

SCIENTIFIC OPINION

Inability to assess the safety of copper-enriched yeast added for nutritional purposes as a source of copper to food supplements, based on the supporting dossiers¹

Scientific Statement of the Panel on Food Additives and Nutrient Sources added to Food (ANS)

(Question No EFSA-Q-2005-118, EFSA-Q-2005-188)

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PANEL MEMBERS

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BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

The European Community legislation lists nutritional substances that may be used for nutritional purposes in certain categories of foods as sources of certain nutrients.

The Commission has received a request for the evaluation of copper-enriched yeast added for nutritional purposes to food supplements. The relevant Community legislative measure is:

- Directive 2002/46/EC of the European Parliament and of the Council on the approximation of the laws of the Member States relating to food supplements².

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

In accordance with Article 29 (1) (a) of Regulation (EC) No 178/2002, the European Commission asks the European Food Safety Authority to provide a scientific opinion, based on its consideration of the safety and bioavailability of copper-enriched yeast added to food supplements.

² OJ L 183, 12.7.2002, p.51.

STATEMENT

1. Introduction

Following a request from the European Commission to the European Food Safety Authority (EFSA), the Scientific Panel on Food Additives and Nutrient Sources added to Food (ANS) was asked to provide a scientific opinion on the safety of copper-enriched yeast added for nutritional purposes as a source of copper to food supplements and on the bioavailability of copper from this source.

This statement is based on the information on copper-enriched yeasts provided by two petitioners.

2. Summary of the information provided in the supporting dossiers on copper-enriched yeasts

Copper-enriched yeasts are derived from cultures of specified strains of *Saccharomyces cerevisiae* in the presence of natural substrates and a source of copper (copper sulphate).

According to one of the petitioners, copper in copper-enriched yeast is naturally integrated by the growing yeast into its own structure and occurs therefore in the way copper would be present in any food material. According to the other petitioner, copper-enriched yeast is “a complex of proteins, peptides and amino acids, resulting from the hydrolysis of *Saccharomyces cerevisiae*, which are bound to copper”.

Copper-enriched yeast has no specific chemical identity (name, CAS No., molecular weight) but is chemically defined in terms of its copper content, following culture of the yeast in the presence of a source of copper (copper sulphate, $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$).

Copper-enriched yeast is described by one of the petitioners as a dark green powder with a characteristic yeast odour. The other petitioner describes the product as an off-white powder with a characteristic yeast-like odour and taste. Both petitioners provided various data on the chemical and microbiological specifications for copper-enriched yeast. The petitioners indicate a copper content ranging from 1.0 to 1.1% of the source. The remaining 99% of the material is made up of enzymatically ruptured yeast cells.

Microbiological specifications were provided by both petitioners in their application dossiers, but specifications for arsenic, cadmium, lead and arsenic were provided by only one petitioner.

Additional information is given by both petitioners on the analytical methods used for the identification and characterisation of the copper-enriched yeast, its purity and the residual impurities of the end product. One petitioner provides Fourier Transform Infrared Spectroscopy (FTIR) spectra of samples of the starter yeast (as reference) and the copper-enriched yeast. A comparative elemental analysis for carbon, hydrogen, and nitrogen (C:H:N analysis) of the starter yeast and the copper-enriched yeast is provided by one petitioner. The same petitioner states that “Confirmation that bio-transformed copper differs from the starter material has been achieved using X ray photoelectron spectroscopy (XPS)”. According to this petitioner, the difference in the C:H:N ratio between the starter yeast and the copper-enriched yeast supports the hypothesis that changes within the yeast due to the incorporation of the mineral into the internal structure of the yeast may have modified the overall composition of

the yeast. Furthermore it is stated that the “XPS data indicate that copper is not present in its elemental form nor in its pre-fermented form but that some incorporation into the yeast structure itself has taken place. The binding energies from the XPS analysis would suggest that copper is present as oxides, chlorides or more complex entities such as $[\text{N}(\text{C}_2\text{H}_5)]_2$ $[\text{CuCl}_4]$ none of which are the sulphate used as starter material”.

The manufacturing process is adequately described by one of the petitioners; the other petitioner provided only brief details on the manufacturing process.

Analytical methods for the determination of the source in food are based on the determination of total copper by Atomic Absorption Spectroscopy (AAS).

Both petitioners state that copper-enriched yeast is stable in foods and food supplements for a minimum of three years.

Copper-enriched yeast is stated to be used as an ingredient in tablets, caplets, capsules, chewable tablets, effervescent powders and liquids that are food supplements. One petitioner mentions products containing enriched yeast, providing an intake of between 80 and 1000 µg copper/day. According to the other petitioner, copper-enriched yeast is incorporated into supplements at levels providing daily intakes of copper up to 2000 µg copper/day.

No estimation was provided by one of the petitioners on the bioavailability of copper from copper-enriched yeast, other than a statement that “copper-enriched yeast is more bioavailable than copper sulphate provided to the yeast strain during the production process”. According to the other petitioner, “studies have indicated that copper from copper-enriched yeast is more readily bioavailable than inorganic or organic copper salts”. The same petitioner provided a summary of data on the bioavailability of copper from the source.

