ALIMENTOS A BASE DE CEREALES Y MICOTOXINAS

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Grupo de Investigación CoaL CONTAMINACIÓN DE ALIMENTOS

30 AETC 2018 Jornadas Técnicas

MICOTOXINAS

- ✓ Las **micotoxinas** son compuestos producidos por el metabolismo secundario de distintos tipos de **hongos filamentosos**.
- ✓ Poseen un peso molecular medio/bajo y gran variedad de estructuras químicas.
- ✓ Son sustancias naturales **tóxicas** con un **elevado impacto económico** mundial debido a su relación con la **salud humana** y **animal**.

Aspergillus

Penicillium

Fusarium

Claviceps

Alternaria

MICOTOXINAS

Principales especies productoras	Micotoxinas
Aspergillus parasiticus y *Aspergillus flavus*	Aflatoxinas B_1, B_2, G_1 y G_2
Penicillium verrucosum y *Aspergillus ochraceus*	Ocratoxina A
Fusarium moniliforme (F. verticillioides)	Fumonisinas B_1 y B_2
Fusarium graminearum	Deoxinivalenol
Fusarium graminearum	Zearalenona
Penicillium, Aspergillus y *Byssochylamys*	Patulina

Aspergillus

Penicillium

Fusarium

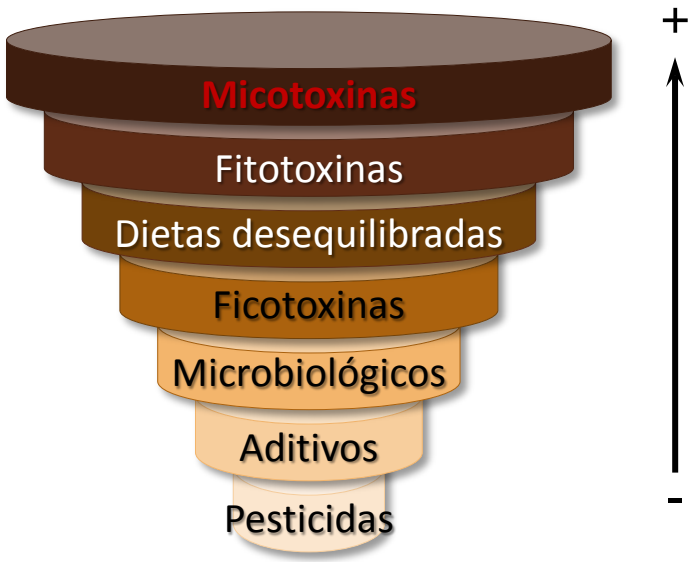
Claviceps

Alternaria

RIESGOS PARA LA SALUD HUMANA

Efectos agudos

Efectos crónicos



RIESGOS PARA LA SALUD HUMANA

Micotoxinas	Efectos tóxicos
Aflatoxinas	Carcinogénicos, hepatotóxicos, genotóxicos e inmunosupresores.
Ocratoxina A	Nefrotóxicos, teratogénicos e inmunosupresores.
Fumonisinas	Neurotóxicos y citotóxicos
Zearalenona	Disrupción hormonal
Tricotecenos	Inmunosupresores y dermatotóxicos
Patulina	Hepatotóxicos, inmunosupresor y citotóxicos
Ácido ciclopiazónico	Neurotóxicos
Citrinina	Neurotóxicos
Esterigmatocistina	Hepatotóxicos

PROBLEMAS MICROBIOLÓGICOS DE LOS CEREALES

Los principales problemas microbiológicos de los cereales son causados por la contaminación por hongos.

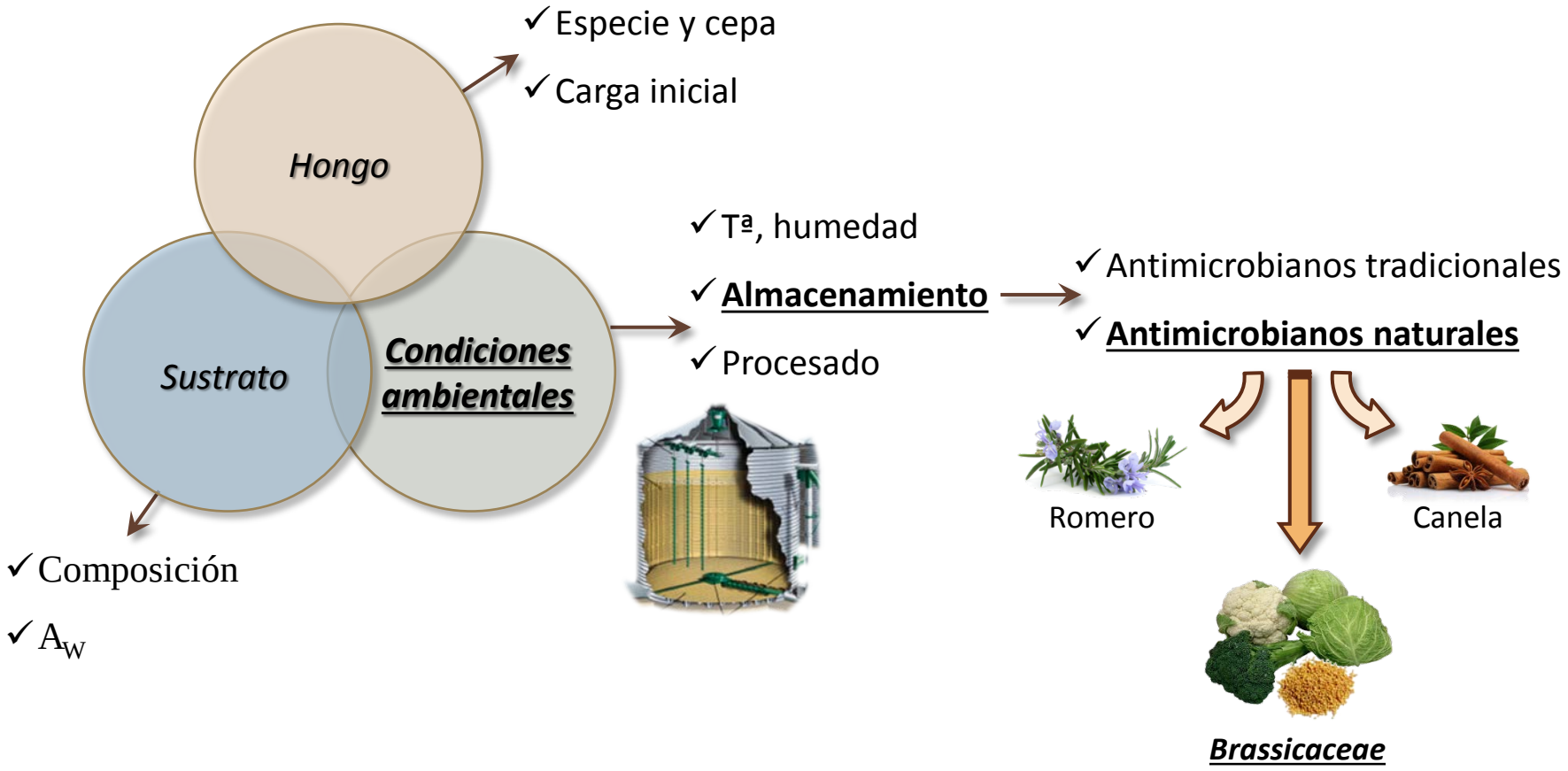
El **FHB (Fusarium head blight)** es una enfermedad del **trigo**, la **cebada** y **otros cereales**, causada por varias especies de ***Fusarium***.

