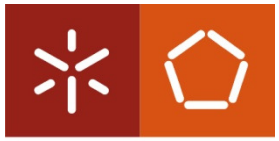

Universidade do Minho

Current situation on the occurrence of mycotoxins and toxigenic fungi in Portugal

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PRESENTATION - OBJECTIVES

- Climate and Food market characteristics of Portugal
- Data about the mycotoxin levels found in some Portuguese food and feeds
- Data about the FB_2 production by *A. niger* from Portuguese wine grapes

Portugal in the Mediterranean context

- Portugal is at the most western point of the Mediterranean Sea and has no direct borders with it

PORTUGAL IN THE MEDITERRANEAN CONTEXT

CLIMATE

- Nevertheless, its climate is considered to be Mediterranean, even if the influence of the Atlantic Sea changes its typical caracteristics

Figure 1. Mediterranean climate regions [1]

[1] http://en.wikipedia.org/wiki/Mediterranean_climate

PORTUGAL IN THE MEDITERRANEAN CONTEXT

CLIMATE

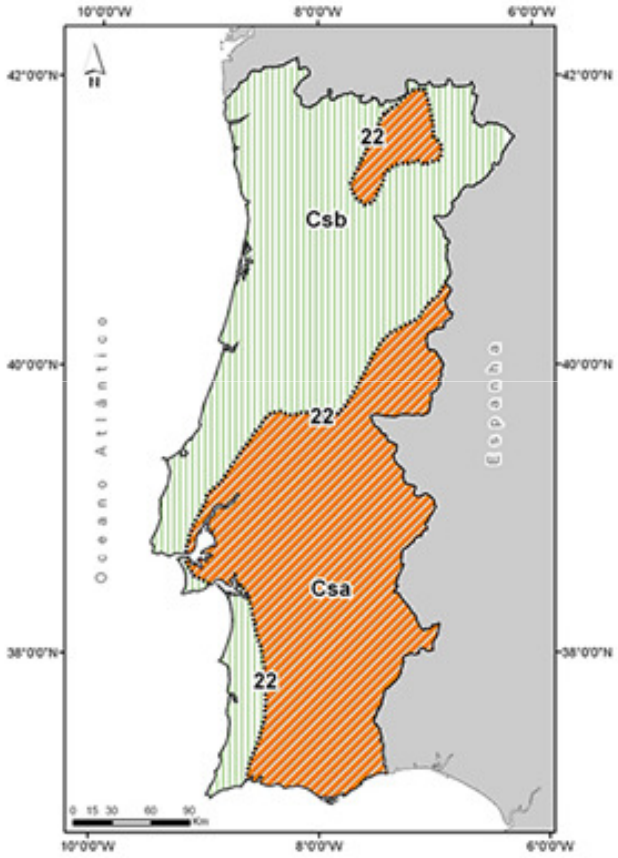

Figure 2. Climate of Portugal, according to Köppen classification [2]

In particular, the climate of Portugal, according to Köppen classification, is divided in two regions:

1. One at North with wet winters and summers less dry and hot (Csb).

2. Another at South with a clear Mediterranean influence that has drier winters and hot dry summers (Csa)

[2] Institute of Meteorology (1961-1990). http://www.meteo.pt/pt/areaeducativa/otempo.eoclima/clima.pt/index.html

Mycotoxins in Portuguese market

Food Market

- Presently, Portugal imports almost 54% of the consumed food products, being far more an importer country than an exporter one in some types of foods, *e.g.*

	Imported [3]
Cereals	73%
Pulses	87%
Fresh Fruits	26%
Meat products	31%
Vegetable oils	87%
Dried fruits	67%
Milk products	6%

[3] INE (2008). Agricultural Statistics of 2007. National Institute of Statistics, Portugal.

Mycotoxins in Portuguese market

Food Market

- Therefore, we can say that the mycotoxins levels found in the Portuguese market express in some situations, far more the levels found in imported products than in local commodities.

	Imported [3]
Cereals	73%
Pulses	87%
Fresh Fruits	26%
Meat products	31%
Vegetable oils	87%
Dried fruits	67%
Milk products	6%

[3] INE (2008). Agricultural Statistics of 2007. National Institute of Statistics, Portugal.

Mycotoxins in Portuguese market

Baby food

- 56% were found to be positive for AFB_1, AFM_1 and OTA
- Levels found were between 0.009 – 0.212 µg/kg
- with 1 sample exceeded the established EC limits

Mycotoxin	Analyzed Samples	Positive Samples	Levels (µg/kg)	EC limit (µg/kg)	Ref.
AFB_1	27	1 (4%)	0.009	0.10	[4]
AFM_1	27	4 (15%)	0.017 – 0.041	0.025	[4]
OTA	27	10 (37%)	0.034 – 0.212	0.50	[4]

[4] Alvito, P.C. et al. *Food Analytical Methods* **2010**, *3*, 22-30.