An 8-week study was conducted to assess the bioaccumulation of copper in eggs, blood from wing vein and feathers of laying hens (Dobrzański *et al.*, 2008). Sixty Lohmann-Brown layers were divided into five groups, three of them were supplemented with 10 mg copper/kg diet (approximately 1.25 mg/kg bw/day) either as part of an inorganic mixture containing copper, iron and manganese (considered as control group), as a copper-enriched yeast source, or as a copper-enriched yeast source in combination with manganese- and iron-enriched yeasts. Feed was standardised to contain a constant amount of copper during the study (11.2 mg copper/kg diet). Eggs for analysis (12 per group) were collected after 25, 40 and 55 days of supplementation, and copper was measured by Inductively Coupled Plasma-Mass Spectroscopy (ICP-MS). Results showed that copper concentration in the egg contents was statistically significantly higher ($p < 0.05$) in animals fed copper-enriched yeast only, as compared to the other groups. The mean copper concentration in egg shells was also statistically significantly higher ($p < 0.05$) in animals fed copper-enriched yeast and in animals fed the mixture of copper-, iron- and manganese-enriched yeasts, in comparison to the control group. Results of blood analysis also showed a statistically significant increase ($p < 0.05$) in copper concentration in animals fed copper-enriched yeast alone. Results from hens’ feathers were difficult to interpret due to environmental interferences.

A study with 64 copper-deficient Charles-River male weanling rats compared the bioavailability of copper from copper gluconate and from copper-enriched yeast (Vinson *et al.*, 2007). The rats were divided into six groups and fed copper-enriched diets containing 4, 8 or 20 mg copper/kg diet (equivalent to 0.2, 0.4 and 1 mg/kg bw/day) as copper-gluconate or copper-enriched yeast for 6 weeks. Copper concentrations in liver samples were measured by Atomic Absorption Spectroscopy (AAS). Results showed that copper-gluconate and copper-enriched yeast statistically significantly ($p < 0.01$) increased copper liver concentrations in

animals fed these diets. At the highest dose, animals showed higher copper concentrations in liver when fed copper-gluconate as compared to copper-enriched yeast. The authors concluded from the comparison of the dose-response curves in this study that copper from copper-enriched yeast was 43.5% more bioavailable than from copper-gluconate.

In a study with 25 piglets, groups of five animals were treated to compare the relative absorption of copper from copper-enriched yeast and from copper sulphate (Korniewicz *et al.*, 2007). Relative absorption was calculated by comparing faeces and urine copper excretion every four days during a 3-week feed supplementation period with 20 mg copper/kg diet (equivalent to 0.8 mg copper/kg bw/day) either as copper-enriched yeast or as copper sulphate. Relative absorption of copper in animal groups fed diets containing copper-enriched yeast (~28%) was statistically significantly higher ($p < 0.05$) when compared to those from the control group fed diets containing copper sulphate (~24%). By subtracting copper intake from excreted copper, the authors concluded that copper retention in animals fed copper-enriched yeast statistically significantly ($p < 0.05$) increased up to approximately 26% compared to those from the control group fed copper sulphate (increased up to approximately 21%).

No toxicological data were provided on the sources. According to both petitioners copper-enriched yeasts are safe.

Although not explicitly stated in the dossiers, the argument for the safety of copper-enriched yeast appears to be based on copper being a normal constituent of the diet, and the long history of use of *Saccharomyces cerevisiae* in fermented food and beverages. The assumption is that, provided there is no overload of normal metabolic pathways, fermentation within eukaryotic cells will produce copper compounds, not further defined but with a metabolic fate and distribution similar to that of other sources of copper in the diet.

3. Assessment

Chemical information

The Panel notes that *Saccharomyces cerevisiae* has a qualified presumption of safety (EFSA, 2008) but considers that this presumption of safety might not be applicable to the specific conditions of culture of the yeasts in presence of a high quantity of copper.

Analytical results on three mineral-enriched yeasts would suggest that in general the crude chemical composition in terms of ash, protein, fat, minerals (except those added to enrich the yeast) and amino acids composition are comparable to the non-enriched yeast (Dobrzański *et al.*, 2003).

However, the Panel considers that the C:H:N analysis is not relevant to compare the starter yeast and the copper-enriched yeast and that such a difference in the C:H:N ratio would not in any case provide clear evidence of incorporation of copper or change in the structure of the yeast. The Panel considers that the XPS spectra provided can give some information on the crystallinity of copper-enriched yeasts, but do not provide a significant contribution to its chemical characterisation. Also, the Panel considers also that the FTIR spectra provided do not demonstrate the existence of coordinate bonds between copper and the yeast biomass.