MICOTOXINAS EN CEREALES

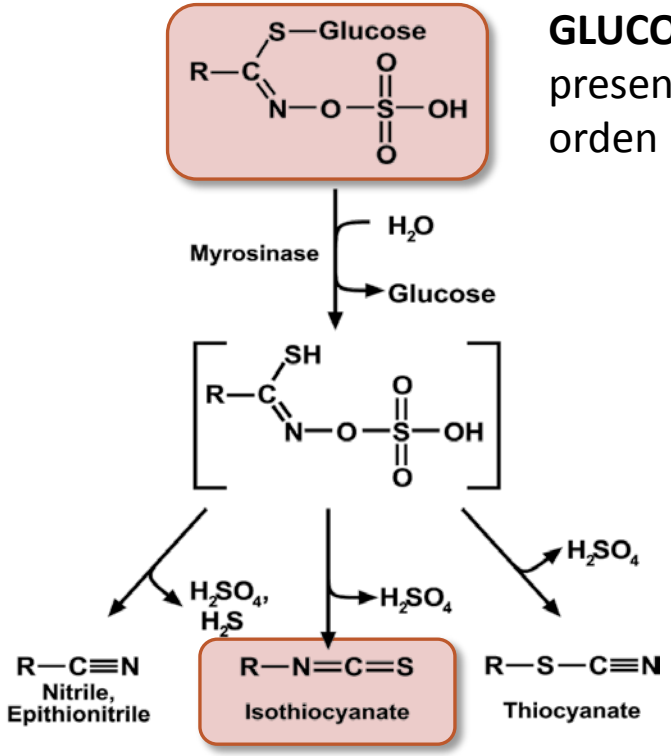
Límite de micotoxinas para productos alimenticios a base de cereales	
Aflatoxina B_1	*2 µg/Kg*
Total Aflatoxinas	*4 µg/Kg*
Ochratoxina A	*5 µg/Kg*
Fumonisinas B_1 and B_2	*400 µg/Kg*
Deoxinivalenol	*750 µg/Kg*
Zearalenona	*75 µg/Kg*

European Commission 1881/2006

FORMACIÓN DE LAS MICOTOXINAS



GLUCOSINOLATOS E ISOTIOCIANATOS



GLUCOSINOLATOS (GSs): Metabolitos secundarios presentes en algunos vegetales, mayoritariamente del orden ***Brassicales***.

ISOTIOCIANATOS (ITCs): Sustancias con propiedades antioxidantes, anticancerígenas y antimicrobianas (bactericida, **fungicida** y sobre insectos y pequeños invertebrados).

GLUCOSINOLATOS E ISOTIOCIANATOS

Broccoli
(*Brassica oleracea italica*)

GLUCORAFANINA

Coles de Bruselas
(*Brassica oleracea gemmifera*)

PROGOITRINA

Coliflor
(*Brassica oleracea botrytis*)

GLUCONAPINA

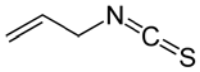
Mostaza amarilla
(*Sinapsis alba/ Brassica hirta*)

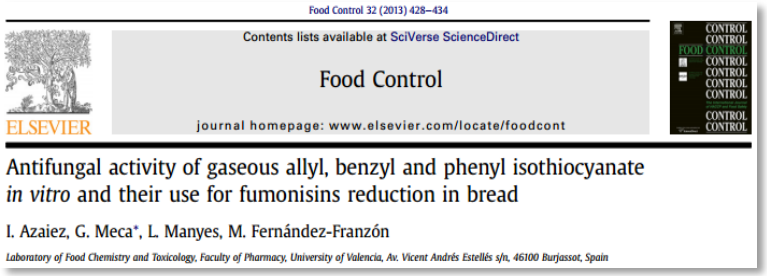
SINALBINA

Mostaza oriental
(*Brassica juncea*)

SINIGRINA ➡ ALIL ISOTIOCIANATO (AITC)

GRAS





Food Control 32 (2013) 428–434

Contents lists available at SciVerse ScienceDirect

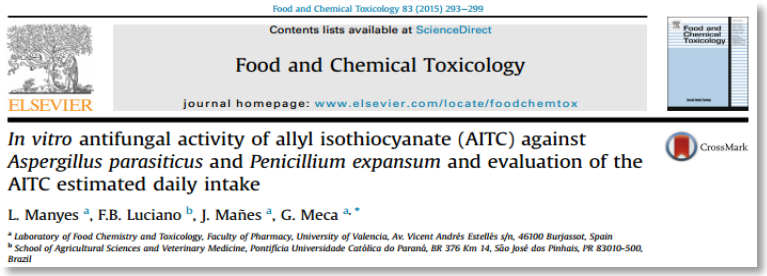
Food Control

journal homepage: www.elsevier.com/locate/foodcont

Antifungal activity of gaseous allyl, benzyl and phenyl isothiocyanate *in vitro* and their use for fumonisins reduction in bread

I. Azaiez, G. Meca*, L. Manyes, M. Fernández-Franzón

Laboratory of Food Chemistry and Toxicology, Faculty of Pharmacy, University of Valencia, Av. Vicent Andrés Estellés s/n, 46100 Burjassot, Spain



Food and Chemical Toxicology 83 (2015) 293–299

Contents lists available at ScienceDirect

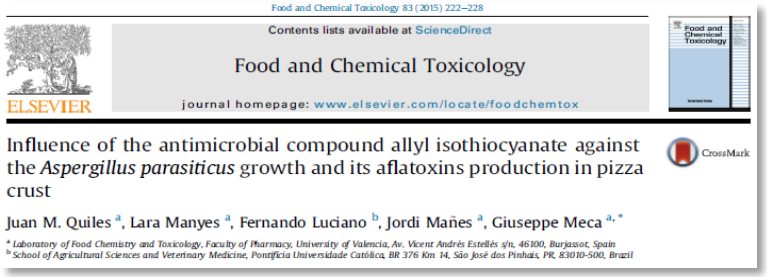
Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

In vitro antifungal activity of allyl isothiocyanate (AITC) against *Aspergillus parasiticus* and *Penicillium expansum* and evaluation of the AITC estimated daily intake

L. Manyes [a], F.B. Luciano [b], J. Mañes [a], G. Meca [a,*]

[a] *Laboratory of Food Chemistry and Toxicology, Faculty of Pharmacy, University of Valencia, Av. Vicent Andrés Estellés s/n, 46100 Burjassot, Spain*
[b] *School of Agricultural Sciences and Veterinary Medicine, Pontifícia Universidade Católica do Paraná, BR 376 Km 14, São José dos Pinhais, PR 83010-500, Brazil*



Food and Chemical Toxicology 83 (2015) 222–228

Contents lists available at ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

Influence of the antimicrobial compound allyl isothiocyanate against the *Aspergillus parasiticus* growth and its aflatoxins production in pizza crust

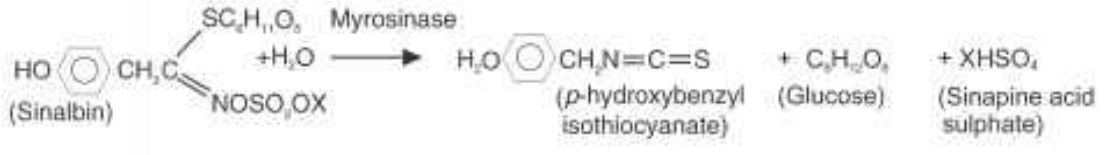
Juan M. Quiles [a], Lara Manyes [a], Fernando Luciano [b], Jordi Mañes [a], Giuseppe Meca [a,*]

[a] *Laboratory of Food Chemistry and Toxicology, Faculty of Pharmacy, University of Valencia, Av. Vicent Andrés Estellés s/n, 46100, Burjassot, Spain*
[b] *School of Agricultural Sciences and Veterinary Medicine, Pontifícia Universidade Católica, BR 376 Km 14, São José dos Pinhais, PR, 83010-500, Brazil*

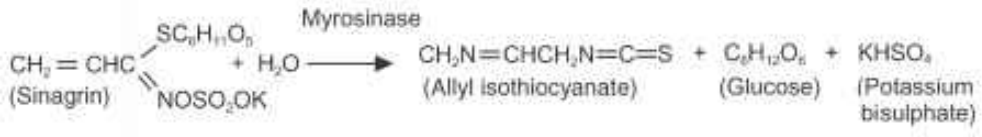
GLUCOSINOLATOS E ISOTIOCIANATOS

Sinapsis alba

MOSTAZA AMARILLA: Su **GS** e **ITC** principales son la **SINALBINA** y el **HIDROXIBENCIL ISOTIOCIANATO (HBITC)**. El **HBITC** es **poco volátil** en comparación con el resto de los ITCs y sus efectos son también son menores.