Mycotoxins in Portuguese market

Cereals and cereals products - OTA

- 34% of the samples were found to be positive
- Levels found were between 0.02 – 7.97 µg/kg
- with 4 samples exceeded the established EC limits

OTA	Analyzed Samples	Positive Samples	Levels (µg/kg)	EC limit (µg/kg)	Ref.
Cereals	38	6 (16%)	0.27 – 7.97	5.0	[5]
Rice	42	6 (14%)	0.09 – 3.52	5.0	[6]
Bread	50	28 (56%)	0.02 – 0.490	3.0	[7]
Bread	61	25 (41%)	0.033 – 5.86	3.0	[8]
Total	**191**	**65 (34%)**			

[5] Juan, C. et al. Food Chem. 2008, 107, 525-530; [6] Pena, A. et al. Anal Bioanal Chem 2005, 382, 1288-1293; [7] Bento, J.M.V. et al. Microchemical Journal 2009, 91, 165-169; [8] Juan, C. et al. Int. J. Food Microbiol. 2008, 127, 284-289.

Mycotoxins in Portuguese market

DAIRY PRODUCTS – AFM_1

- 63% of the samples were found to be positive
- Levels found were between 0.005 – 0.8 µg/kg
- with 49 (7%) samples exceeded the established EC limits

AFM_1	Analyzed Samples	Positive Samples	Levels (µg/kg)	EC limit (µg/kg)	Ref.
Dairy products	598	394 (66%)	0.005 – 0.8	0.050	[9]
Dairy products	74	29 (39%)	0.060 – 0.065	0.050	[10]
Total	**672**	**423 (63%)**			

[9] Martins, H. et al. Mycot. Res. 2005, 21, 192-195; [10] Ouakinin J. et al. Repositorio de Trabalhos Instituto Nacional de Veterinaria XIV. 1982, 75-78

Mycotoxins in Portuguese market

APPLE-PRODUCTS - PATULIN

- 55% of the samples were found to be positive
- Levels found were between 3.0 – 80.5 µg/kg
- with some of the analysed apples exceeding the EC limits

Patulin	Analyzed Samples	Positive Samples	Levels (µg/kg)	EC limit (µg/kg)	Ref.
Apple juice	68	28 (41%)	3.9 – 42.0	50.0	[11]
Purees for infant	76	5 (7%)	3.9 – 5.7	10.0	[11]
Apples	351	241 (69%)	3.0 – 80.5	25.0	[12]
Total	**495**	**274 (55%)**			

[11] Barreira, M.J.et al. Food Chem. 2010, 121, 653-658; [12] Martins, M.L.G. Food Addit. Contam. 2002, 19, 568-574

Mycotoxins in Portuguese market

Feed and Feedstuffs - AFB_1

- 21% of the samples were found to be positive

AFB_1	Analyzed Samples	Positive Samples	Levels (µg/kg)	EC limit (µg/kg)	Ref.
Bovine fedd	399	34 (4%)	5 – 15	20	[13]
Poultry feed	85	16 (93%)	1 – 20	20	[13]
Swine feed	74	7 (71%)	1 – 2	20	[13]
Raw materials	513	63 (12%)	1 – 45	20	[14]
Feed	1584	436 (28%)	1 – 80	20	[14]
Total	**2655**	**556 (28%)**			

[13] Peito, A.; Venâncio, A. in Overview on Toxigenic Fungi and Mycotoxins in Europe 2004, 173-184
[14] Martins, H. et al. Mycot. Res. 2008, 24, 19-23

Mycotoxins in Portuguese market

Feed and Feedstuffs - AFB_1

- Levels found were between 1 – 80 µg/kg

AFB_1	Analyzed Samples	Positive Samples	Levels (µg/kg)	EC limit (µg/kg)	Ref.
Bovine fedd	399	34 (4%)	5 – 15	20	[13]
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[14] Martins, H. et al. Mycot. Res. 2008, 24, 19-23

Mycotoxins in Portuguese market

Feed and Feedstuffs - AFB_1

- Some of the samples exceeded the EC limits

AFB_1	Analyzed Samples	Positive Samples	Levels (µg/kg)	EC limit (µg/kg)	Ref.
Bovine fedd	399	34 (4%)	5 – 15	20	[13]
Poultry feed	85	16 (93%)	1 – 20	20	[13]
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[14] Martins, H. et al. Mycot. Res. 2008, 24, 19-23

