

Production of ochratoxin A in dry-cured ham based-media by *Aspergillus westerdijkiae*

Ziva Vipotnik¹, Alicia Rodríguez², Paula Rodrigues^{1,*}

¹Mountain Research Centre (CIMO), ESA, Polytechnic Institute of Bragança, Campus de Santa Apolónia, 1172, 5300-253 Bragança, Portugal

² University of Extremadura, Food Hygiene and Safety, Meat and Meat Products Research Institute, Cáceres, Spain

* prodrigues@ipb.pt

Introduction

Ochratoxin A (OTA) is a secondary metabolite produced by *Penicillium* and *Aspergillus* genera and is considered one of the most important mycotoxins occurring in animal and human food chains. Oxidative stress, inhibition of protein synthesis, disruption of calcium homeostasis, inhibition of mitochondrial respiration and DNA damage are some of OTA's mechanisms of action causing teratogenicity, immunotoxicity, neurotoxicity and mostly nephrotoxicity. In dry-cured and fermented meat products, OTA is strongly associated with *Penicillium nordicum* (PN), but *Aspergillus westerdijkiae* (AW), a strong OTA producer usually associated with contamination of coffee beans, has also been found to be responsible for high OTA levels in cured meat products.

Fungal growth and OTA production in meat products can be influenced by environmental conditions, physico-chemical characteristics of the matrix, and its endogenous flora. OTA is highly stable, so its destruction during normal food processing is very difficult to achieve.

Objective

The aims of this study was to evaluate potential risk of *Aspergillus westerdijkiae* in dry-cured ham compared to the ham-adapted OTA producer *Penicillium nordicum*.

Materials and methods

Fungal strains:

Aspergillus westerdijkiae, AW 6B/131 and type-strain AW CECT 2948; *Penicillium nordicum*, PN 44 and the type-strain PN CBS 110.769

Culture media:

3 % (w/v) of lyophilised dry-cured ham, 2 % agar, glycerol and NaCl as shown in table.

OTA evaluation: OTA was extracted from agar plugs with methanol and quantified by HPLC-FLD (λ_{ex} 330 nm and λ_{em} 463 nm) with a RP-C18 column (100 x 4.6 mm), with isocratic elution in water: acetonitrile: acetic acid (29.5 : 70 : 0.5) at 0.8 mL/min.

a_w	glycerol (%)	NaCl (%)
0.99	0	0
0.97	2	3
0.93	8	6
0.90	16	8
0.85	20	10

Results and Discussion

- *A. westerdijkiae* was able to produce the toxin in a wider set of conditions and at significantly higher levels than *P. nordicum* under all tested conditions.
- The highest amounts of OTA by *A. westerdijkiae* were detected at 20 °C and 0.97 a_w (1934 ng/g) and 0.93 a_w (155 ng/g). At 15 °C OTA was detected only at low levels only for the highest a_w values (0.99 and 0.97).
- *A. westerdijkiae* type-strain CECT 2948 was able to produce OTA at all tested temperatures, and produced higher amounts of OTA at higher a_w .
- *A. westerdijkiae* native strain AW 6B/131 was able to produce higher amounts of the toxin at lower a_w conditions (a_w 0.90).
- *A. westerdijkiae* produced significantly higher amounts of OTA than *P. nordicum* ($p = 0.014$), except at 15 °C and 0.97 a_w .
- *P. nordicum* CBS 110769 was able to produce detectable amounts of OTA at all temperatures except 30 °C, with the maximum (712 ng/g) being produced at 20 °C and 0.97 a_w .
- *P. nordicum* native strain PN 44 produced OTA at 30 °C at 0.97 a_w , and at 20 – 25 °C between 0.99 and 0.93 a_w . At 15 °C OTA was produced only at 0.97 a_w .