MOSTAZA ORIENTAL: Su **GS** e **ITC** principales son la **SINIGRINA** y el **ALIL ISOTIOCIANATO (AITC)**. El **AITC** es **muy volátil** en comparación con el resto de los ITCs y sus efectos son muy potentes y están muy demostrados



Brassica juncea

DISEÑO DE GEL BIOACTIVO

EXAMPLES OF GELLING AGENTS

- ✓ Alginic acid (E-400), sodium alginate (E-401), potassium alginate (E-402), ammonium alginate (E-403), calcium alginate (E-404)
- ✓ Agar (E-406)
- ✓ Carrageenan (E-407)
- ✓ Locust bean gum (E-410)
- ✓ Pectin (E-409)
- ✓ Gelatin (E-441)

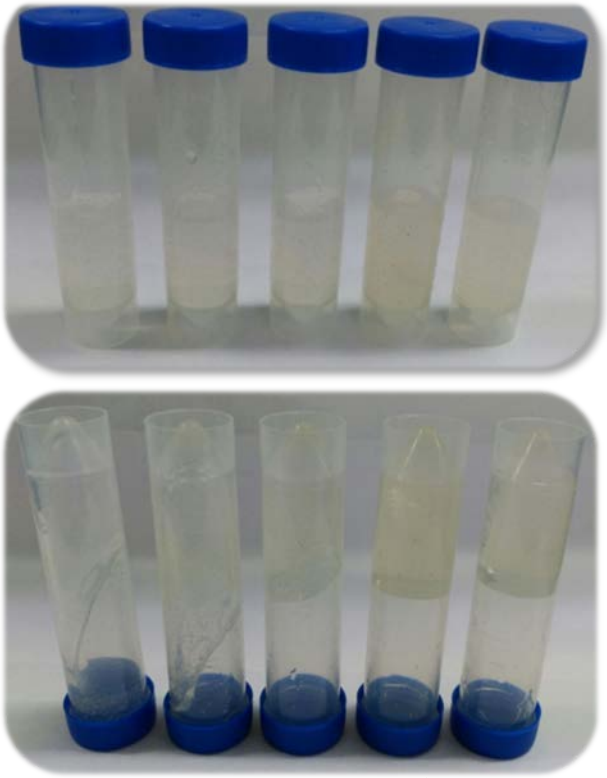
!THEY NEED HEAT TO GELLING!

HYDROXYETHYLCELLULOSE

- ✓ It is characterized by forming viscous water gels **useful for making dietary products**, cosmetics and detergents
- ✓ **Cold gelling**
- ✓ **Chip**

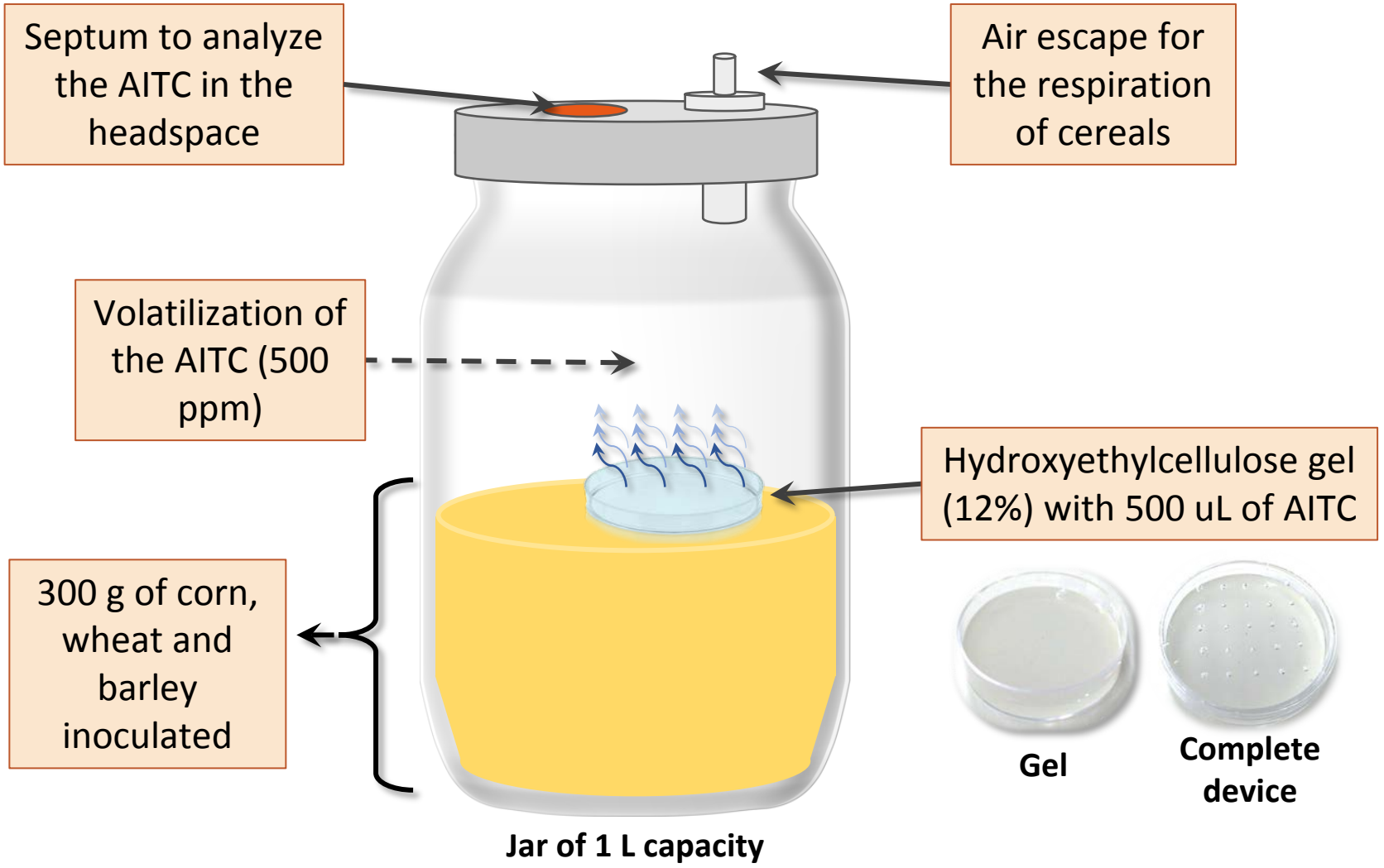
% HYDROXYETHYLCELLULOSE

6% 8% 10% 12% 14%



X X X ✓ ✓

DISEÑO DE MINISILO SIMULADO



ENSAYO EN MINISILO SIMULADO

Control**, and **500 ppm** of **AITC

300 g** of **corn**, **wheat** and **barley

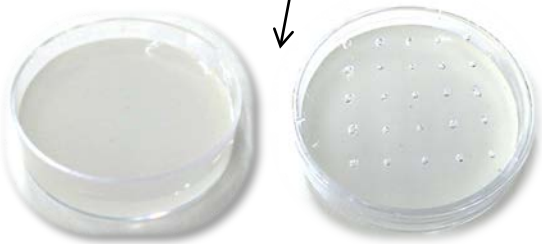
Container with 1 liter capacity

30 days at room temperature and darkness

***Corn**: Inoculated with 1x104 conidia/g **A. flavus** (ISPA 8111).*

***Wheat** and **barley**: Inoculated with 1x104 conidia/g **P. verrucosum** (D-01847 VTT).*

x 3



GEL PREPARATION:

- ✓ 10 mL of water, **1.2 g** of **hydroxyethylcellulose** (**12** %) and **500 uL** of **AITC**
- ✓ Mixture at **25°C**

AITC analysis in head space

Days 1 and 30

AITC concentration in cereals

Microbiological analysis

Mycotoxins analysis

ANALISIS

AITC analysis in head space

a) Injection of **100 µl** of **headspace** air into a **GC-FID**.
b) Daily sampling in triplicate from day **1** to **30**.