Mycotoxins in Portuguese market

Feed and Feedstuffs - FB_1

- 10% of the samples were found to be positive

FB_1	Analyzed Samples	Positive Samples	Levels (mg/kg)	EC limit (mg/kg)	Ref.
Corn	12	8 (67%)	0.025 – 32.20	60	[13]
Oat	5	2 (40%)	0.132 – 0.421	60	[13]
Raw materials	208	19 (9%)	0.010 – 0.040	60	[14]
Poultry feed	22	20 (90%)	0.031 – 7.437	20	[13]
Horse feed	7	6 (86%)	0.06 – 0.500	5	[13]
Feed	357	6 (2%)	0.012 – 0.034	20	[14]
Total	**611**	**61 (10%)**			

[13] Peito, A.; Venâncio, A. in Overview on Toxigenic Fungi and Mycotoxins in Europe 2004, 173-184
[14] Martins, H. et al. Mycot. Res. 2008, 24, 19-23

Mycotoxins in Portuguese market

Feed and Feedstuffs - FB_1

- Levels found were between 0.01 – 7.4 mg/kg

FB_1	Analyzed Samples	Positive Samples	Levels (mg/kg)	EC limit (mg/kg)	Ref.
Corn	12	8 (67%)	0.025 – 32.20	60	[13]
Oat	5	2 (40%)	0.132 – 0.421	60	[13]
Raw materials	208	19 (9%)	0.010 – 0.040	60	[14]
Poultry feed	22	20 (90%)	0.031 – 7.437	20	[13]
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[14] Martins, H. et al. Mycot. Res. 2008, 24, 19-23

Mycotoxins in Portuguese market

Feed and Feedstuffs - FB_1

- with no samples exceeding the EC limits

FB_1	Analyzed Samples	Positive Samples	Levels (mg/kg)	EC limit (mg/kg)	Ref.
Corn	12	8 (67%)	0.025 – 32.20	60	[13]
Oat	5	2 (40%)	0.132 – 0.421	60	[13]
Raw materials	208	19 (9%)	0.010 – 0.040	60	[14]
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[14] Martins, H. et al. Mycot. Res. 2008, 24, 19-23

MICOTOXIGENIC FUNGI

OTA IN BLACK ASPERGILLI FROM WINE GRAPES

- Some years ago, we have participated in the Europpean project that studied the OTA problematic in wine grapes
- At that time, studies were conducted to find the main fungi responsable for OTA presence in wines [15]

	Isolated strains	**OTA production**	**Mean levels (µg/kg)**
A. carbonarius	68	100%	1129
A. niger aggregate	571	4%	137
Other aspergilli	131	0%	-

[15] Serra, R. et al. Res. Microbiol. 2005, 156, 515-521.

MICOTOXIGENIC FUNGI

OTA IN BLACK ASPERGILLI FROM WINE GRAPES

- OTA in grapes was due to the presence of *A. carbonarius*
- Nevertheless, most of the black aspergilli found were from the *A. niger* aggregate

	Isolated strains	**OTA production**	**Mean levels (µg/kg)**
A. carbonarius	68	100%	1129
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[15] Serra, R. et al. Res. Microbiol. 2005, 156, 515-521.

MICOTOXIGENIC FUNGI

OTA IN BLACK ASPERGILLI FROM WINE GRAPES

- At that time, all those strains were also preserved and they are still representative of the local black aspergilli present in Portuguese wine grapes

	Nº of isolates	OTA production	Mean levels (µg/kg)
A. carbonarius	68	100%	1129
A. niger aggregate	571	4%	137
Other aspergilli	131	0%	-

[15] Serra, R. et al. Res. Microbiol. 2005, 156, 515-521.

MICOTOXIGENIC FUNGI

FB_2 IN BLACK ASPERGILLI FROM WINE GRAPES

- We tested:
  - All the strains of *A. niger* aggregate and of *A. carbonarius* available in the collection
  - *A. niger* Type strain CBS 554.65^T (positive control) [16]
  - *A. niger* CBS 120.49 (positive control) [16]
  - We confirmed FB_2 identity in some strains extracts with IMAC

[16] Frisvad, J.C. et al. J. Agric. Food Chem. 2007, 55, 9727-9732.

MICOTOXIGENIC FUNGI

FB_2 IN BLACK ASPERGILLI FROM WINE GRAPES

- Strains were grown in CYA for 8 days at 25 ºC
- 5 plugs extracted with methanol [16]
- We optimized an isocratic HPLC-FL method to detect and quantify FB_2 in black aspergilli strains extracts
- The method uses NDA derivatization and was adapted from [17]

[16] Frisvad, J.C. et al. J. Agric. Food Chem. 2007, 55, 9727-9732.
[17] Bennett, G.A.; Richard, J.L. Journal of Aoac International 1994, 77, 501-506.

