PREPARATIVE PURIFICATION OF OCHRATOXIN A BY LIQUID-LIQUID CHROMATOGRAPHY

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Abstract
Ochratoxin A is an important mycotoxin, produced by different *Aspergillus* and *Penicillium* species hence there are strict regulations on its level in foods and feeds. Qualitative and quantitative measurements of this compound require relatively high amounts of pure ochratoxin A as a standard. This large amount of pure compound can be obtained by purifying the fermentation medium of the producing microorganisms. Liquid-liquid chromatography (LLC) seems to be a suitable method, which is becoming more common in the purification of natural compounds.

Introduction
Mycotoxins are toxic secondary metabolites produced by certain filamentous fungi. Among them ochratoxins have outstanding importance due to their high-level toxicity, which could cause remarkable problems in food and feed industry [1,2]. One of the most important member of this metabolite group is ochratoxin A, which was classified as possible human carcinogen (group 2B) by IARC [3]. In food safety laboratories, numerous methods are available for measuring these compounds from various matrices [4-6] requiring relatively high amounts of pure ochratoxins as standard compounds for both qualification and quantification. Generally, the chemical synthesis of ochratoxins can be accomplished with low yield [7], but higher amount of pure compound can be obtained by the purification of the fermentation environment of the producer microorganisms. Liquid-liquid chromatography may be a suitable method, whose application is becoming frequently used in the purification of natural compounds [8,9]. One of the technical implementations of this technique is the Centrifugal Partiton Chromatography (CPC), which was applied in our work for the separation of ochratoxins from the fermentation product and from each other.

Experimental
Aspergillus albertensis (SZMC 2107) was cultivated on yeast extract, sucrose media in dark at 28 °C. The incubation time and the sucrose content of the culture media was optimized for maximum ochratoxin production. For sample preparation a three-step acid-base extraction was used with ethyl acetate and sodium-bicarbonate as solvents. The crude extract was dispensed into 1,5 ml vials and were evaporated to dryness. For solvent system testing numerous three- and four-component biphasic systems were examined with the “Shake-flask” method. The selected systems were assembled and mixed in a test tubes, thereafter equal volumes of the phases were added to the vials containing the extract. The concentrations in the upper- and lower phase were measured by HPLC-UV technique. Partition coefficients (P) and separation factors (α) were calculated based on the concentrations, and the best system was selected for the purification procedure. During instrumental optimization suitable flow direction, flow rate and rotational speed was chosen. The purity of ochratoxin A and B in the collected fractions was calculated based on the areas of HPLC-UV chromatogram on 333 nm.

Results and discussion

At the beginning of our work the cultivation parameters of *Aspergillus albertensis* (SZMC 2107) were optimized. The maximum amount of ochratoxin A was measured after an incubation period of 8 days on liquid media containing 2 % yeast extract and 15 % sucrose. The crude extract obtained after liquid-liquid extraction contained ochratoxin A (OTA), ochratoxin B (OTB) and 9 major impurities. The extract was used for solvent system testing to find an appropriate biphase for the purification. Several compositions of one quaterner (hexane-ethyl-acetate-methanol-water) and 19 different type ternary systems were studied. The distribution coefficients and the separation factors of both OTA and OTB were in the proper range in a hexane-isopropanol-water system. After instrumental optimization the separation was carried out in ascendent mode at 10 ml/min flow rate and 2000 rpm rotational speed. The purities of OTA and OTB were more than 99% and 55 %, respectively.

Conclusion

The separation of ochratoxin A and B was accomplished using Centrifugal Partition Chromatography with the purities of more than 99 % and 55 %, respectively. Based on the results the developed method may be suitable for large scale purification of ochratoxin A in high purity, which is required for quantitative and qualitative measurements. Further investigation is necessary in order to increase the purity of OTB, and to confirm the purities by HR-MS and NMR techniques.

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