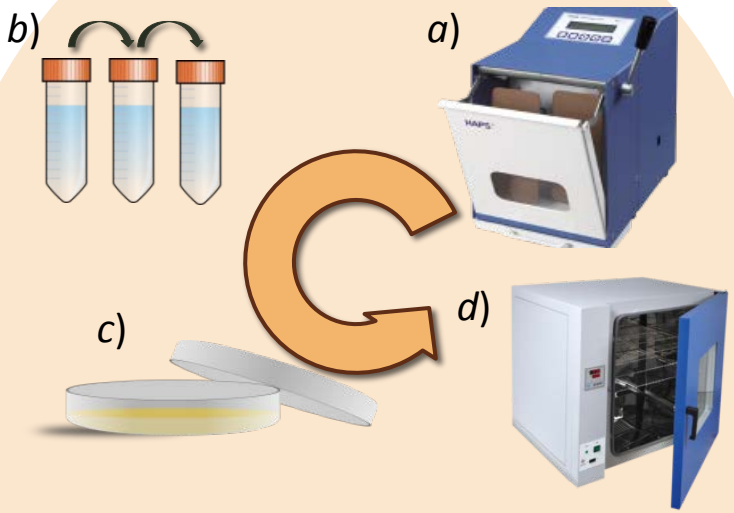
AITC concentration in cereals

(Tracz *et al*, 2017)

a,b) **5 g** of sample whit **10 mL** of **methanol** was **shaken** for **30 min** in water bath (**40 ºC**) and **10 min** in an ultrasonic bath.
c) Centrifuged **4000 rpm**, **20 °C**, **5 min**.
d) Filter (**0.22 uM**), vialized and determination by **HPLC-UV/VIS**.

DETERMINACIONES

Microbiological analysis



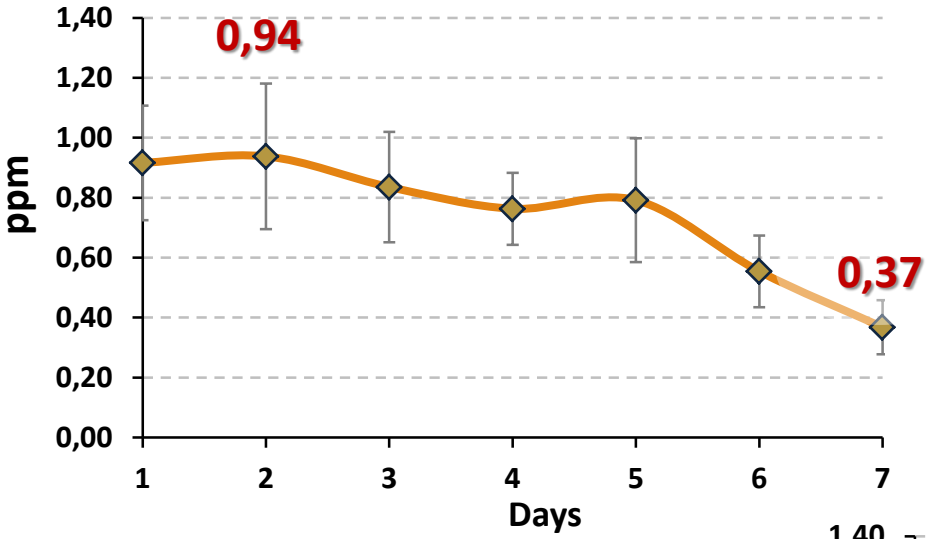
a) Homogenization by **stomacher**.
b) Decimal serial dilutions in **peptone water**.
c) Sowing in triplicate of 100 uL on plates with **PDA** medium.
d) Count after **48 hours at 25 ° C**.

Determination of mycotoxins

(Saladino *et al*, 2017)

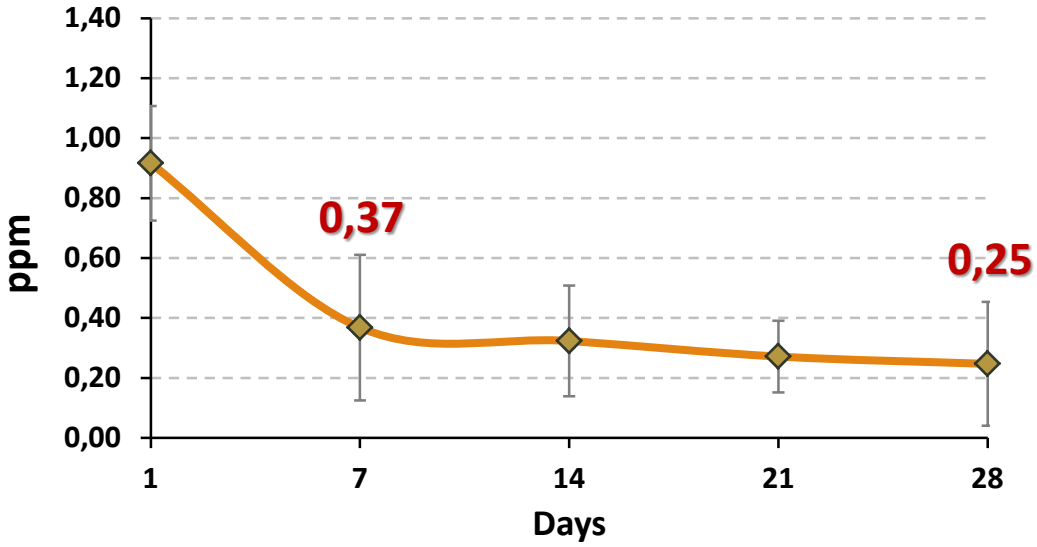
a) The samples were grounded.
b) 5 g + 25 mL of **methanol** were homogenized by **ultraturrax.**
c) Centrifuged **4000 rpm**, **5 °C**, **5 min**.
d) Evaporation by **Rotavapor** and **Turbovap.**
e) Filter (**0.22 uM**), vialized and determination by **LC-MS/MS.**

AITC EN EL ESPACIO DE CABEZA

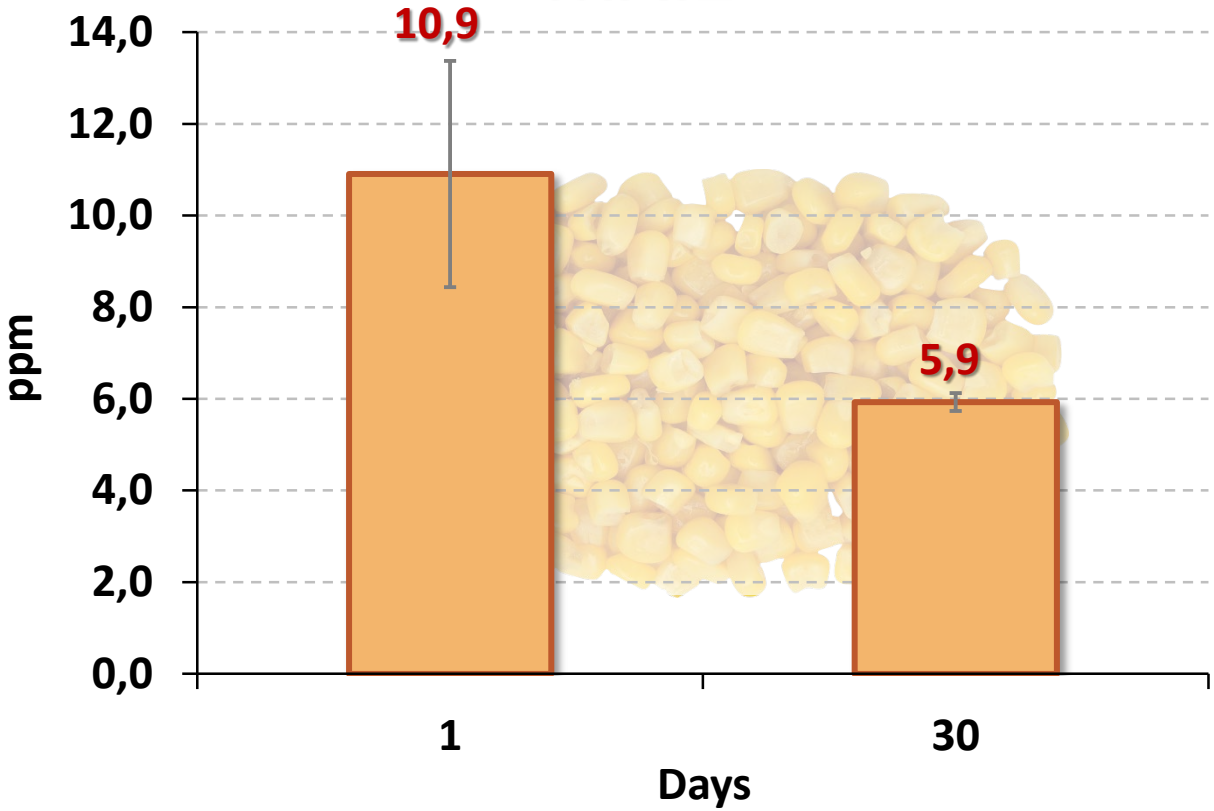


AITC values in the headspace decreased from a maximum at **day 2** of **0.94 ppm** to **0.37 ppm** at **day 7**