MICOTOXIGENIC FUNGI

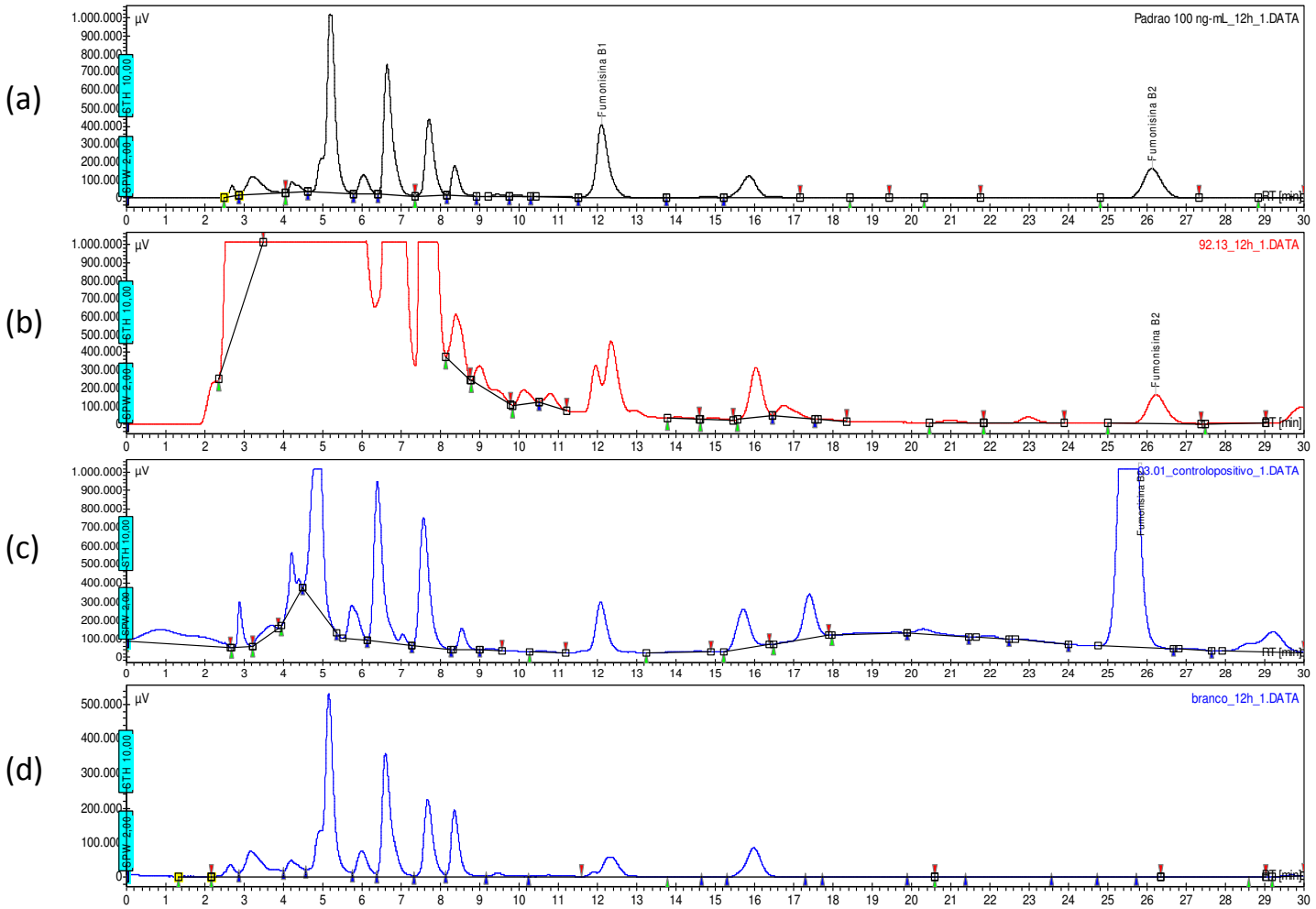


Figure 4. Chromatograms: a) 100 ng/mL standard; b) control strain *A. niger* CBS 120.49; c) control strain with IMAC purification ; d) Blank

