studies and mutagenicity studies on mannitol as such, which were considered by the Committee as sufficient evidence for safety-in-use, when mannitol was evaluated previously by the SCF and the Joint FAO/WHO Expert Committee on Food Additives (1,7).

The only new by-product reported to be present in mannitol produced by fermentation is arabitol. While there are no toxicological studies on arabitol itself, as a 5-carbon sugar alcohol, isomeric with xylitol, occurring naturally in, for example, mushrooms, the Committee considered that a content up to 0.3% in mannitol would not be of health concern.

Conclusion

Zygosaccharomyces rouxii is a spoilage organism of low water activity foodstuffs and has a long history of use in traditional fermented foods. There is no evidence that the organism is pathogenic and the consumer will not be exposed to the organism itself in the final product. The toxicological studies with the fermentation broth and derived test preparations did not indicate that the examined strain of the yeast *Zygosaccharomyces rouxii* produces toxic or mutagenic fermentation products. Mannitol itself has been previously evaluated by the Committee as safe-in-use.

Thus there are no toxicological or microbiological objections to the use of mannitol made by fermentation by *Zygosaccharomyces rouxii*. However, the Committee recommends that the present specification on mannitol is modified to include a maximum limit for arabitol and for microbiological parameters as specified in the text.

References:

1. Commission of the European Communities (1985). Reports of the Scientific Committee for Food, 16th Series. CEC, Luxembourg. ISBN 92-8255773-1.
2. European Parliament and Council Directive 94/35/EC of 30 June 1994 on sweeteners for use in foodstuffs. Official Journal of the European Communities No. L 237, 10.09.94, pp 3-12.
3. European Parliament and Council Directive 95/2/EC of 20 June 1994 on food additives other than colours and sweeteners. Official Journal of the European –Communities No. L 61,18.03.95, pp 1-40.
4. Petition for authorisation of mannitol produced by fermentation process. Unpublished Dossier No. 133.01, submitted to the European Commission by Roquette Frères, 22 February 1996.
5. Commission Directive 95/31/EC of 5 July 1995 laying down specific criteria of purity concerning sweeteners for use in foodstuffs. Official Journal of the European Communities No. L 257, 19.09.98, p. 35.
6. Council Directive 93/43/EEC of 14 June 1993 on the hygiene of foodstuffs. Official Journal No. L 175, 19.07.93.
7. Joint FAO/WHO Expert Committee on Food Additives (1987). Toxicological evaluation of certain food additives and contaminants. WHO Food Additives Series 21, pp 123-136, Cambridge University Press.