After decreasing to **0.37 ppm** in the **first week**, the AITC values remained stable until reaching a minimum of **0.25 ppm** at the end of treatment

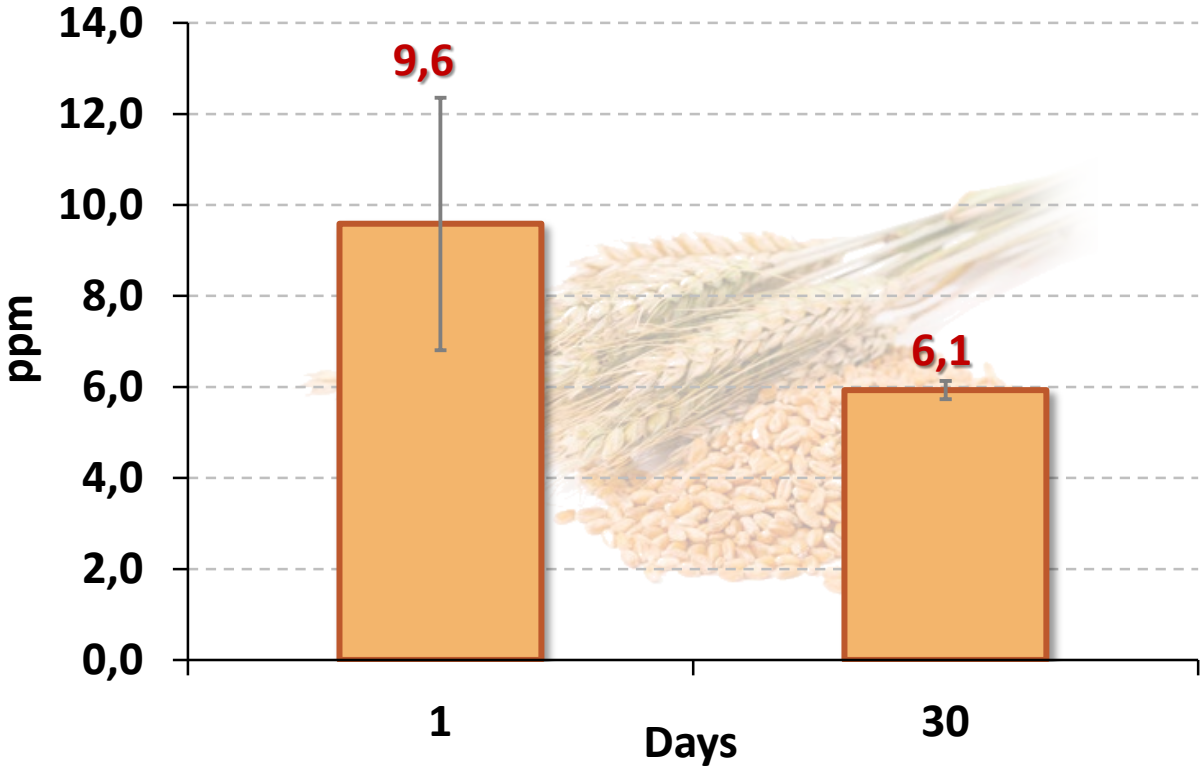


CONCENTRACIÓN DE AITC EN MAIZ



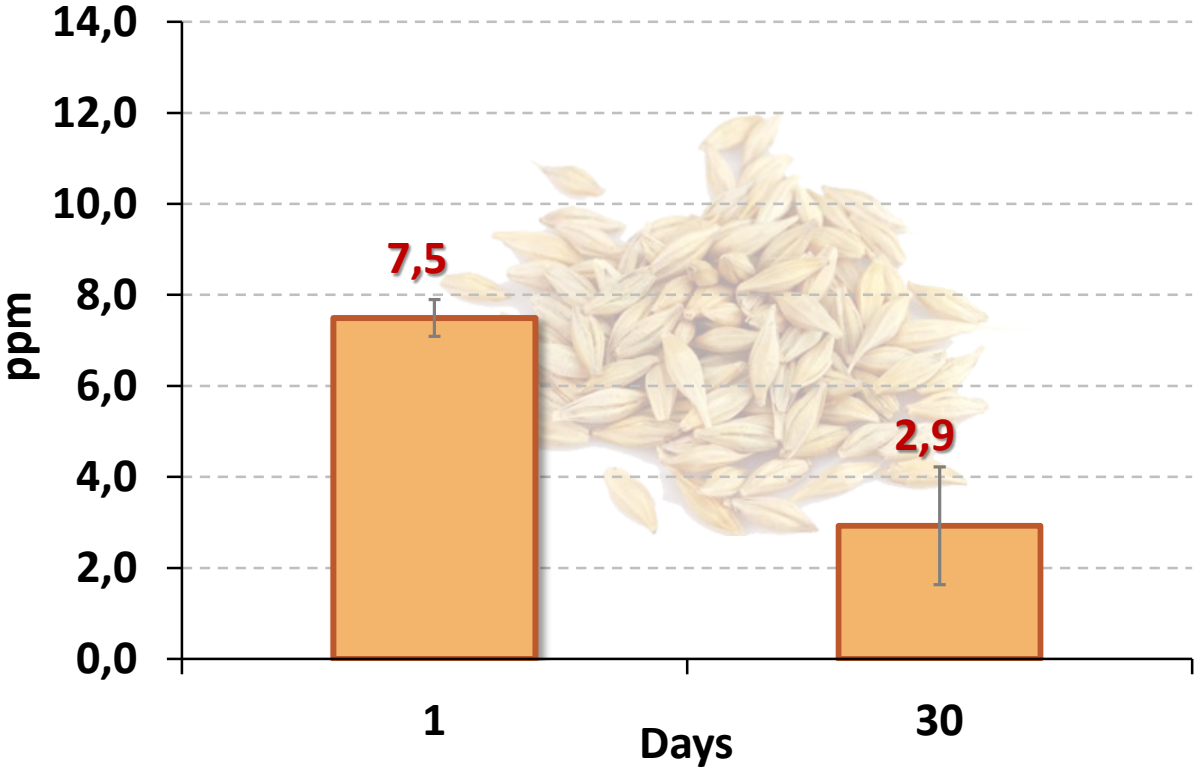
In agreement with the decrease of the **AITC** in the headspace, the concentration in the **corn** drops to a final value at **day 30** of **5.9 ppm**, the equivalent **0.36%** of the **AITC used in the gel**.