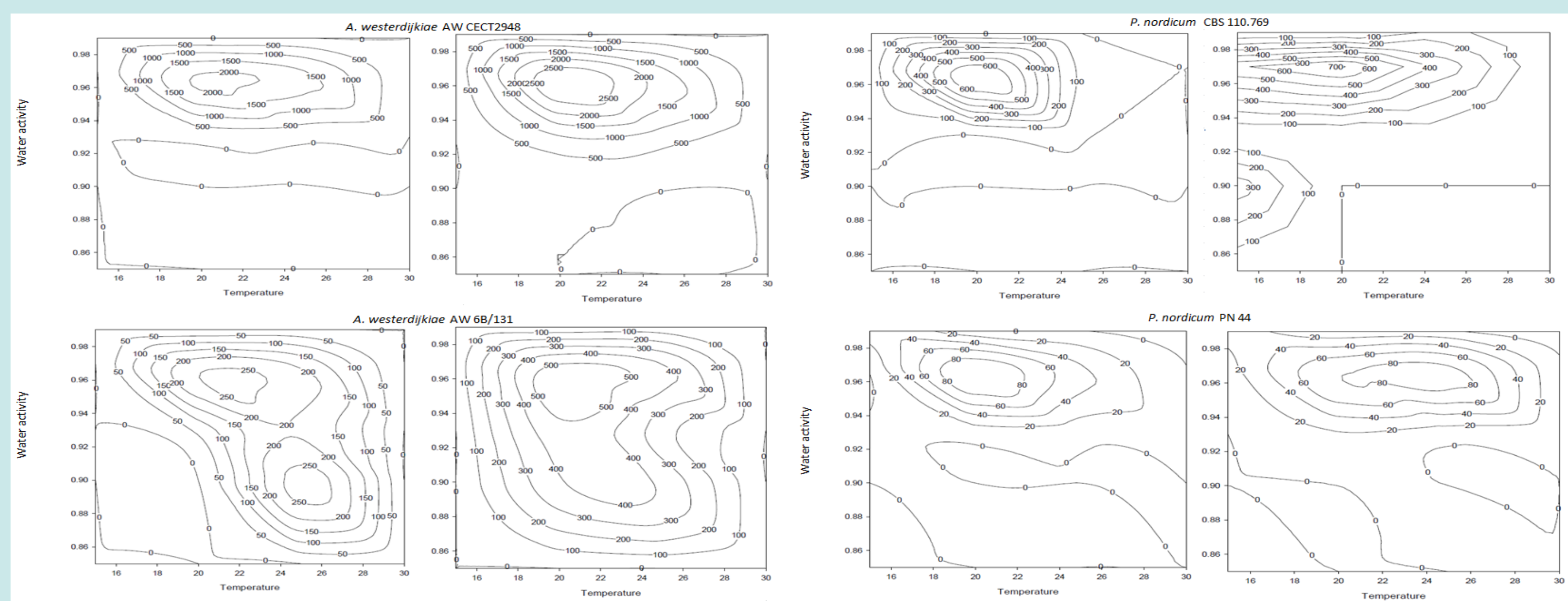


Figure 1: Two dimensional contour maps of OTA production of *A. westerdijkiae* (left) and *P. nordicum* (right) on dry-cured ham based-media in relation to water activity and temperature by day 10 (left) and 15 (right). The numbers on the isopleths represent conditions where the same OTA (ng/g agar) production occurred over a range of a_w x temperature conditions.

