Ochratoxin A in Brewer's Yeast Used as Nutrient Supplement

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Abstract

Brewer's yeast comprises different strains of *Saccharomyces cerevisiae* used for beermaking. It is additionally used as a nutrient supplement to increase the intake of B vitamins and is recommended primarily for children in growth, women during pregnancy and lactation and persons during convalescence. A total of 51 samples of brewer's yeast from the German market were analysed for the occurrence of ochratoxin A (OTA) by means of immunoaffinity clean up and HPLC with fluorescence detection. Thirty-two samples (63%) were found to be naturally contaminated with OTA in the range from the detection limit (0.03) to 1.53 ng/g. Mean values of the positive samples varied between 0.10 ng/g (powder) and 1.2 ng/g (drages). In a worst case scenario, the consumption of brewer's yeast could enhance the calculated daily intake for the German population by 10 to 14 ng OTA/day and person and increase the intake particularly for children from 1.3 up to about 1.9 ng/kg body weight.

Thus, the results document that food supplements consisting of natural brewer's yeast from the brewing process are a yet unknown source for the intake of ochratoxin A and a potential exposure risk. The screening of brewer's yeast food supplements for OTA is therefore recommended in the context of food safety and quality control.

Keywords: Mycotoxin, ochratoxin A, food supplement, *Saccharomyces cerevisiae*

Introduction

Brewer's yeasts are special non-leavening yeasts used in beermaking. The different strains used for different beers belong taxonomically to *Saccharomyces cerevisiae* [1]. Because it is a rich source of B vitamins and other nutritional factors, brewer's yeast originating from the brewing process is also used as a dietary supplement and has been a staple of the health food industry since its inception. The consumer target...
groups for the use of brewer's yeast are primarily children in the growth, women in the pregnancy and lactation period as well as persons during convalescence and with high levels of physical activity. The yeasts are available in flake, powder, tablet, dragee, capsule and liquid forms.

It is known that brewing barley and malts as well as beer can be contaminated with ochratoxin A \([7-L-\beta\text{-phenylalanylcarbonyl-5-chloro-8-hydroxy-3,4-dihydro-3-R-methylisocumarin}]\) (OTA) [2]. For this reason the brewing process can possibly lead to a contamination of brewer's yeast with this mycotoxin. Aim of this study was therefore to investigate the natural occurrence of OTA in food supplements which consist of brewer's yeast.

**Materials and Methods**

A total of 51 samples of brewer's yeast from about 20 different suppliers were bought during 2001 in pharmacies, drugstores and health food stores in different towns of Germany (Berlin, Munich, Braunschweig, Gießen, Bayreuth, Kulmbach, Münchenberg).

Samples were finely ground and to aliquots of 10 g 20 ml of a 0.4 M magnesium chloride solution and 20 ml 20% trichloroacetic acid were added. Solutions were extracted on a magnet stirrer with 40 ml of chloroform for 30 min and then centrifuged at 10.000 rpm and 15°C for 20 minutes. 20 ml of a 0.13 M solution of sodium hydrogen carbonate were added to a 20 ml aliquot of the chloroform extract and mixed by stirring for 20 min. The mixture was centrifuged for 15 min at 4.000 rpm and 15°C. Ten ml of the (upper) aqueous phase were adjusted to pH ≤ 8.2 with 0.25 M hydrochloric acid. For clean up purpose immunoaffinity columns (R-Biopharm, Darmstadt) were used as reported previously [3]. After equilibration of the columns with 10ml PBS buffer, 10 ml of the sodium hydrogen carbonate phase were applied to the column at a flow rate of <1.5 ml/min. After washing with 10 ml of a 10% methanol/PBS solution and subsequently with 10ml PBS buffer OTA was eluted from the immunoaffinity column) with 3 ml of a 30% methanol/0.1 M glycine buffer (<1.5ml/min). The eluate was extracted twice with 3 ml methylene chloride. The solvent phases were then combined and dried.

The solid residue was dissolved in 100µl methanol and applied at 10µl aliquots to the Altima C18-high pressure liquid chromatography column of the HPLC system (Kontron, Garching). The isocratic separation was performed with a mixture of acetoniitrile/ water/acetic acid (570/410/20) at 25°C and a flow rate of 1ml/min. Measurements were performed by fluorescence detection at wavelengths of 440nm (emission) and 330nm (excitation). Under these conditions OTA exhibits a retention time of 9.6 min. For confirmation the positive samples were re-chromatographed following esterification with boron trifluoride and methanol in analogy to a previously described method [4].

**Results and Discussion**

Recoveries of OTA varied between 64% and 150% (Tab.1). The detection limit was found to be 0.03 ng OTA /g of sample.