CONCENTRACIÓN DE AITC EN TRIGO



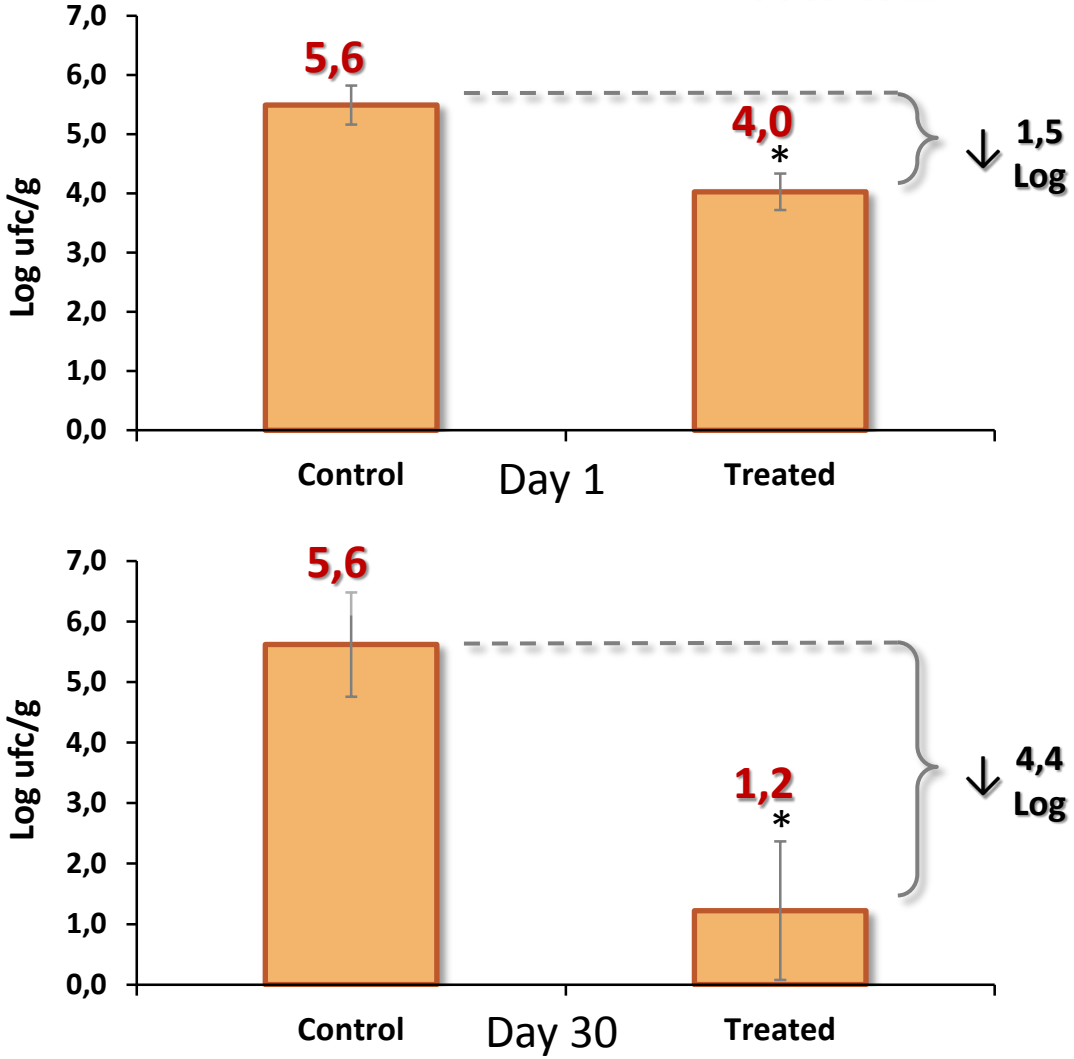
In agreement with the decrease of the **AITC** in the headspace, the concentration in the **wheat** drops to a final value at **day 30** of **6.1 ppm**, the equivalent **0.37%** of the **AITC used in the gel**.

CONCENTRACIÓN DE AITC EN CEBADA



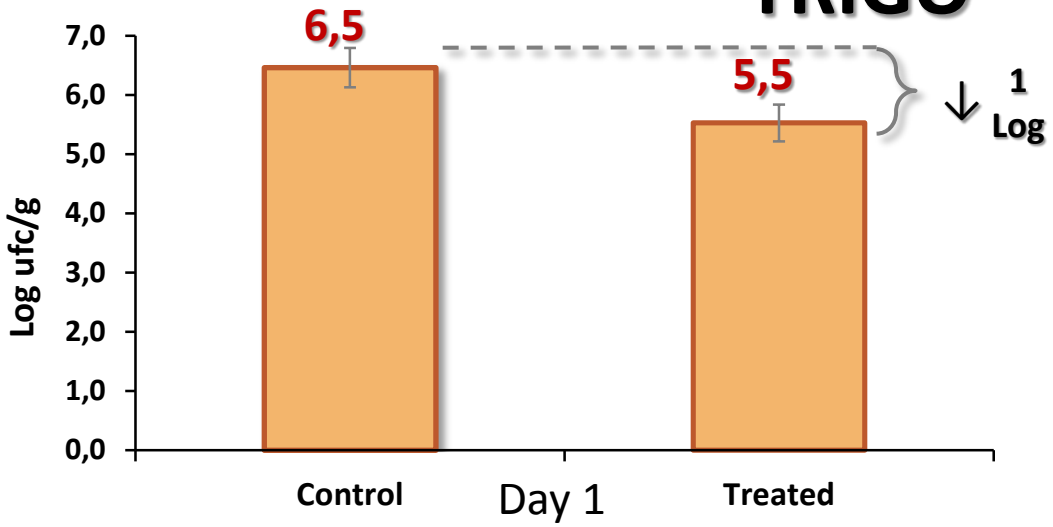
In agreement with the decrease of the **AITC** in the headspace, the concentration in the **barley** drops to a final value at **day 30** of **2.9 ppm**, the equivalent **0.18%** of the **AITC used in the gel**.

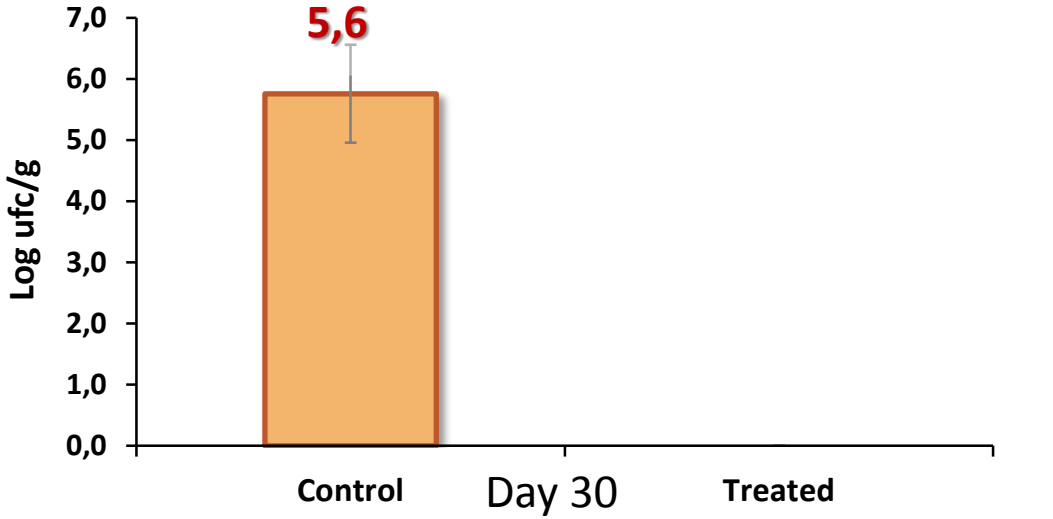
ANÁLISIS MICROBIOLÓGICO DEL MAIZ



The maximum growth of ***A. flavus*** was **5.6 log cfu/g** in the **control silo**, **1 log** higher than the inoculated dose. After only **24 hours** of exposure to the **AITC**, the amount of fungus was reduced by **1.5 log**, having **practically disappeared** with the treatment at **30 days**.