MICOTOXIGENIC FUNGI

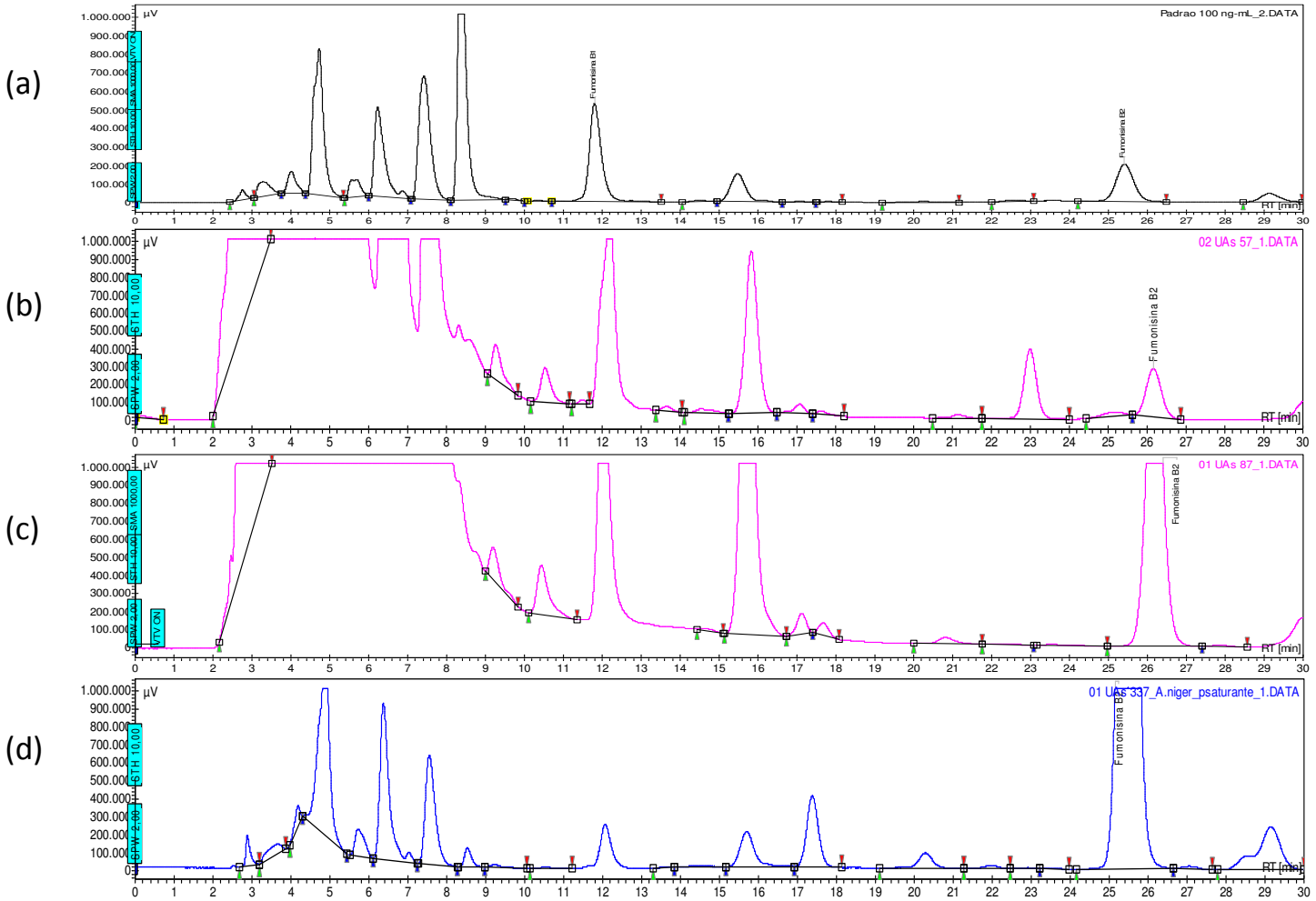


Figure 5. Chromatograms: a) 100 ng/mL standard; b) strain 02UAs57; c) strain 01UAs87; d) strain 01UAs307 with IMAC purification.

