

Spectroscopic properties of Ochratoxin A and B

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Introduction

Ochratoxin A (OTA) is an ubiquitous mycotoxin and natural food contaminant which is produced by several storage fungi such as *Aspergillus ochraceus*, *Aspergillus niger* and *Penicillium verrucosum*. OTA can occur in a large variety of food, e.g. in cereals, beans, nuts, spices, fruits, coffee, beer or wine. Because of carry-over effects it can also be found in meat especially in kidneys of animals fed with contaminated feed. Moreover, in animal experiments this toxin was identified as immunotoxic, genotoxic, teratogenic, hepatotoxic, and potentially carcinogenic for humans.

Results

OTA (see Figure 1.) is composed of an isocoumarin unit connected to a phenylalanine unit. The photophysics of OTA is determined by intra- and intermolecular proton transfer reactions. These dynamic characteristics can be investigated using absorption, steady state and time resolved fluorescence spectroscopy.^[1]

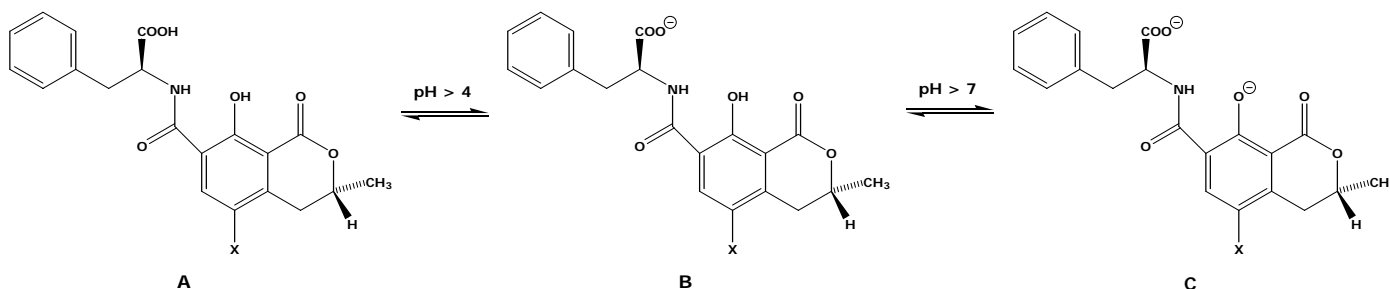


Figure 1: Deprotonation of Ochratoxin A (X=Cl) and B (X=H)

Structure A and B are showing an absorption maximum at $\lambda_{\text{abs}} = 333$ nm. In ethanolic solution (pH > 4) the phenolic group of the isocoumarin moiety is stabilized by a keto-enol-tautomerism (B). After the addition of a base the dianion is established (C) and the absorption maximum is red-shifted, while the fluorescence emission maximum shifts from 455 to 427 nm. Furthermore, in time-resolved fluorescence experiments non-monoexponential decay kinetics are observed (table 1).

	λ_{em} [nm]	τ_1 [ns]	τ_2 [ns]	τ_3 [ns]
OTA in EtOH	360	2.3	4.6	7.3
OTA in DMF	370 / 390	3.7	-	-
OTA in H ₂ O	360	2.9	5.2	-
OTB in EtOH	360	2.0	4.8	6.8
OTB in DMF	360	2.9	-	-
OTB in H ₂ O	360	2.4	3.8	6.3

Table 1: Fluorescence life times of OTA and OTB in different solvents (global fits)

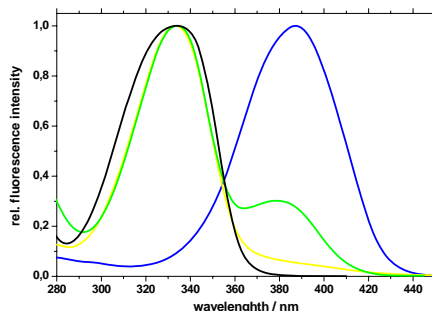


Figure 2: Fluorescence excitation spectra of Ochratoxin A in ethanol (light green), DMF (blue), water (yellow) and water pH<2 (black)

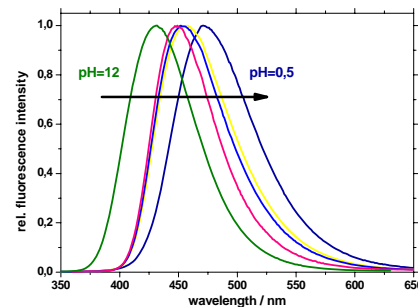


Figure 3: Fluorescence emission spectra of Ochratoxin A in water with different pH-values

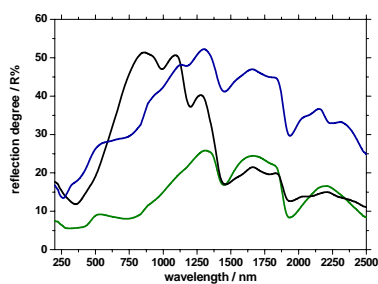


Figure 4: Reflection spectra of wheat with fungal contamination (blue), the pure culture of *Penicillium verrucosum* (green) and wheat with OTA (black)

The fluorescence excitation spectra of OTA in different solvents can be seen in figure 2. In acidic solution the spectral band around $\lambda = 333$ nm is broader than in ethanolic or neutral solution. The ethanolic solution shows two maxima, in DMF only one can be detected at $\lambda = 380$ nm. The red-shift of the emission maxima with decreasing pH-value is pointed out on the right (figure 3).

In addition, diffuse reflectance spectra in the UV/Vis and NIR spectral range from 200 nm < λ < 2500 nm (figure 4) are collected and analysed according to the Kubelka-Munk-Theory.^[2]

Because of the strong absorption of OTA and OTB in the UV range, it is difficult to separate fluorescence signals of the mycotoxins (figure 5) from the intrinsic matrix signals (e.g., proteins in grain and flour). The fluorescence of tryptophan coincides with the OTA fluorescence in the whole spectral range. Therefore, in case of fluorescence measurements the excitation conditions have to be chosen very carefully.

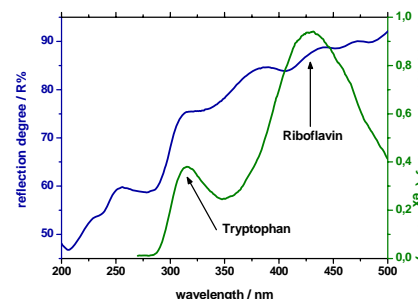


Figure 5: Reflection spectra (blue) of Ochratoxin A on wheat flour and fluorescence emission (green) of uncontaminated wheat flour

Conclusion

The absorption and fluorescence properties of Ochratoxin A and B vary in the different solvents because of proton transfer reactions. Due to the excited-state reactions the photophysical processes are complex and further analytical information are indispensable for a sensitive and selective in-situ fluorescence detection of OTA and related compounds (e.g. OTB) in food and feed.

References

- [1] Chu, F.S., 1974. Studies on Ochratoxins. CRC Critical Reviews in Toxicology, 2, 499-523
- [2] Kubelka, P., Munk, F., 1931. Ein Beitrag zur Optik der Farbanstriche. Zeitschrift für technische Chemie, 11a, 593-601

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