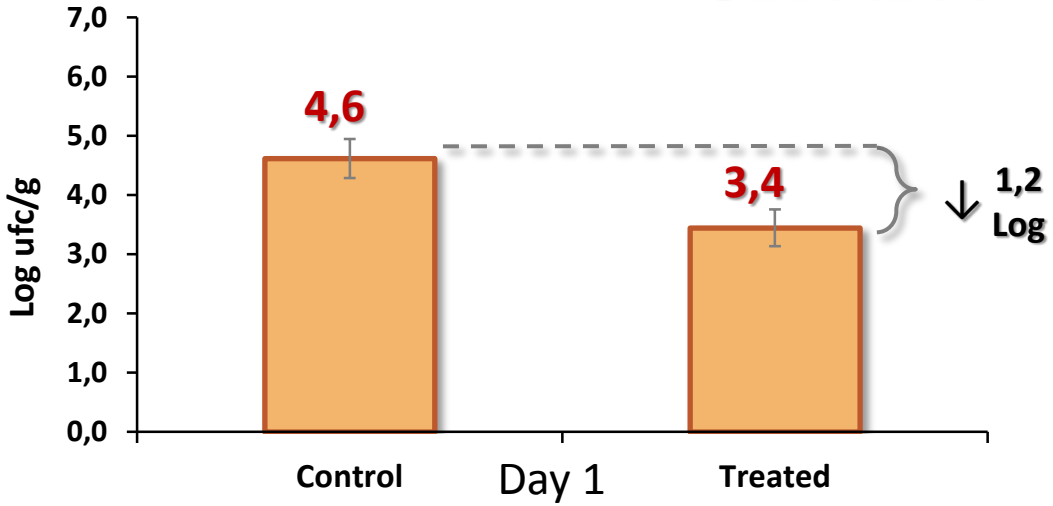
ANÁLISIS MICROBIOLÓGICO DEL TRIGO

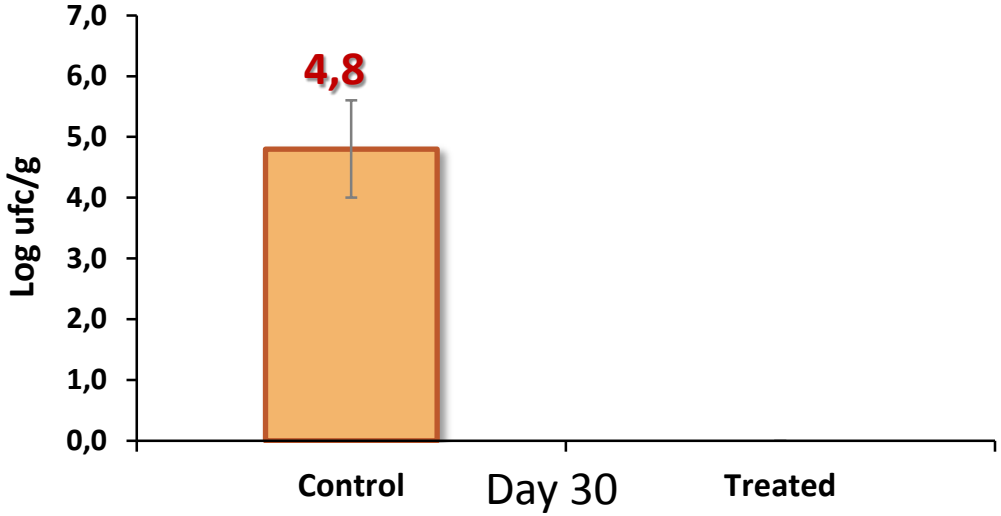




The maximum growth of **P. verrucosum** in **wheat** was **6.5 log cfu/g** in the **control silo**, **2 log** higher than the inoculated dose. After only **24 hours** of exposure to **AITC**, the amount of fungi was reduced by **1 log**, and **disappeared** with the treatment at **30 days**.

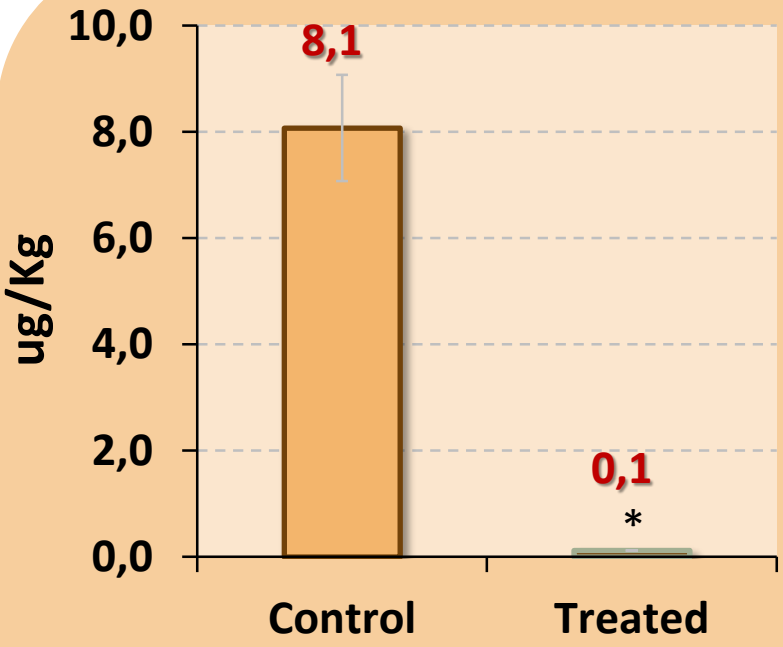
ANÁLISIS MICROBIOLÓGICO DE LA CEBADA



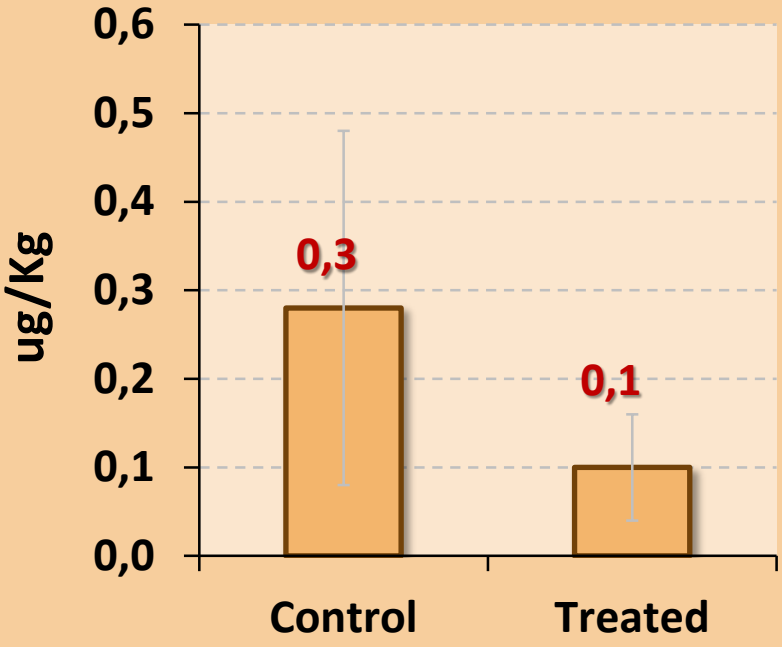


The maximum growth of **P. verrucosum** in **barley** was **4,8 log cfu/g** in the **control silo**, after **30 days**. After only **24 hours** of exposure to **AITC**, the amount of fungi was reduced by **1,2 log**, and **disappeared** with the treatment at **30 days**.

ANÁLISIS DE MICOTOXINAS



AFB_1 at corn, **30 days**.

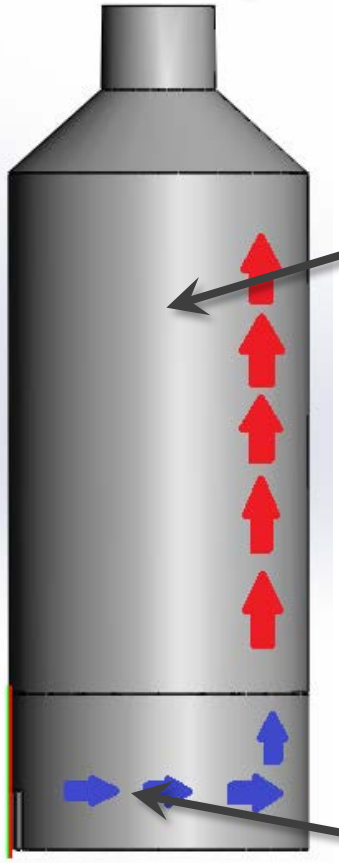


OTA at barley, **30 days**.