MICOTOXIGENIC FUNGI

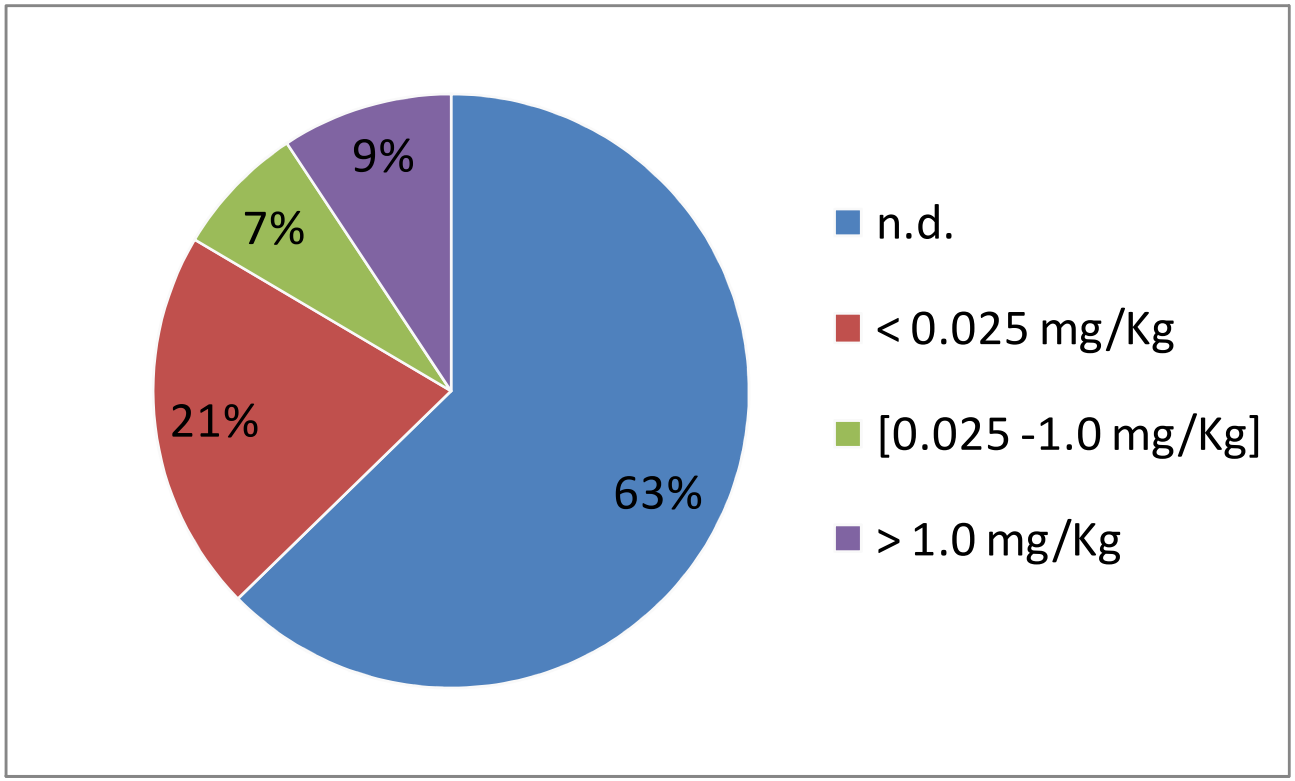
FB_2 IN BLAK ASPERGILLI FROM WINE GRAPES

- FB_2 was not detected in *A. carbonarius* strains tested
- But was detected in 158 (29%) strains from the *A. niger* aggregate
- Levels of production for all the strains:
  - 0.003 – 6.0 mg/kg
  - Mean = 1.0 mg/kg
  - Mediana = 0.022 mg/kg