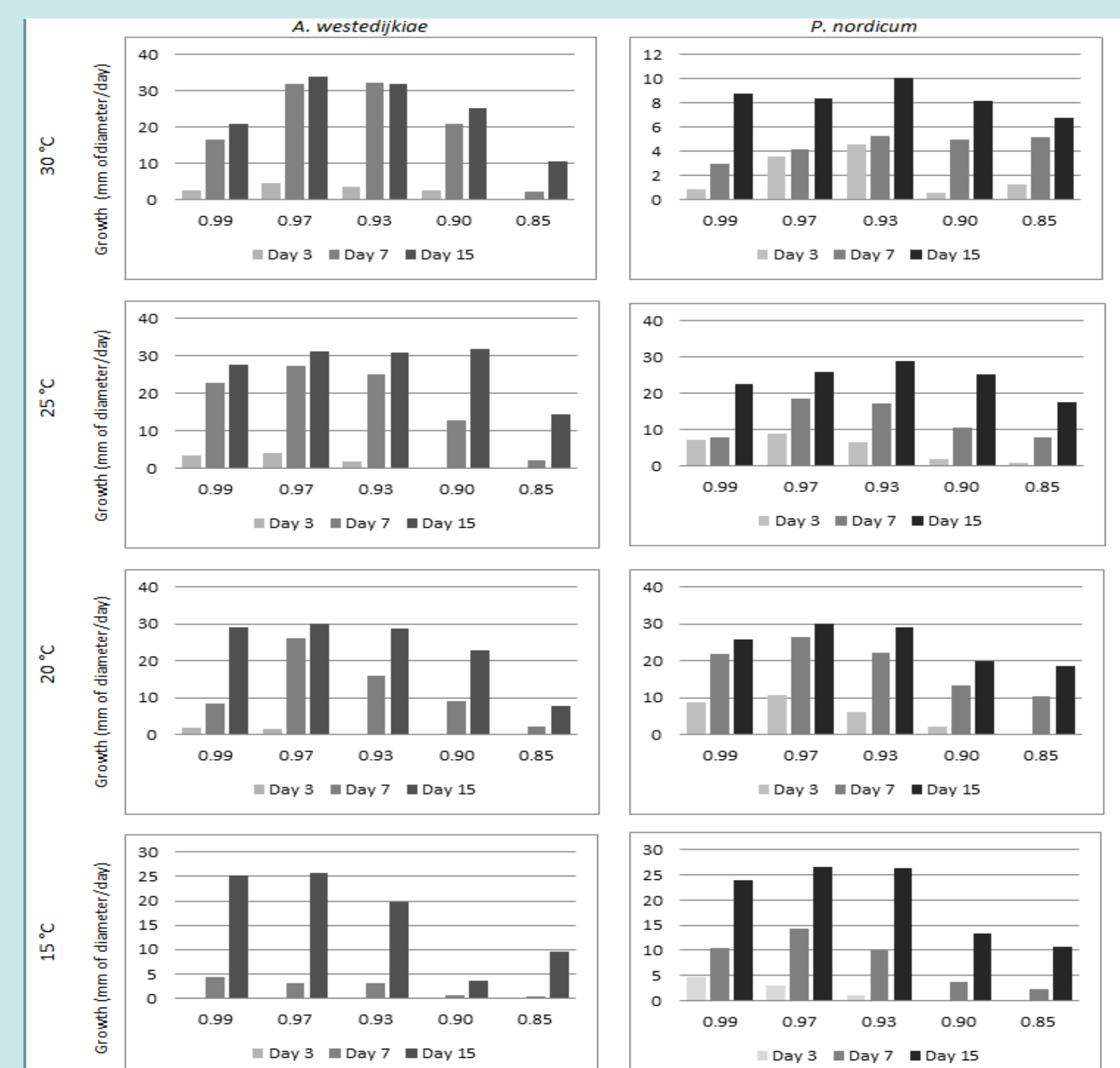


Figure 2: Effect of temperature and water activity on growth of *A. westerdijkiae* and *P. nordicum* on dry-cured ham based media

- The highest growth generally occurred at a_w levels between 0.93 and 0.97 for both species, and it was significantly reduced with lower a_w at all tested temperatures.
- *A. westerdijkiae* growth increased with temperature, while *P. nordicum* growth decreased with increasing temperature, except for 15 °C. Optimal growth occurred at 20 °C.
- Optimal growth for *A. westerdijkiae* occurred at temperatures > 27 °C and 0.93-0.97 a_w , while for *P. nordicum* this was at temperatures ranging between 19-25 °C and 0.93-0.97 a_w .

Conclusions

- ✓ Results shows growth and production of OTA in dry cured ham based matrix by *A. westerdijkiae* and *P. nordicum*. *A. westerdijkiae* produces OTA at higher concentrations and at a larger set of temperature and a_w conditions, and it seems to possess higher risk of OTA contamination in ham than *P. nordicum*.
- ✓ Growth of the fungus is not a good indicator of OTA production in this study.

Reference: Vipotnik Z, Rodríguez A, Rodrigues P, 2016. *Aspergillus westerdijkiae* as a major ochratoxin A risk in dry-cured ham based-media. International Journal of Food Microbiology (in press). doi: 10.1016/j.ijfoodmicro.2016.10.031

Acknowledgements: The authors are grateful to the research center CIMO (strategic project PEst-OE/AGR/UI0690/2014).