Bioavailability information

The Panel notes that copper is an essential element in *Saccharomyces cerevisiae* yeasts serving as cofactor in a variety of enzymes such as cytochrome oxidase, Cu-, Zn- superoxide dismutase, etc, (Linder and Hazegh-Azam, 1996). Copper homeostasis (absorption-mobilisation) is tightly regulated by specific high affinity proteins in yeast (Peña *et al.*, 2000; Rees *et al.*, 2004) that probably also occur in humans (Puig *et al.*, 2002). Copper is readily absorbed by humans from the diet, its bioavailability is reported to be between 55 and 75% (Linder and Hazegh-Azam, 1996).

Overall the Panel concludes that the results of available experimental studies suggest that copper from copper-enriched yeast sources is absorbed and is bioavailable to an extent comparable to that from other copper sources tested.

Copper is naturally found in yeast at reported concentrations of 8 mg/kg (Linder and Hazegh-Azam, 1996) however the copper-enriched yeast sources considered in this opinion contain more than 1000-times this concentration (up to 11 g/kg). The Panel considers therefore that it cannot be assumed that, under these conditions, the normal copper homeostasis in copper-enriched yeast is not disturbed nor that the copper-containing compounds in the copper-enriched yeasts will show a similar metabolic fate to that of other sources of copper in the diet.

CONCLUSIONS

Overall, the Panel concludes that the bioavailability of copper from copper-enriched yeast is at least similar to that from other copper sources (i.e. copper sulphate, copper gluconate).

The Panel also concludes that due to the lack of appropriate dossiers supporting the use of copper-enriched yeast in food supplements, the safety of the copper-enriched yeasts under consideration cannot be assessed.

Key words:

Food supplements, copper, copper sulphate, yeast-transformed copper, copper-enriched yeast

DOCUMENTATION PROVIDED TO EFSA

1. Technical dossier 2005a. Dossier on copper-enriched yeast (copper-enriched *Saccharomyces cerevisiae*) proposed for addition to Annex II of Directive 2002/46/EC of the European Parliament and of the Council Relating to Food Supplements. March 2005. Submitted by Nature's Own Limited, UK.
2. Technical dossier 2005b. Dossier on Bio-transformed copper proposed for addition to Annex II of Directive 2002/46/EC of the European Parliament and of the Council Relating to Food Supplements. Original submission June 2005; Additional information submitted January and October 2008. Submitted by Higher Nature Ltd, UK.

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REFERENCES

- Dobrzański Z, Korniewicz A, Dolińska B, Górecki H, Jamroz D, Ryszka F, 2003. The chemical content and feeding value of enriched with chromium, selenium and zinc yeast *Saccharomyces cerevisiae*. Proceedings XI International Congress ISAH. 23-27 February 2003, Mexico.
- Dobrzański Z, Korczynski M, Chojnacka K, Gorecki H, Opalinski S, 2008. Influence of organic forms of copper, manganese and iron on bioaccumulation of these metals and zinc in laying eggs. *Journal of Elementology* 13, 309-319.
- EFSA (European Food Safety Authority), 2008. Opinion of the Scientific Panel on Biological Hazards on the maintenance of the list of QPS microorganisms intentionally added to food or feed. *The EFSA Journal* 923, 1-48.
- Korniewicz D, Dobrzański Z, Chojnacka K, Korniewicz A, Kolacz R, 2007. Effect of dietary yeasts enriched with Cu, Fe and Mn on digestibility of main nutrients and absorption of minerals by growing pigs. *American Journal of Agricultural and Biological Sciences* 2, 267-275. Available at: <http://www.scipub.org/fulltext/AJAB/AJAB24267-275.pdf>
- Linder MC, Hazegh-Azam M, 1996. Copper biochemistry and molecular biology. *American Journal of Clinical Nutrition* 63, 797S-811S.
- Peña MMO, Puig S, Thiele DJ, 2000. Characterisation of the *Saccharomyces cerevisiae* high affinity copper transporter Ctr3. *Journal of Biological Chemistry* 275, 33244-33251.
- Puig S, Lee J, Lau M, Thiele DJ, 2002. Biochemical and genetic analysis of yeast and human high affinity copper transporters suggest a conserved mechanism for copper uptake. *Journal of Biological Chemistry* 277, 26021-26030.
- Rees EM, Lee J, Thiele DJ. 2004. Mobilization of intracellular copper stores by the Ctr2 vacuolar copper transporter. *Journal of Biological Chemistry* 279, 54221-54229.

Vinson JA, Tompkins TA, Agbor GA, 2007. Comparative bioavailability of mineral-enriched gluconates and yeast in rat liver after depletion-repletion feeding. *Biological Trace Element Research* 118 (2), 104-110.

GLOSSARY / ABBREVIATIONS

AAS	Atomic Absorption Spectroscopy
ANS	Scientific Panel on Food Additives and Nutrient Sources added to Food
CAS	Chemical Abstracts Service
EC	European Commission
EFSA	European Food Safety Authority
FTIR	Fourier Transform Infra Red
ICP-MS	Inductively Coupled Plasma Mass Spectroscopy
XPS	X-ray Photoelectron Spectroscopy