Micotoxigenic fungi

Distribution by wine regions

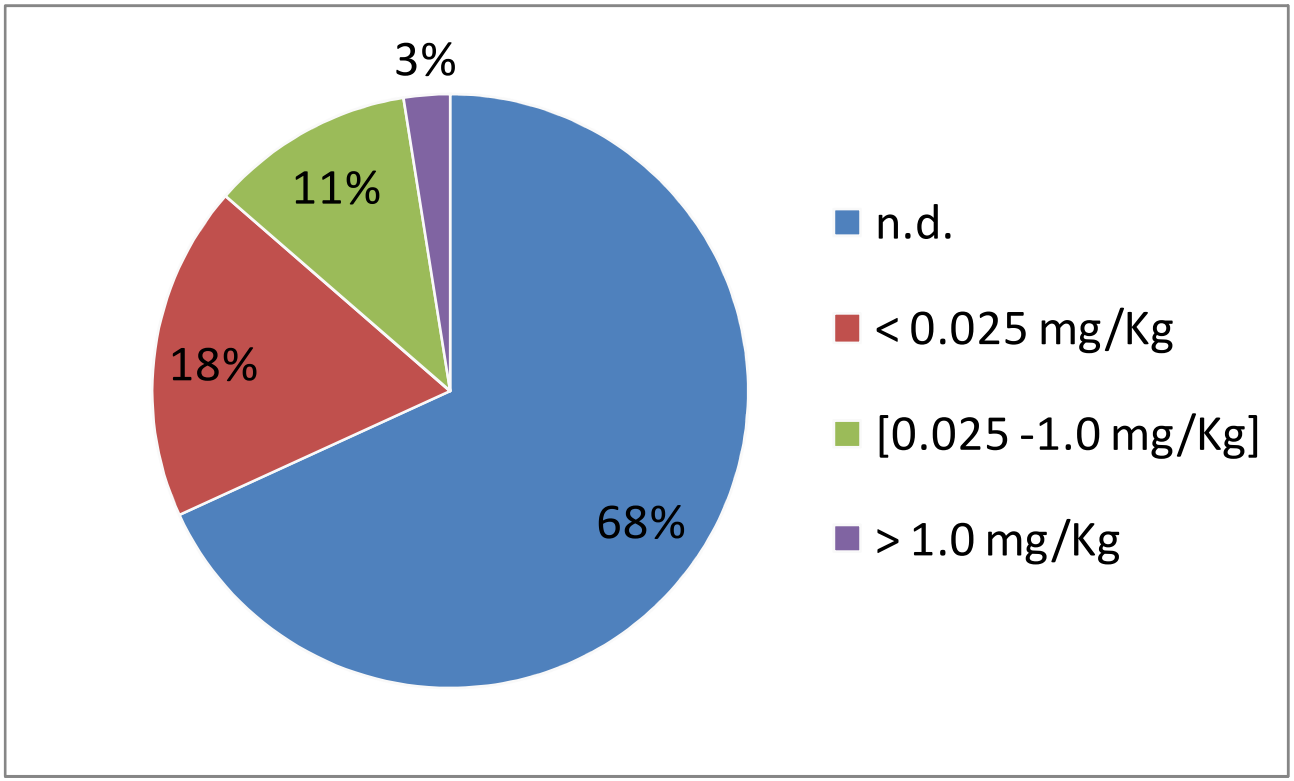
- Douro wine region: mean=0.78 mg/kg



MICOTOXIGENIC FUNGI

DISTRIBUTION BY WINE REGIONS

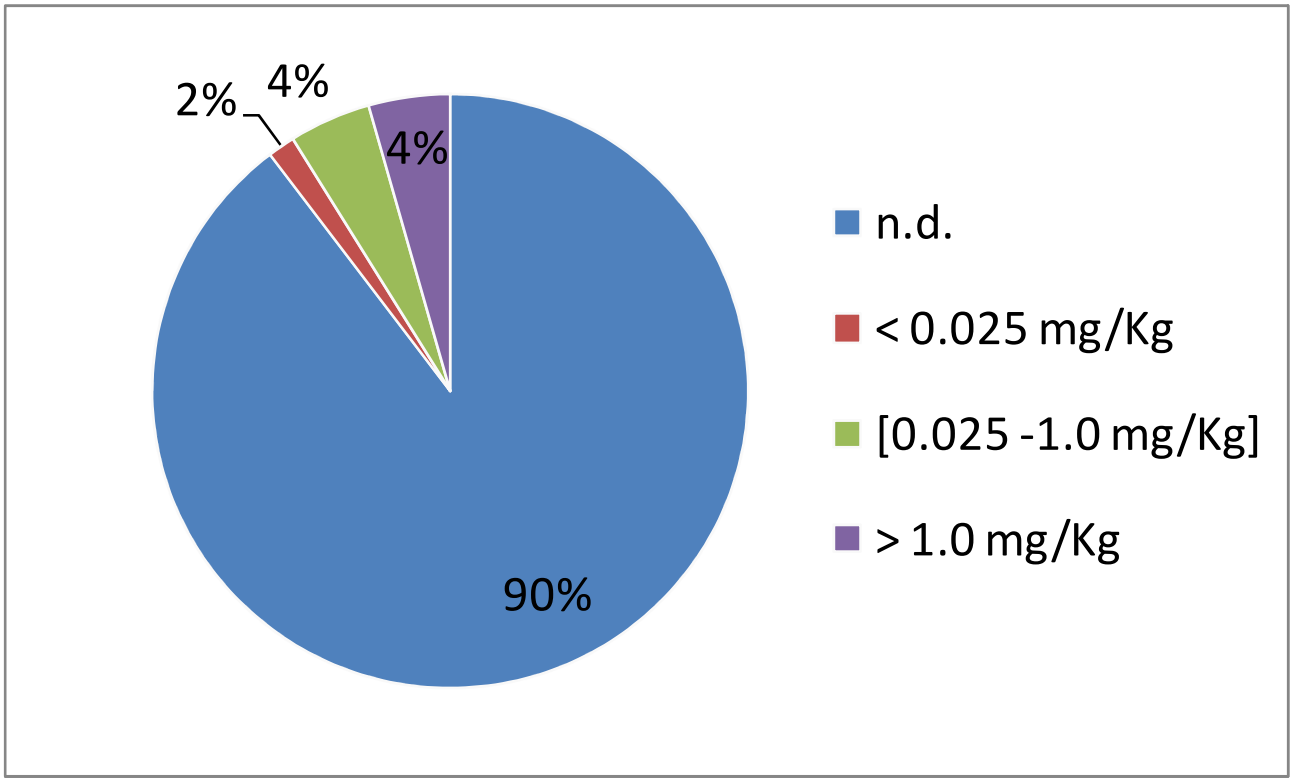
- Ribatejo wine region: mean=0.28 mg/kg



Micotoxigenic fungi

Distribution by wine regions

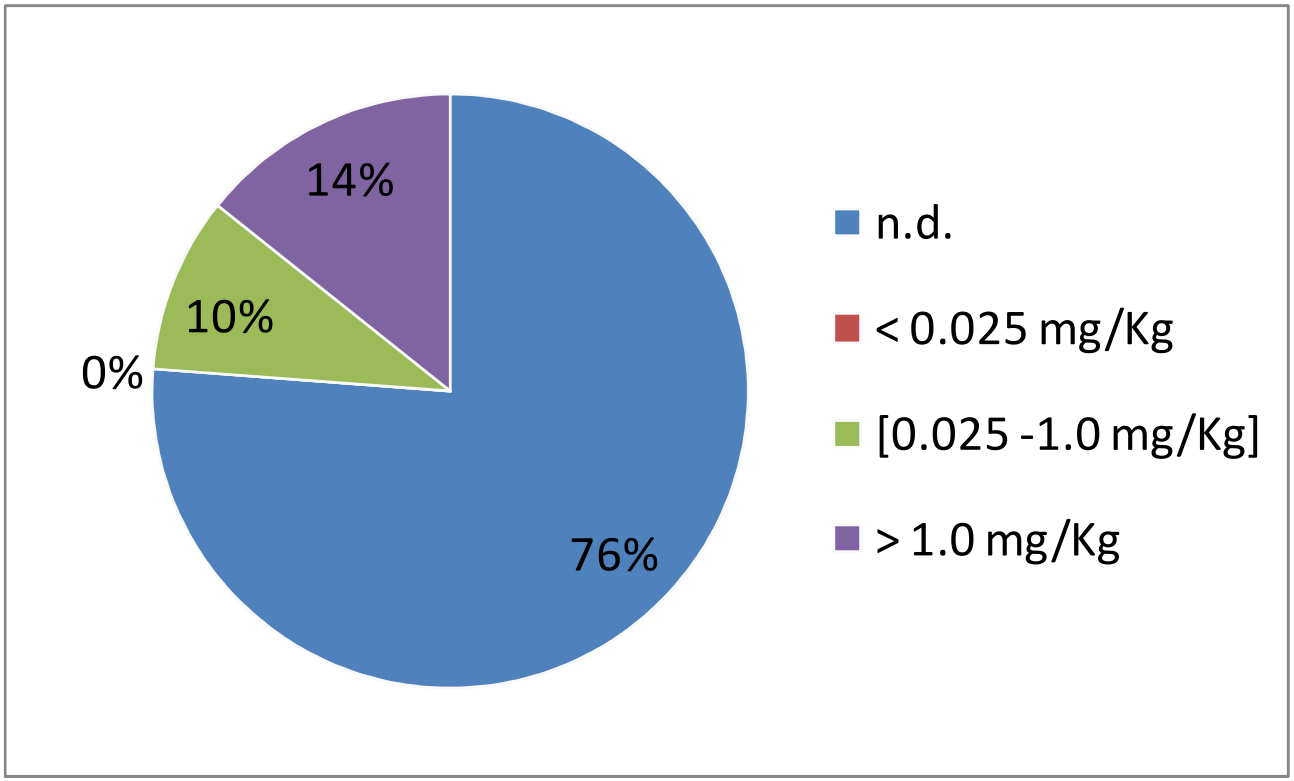
- Alentejo wine region: mean=1.5 mg/kg



Micotoxigenic fungi

Distribution by wine regions

- Vinho Verde wine region: mean=1.1 mg/kg



CONCLUSIONS

- In what concerns mycotoxin levels from published data
  - Levels found in the Portuguese market are not high
- In what concerns local mycotoxigenic fungi
  - We found that 29% of the local *A. niger* from wine grapes were positive for FB_2 production
  - With only 6% of the strains producing more that 1 mg/kg
- Therefore, we can say that the risk for FB_2 presence in Portuguese wines is comparable with the one posed by OTA and *A. carbonarius* - That is low

Acknowledgments

- Thanks to the organization for the invitation to give this presentation and for they nice welcome to Egypt
- Thanks to Thalita Calado to the lab work
- Thanks to all of you also for listening

Shokran