Thermal Stability of Aflatoxin B_1 and Ochratoxin A

LCI Cologne

Marion Raters and Reinhard Matissek

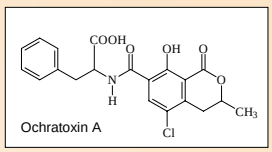
LCI *
Food Chemistry Institute of the Association of the German Confectionery Industry
Cologne/Germany, www.lci-koeln.de

Introduction

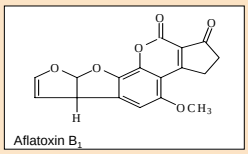
Aflatoxins and ochratoxin A (OTA) are secondary metabolites of toxigenic moulds of the genera Aspergillus and Penicillium. OTA primarily occurs in native starchy cereals [1, 2]. Aflatoxins frequently develop in high-protein products that are grown in humid warm regions, such as nuts (peanuts, pistachios), maize, dried figs, and various spices such as pepper and paprika [2]. The presence of these mycotoxins in foodstuffs is undesired due to their toxicological and carcinogenic potential [3].

In the past it was frequently reported that mycotoxins are predominantly stable, low-molecular compounds that are widely unresponsive to technological measures such as heat and alkali treatment, etc. [4–6]. However, other studies, some being of a more recent date, show a distinct reduction (up to 90 %) of the level of OTA using certain roasting parameters in the manufacture of coffee [7–10]. The scientific literature available to us from the coffee sector shows there is a connection between the initial mycotoxin level of raw coffee beans, the respective type of contamination (natural or artificial), and the degree to which OTA is reduced. As for the thermal stability of aflatoxins, there is hardly any recent data available.

The objective of this research project is to examine the thermal stability of aflatoxins and OTA, both in pure form and in respect of the impact of certain matrix substances, including carbohydrates and proteins, on the thermal degradation behaviour of mycotoxins.



Ochratoxin A



Aflatoxin B_1

Experimentals

The assays carried out to determine the thermal stability of aflatoxins and OTA were performed on so-called mycotoxins in pure form and on model matrices artificially contaminated with those mycotoxins. For the thermal essays performed on pure mycotoxins, standard solutions having defined concentrations were evaporated till dry in a stream of nitrogen and, after thermal treatment, the mycotoxins – clinging in a thin layer to the inside of the glass flask – were rediluted and subsequently analysed. The model matrices used were starch powder, soya protein, and the polyphenol epicatechin spiked with defined amounts of the named mycotoxins. Depending on the required temperature, thermal treatments were performed using a desiccator cabinet and a muffle furnace.

The most frequent method used for analysing mycotoxins is high performance liquid chromatography with fluorescence detection (HPLC-FLD). In this case, the mycotoxins are isolated beforehand using extremely specific immunoaffinity columns. The assays presented here made use of the miniaturised analysis method for the combined determination of aflatoxins and OTA, already presented previously [11].

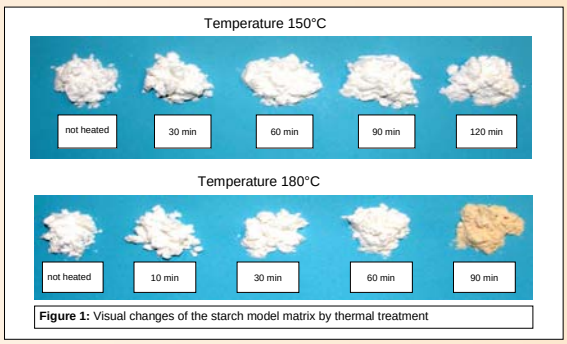


Figure 1: Visual changes of the starch model matrix by thermal treatment

References

[1] Reiß J (1981) Mykotoxine in Lebensmitteln. Fischer, Stuttgart
[2] Deutsche Forschungsgemeinschaft (1990) Ochratoxin A – Vorkommen und toxikologische Bewertung. VCH Verlagsgesellschaft, Weinheim
[3] Märtlbauer E, Usleber E, Dietrich R (1999) Mykotoxine – Eine Übersicht. Der Lebensmittelbrief 10: 131–134
[4] Struder-Rohe I, Dietrich D, Schlatter J, Schlatter C (1994) Ochratoxin A and Coffee. Mitt Gebiete Lebensm und Hygiene 85: 719–727
[5] Tsubouchi H, Yamamoto K, Hisada K, Sakabe Y, Udagawa S (1987) Effect of roasting on Ochratoxin A level in green coffee beans inoculated with Aspergillus ochraceus. Mycopathologia 97: 147–149
[6] Bösenberg H (1970) Untersuchungen über den Nachweis von Aflatoxinen. Arzneim-Forsch (Drug Res) 20, 1157–1167
[7] Van der Stegen GHD, Essens PJM, van der Lijn J (2001) Effect of roasting conditions on reduction of Ochratoxin A in coffee. J Agric Food Chem 49: 4713–4715
[8] Heilmann W, Rehfeldt AG, Rotzoll F (1999) Behavior and reduction of Ochratoxin A in green coffee beans in response to various processing methods. J Agric Food Chem 209: 297–300
[9] Blanc M, Pittet A, Munoz-Box M, Viani R (1998) Behavior of Ochratoxin A during green coffee roasting and soluble coffee manufacture. J Agric Food Chem 46: 673–675
[10] Romani S, Pinnavaia GG, della Rosa M (2003) Influence of roasting levels on Ochratoxin A content in coffee. J Agric Food Chem 51: 5168–5171
[11] Raters M, Matissek R (2004) Zur Verteilungscharakteristik von Mykotoxinen bei Kakaobohnen. Lebensmittelchemie 58: 134

Results

Figures 2 and 3 show the findings of our research for aflatoxin B_1 (AFB_1) and OTA respectively, also showing the two temperature levels applied, namely 150°C and 180°C. Both diagrams reveal a quite contrary degradation behaviour of OTA and AFB_1 when subjected to heat. OTA in pure form is only slightly degraded. A temperature of above 180°C is required to degrade more than 40 % of its initial content level. In the case of AFB_1, however, a heat treatment temperature of only 150°C was needed to reduce its initial content level by 70 %. Furthermore, our research showed that heat treatment levels as of 180°C led to complete degradation of AFB_1 (100%).

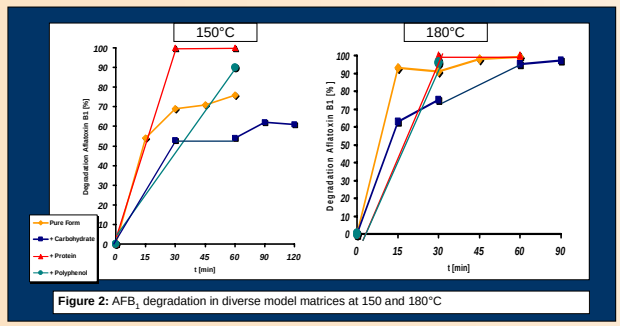


Figure 2: AFB_1 degradation in diverse model matrices at 150 and 180°C

Heat treatment distinctly reduced the mycotoxin content of all analysed model matrices. The highest degradation rates were observed for both mycotoxins in the soya protein matrix. Complete degradation was observed for both AFB_1 and for OTA at heat treatment levels of 150°C and 180°C over a period of 30 minutes. Compared to the behaviour of pure-form mycotoxins, the presence of starch and epicatechin led to increased degradation, especially in the case of OTA and at heat treatment levels of $\geq$ 180°C (cf. Figure 3). AFB_1 is almost completely degraded when subjected to matrix effects and a heat treatment temperature of $\geq$ 180°C.

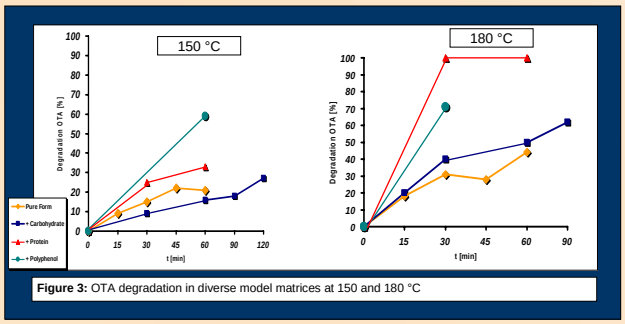


Figure 3: OTA degradation in diverse model matrices at 150 and 180 °C

Conclusion

In sum it can be said that the tests we carried out on the thermal stability of mycotoxins in pure form corroborated the findings we gathered from the relevant literature. OTA could only be degraded to a minor extent by heat impact and only at temperatures of 180°C and above. As regards the test conducted to examine the thermal stability of aflatoxins in pure form, a completely contradictory behaviour was determined compared to the data gathered from the relevant literature. As of heating temperatures of approximately 160°C, AFB_1 was almost completely degraded.

The extent to which this behaviour is also transferable to mycotoxins in matrix was tested on several select model matrices within the scope of this research project. The model assays showed that the degradation of mycotoxins is improved by the existence of certain matrix components. With the presence of proteins, in this case tested on soya protein, heat treatment as of 170°C achieved a complete degradation of OTA and AFB_1. Other matrices, such as carbohydrates and polyphenols, also improve, though to a lesser extent, the degradation of these mycotoxins by way of heat treatment. These findings make it likely that the thermal reaction products from our thermal treatment assays, stemming from the corresponding matrix and the spiked mycotoxins, no longer possess the characteristic fluorescence of mycotoxins. Our research did not include the identification of these reaction products. For this reason, nothing can be said about the toxicity of the reaction products.

This project was backed by the German Cocoa and Chocolate Industry Foundation, Hamburg

Presented at the 30th Mycotoxin Workshop in Utrecht on 28.–30. April 2008

* LCI ⬩ Lebensmittelchemisches Institut des Bundesverbandes der Deutschen Süßwarenindustrie (BDSI) e. V.