DISEÑO DE SILO PEQUEÑO SIMULADO

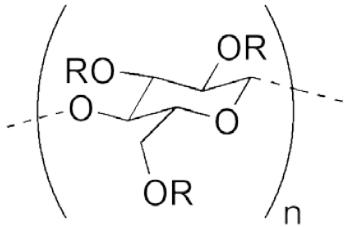
Air escape

AITC** treatment of **70 Kg** of **corn, barley** and **wheat** contaminated with **A flavus,** and **P. verrucosum

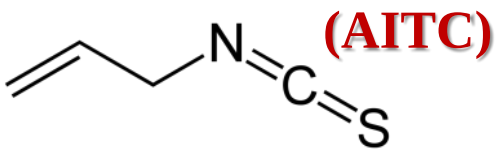


***AITC device** for **volatilization** of the **bioactive** compound in the silo system*

ESCALADO DEL GEL BIOACTIVO



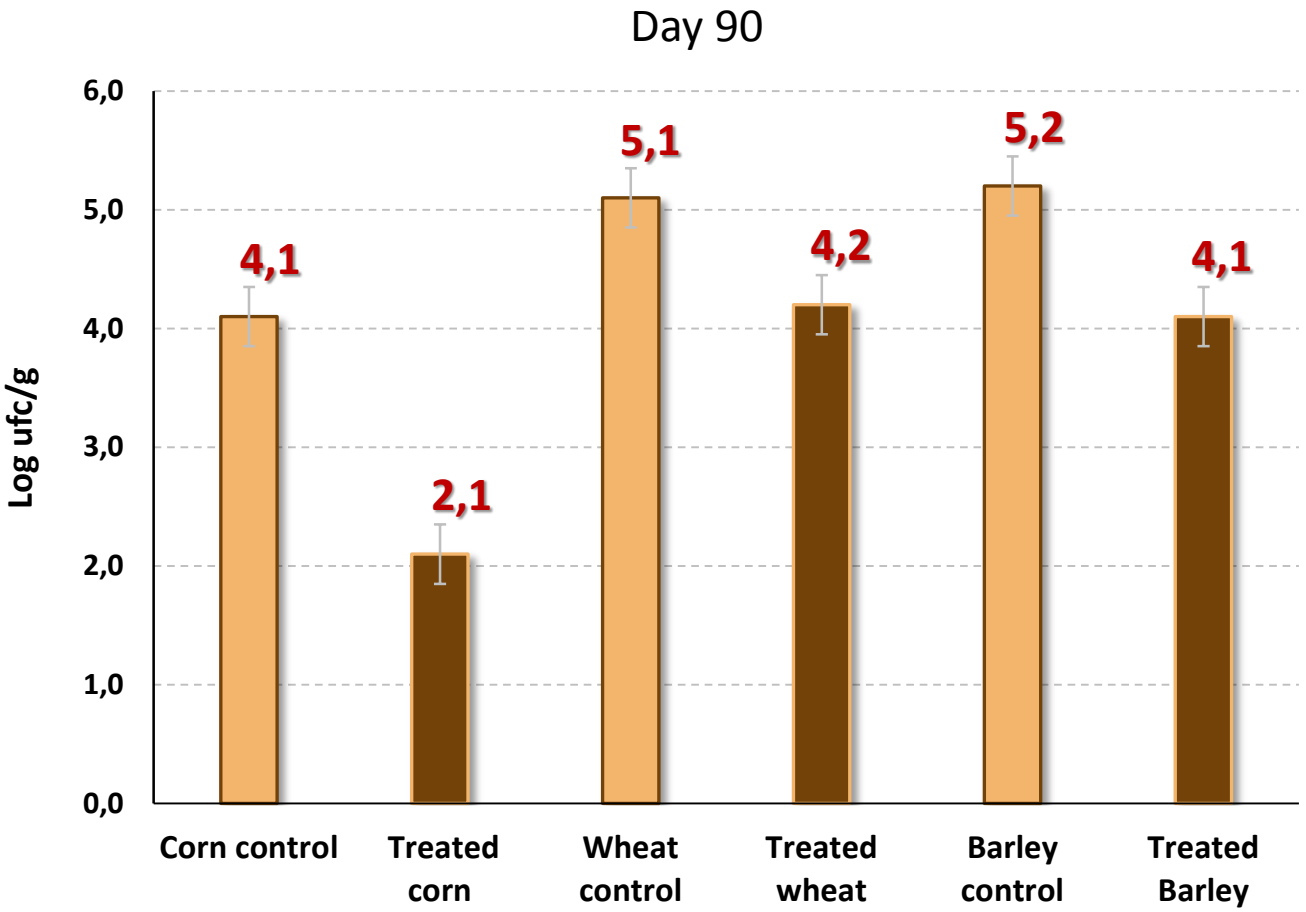
R = H or CH_2CH_2OH



15 ml of **AITC**
hydroxyethylcellulose gel (**12%**)

Release of the **AITC**

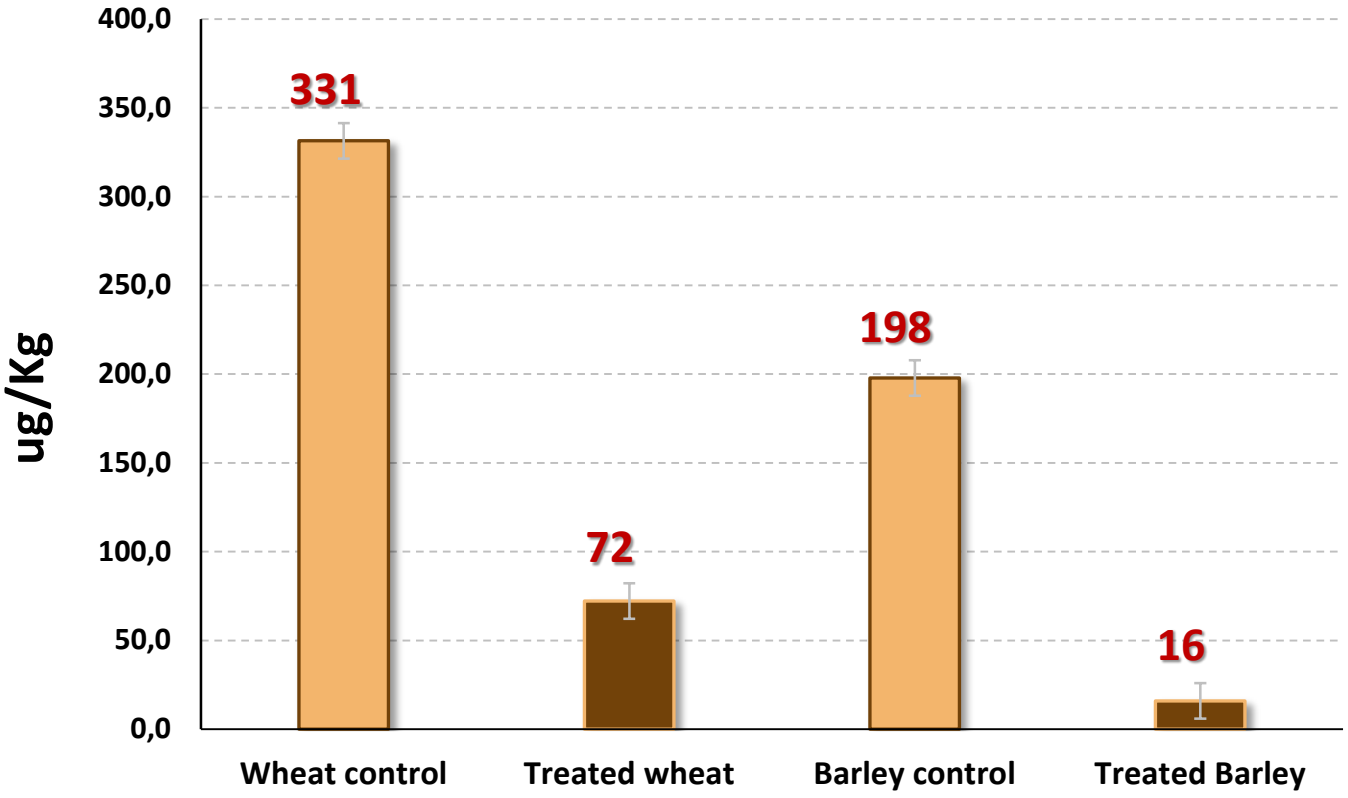
ANÁLISIS MICROBIOLÓGICO



The **reduction** in **fungal growth** at **90 days** varied between a **maximum** of **2 log cfu/g** (**corn**) and a **minimum** of **0.9 log cfu/g** (**wheat**).

ANÁLISIS DE MICOTOXINAS

Day 90



OTA results at **90 days**. **No AFs** were detected in the corn samples.

ESTUDIOS FUTUROS CON CEREALES

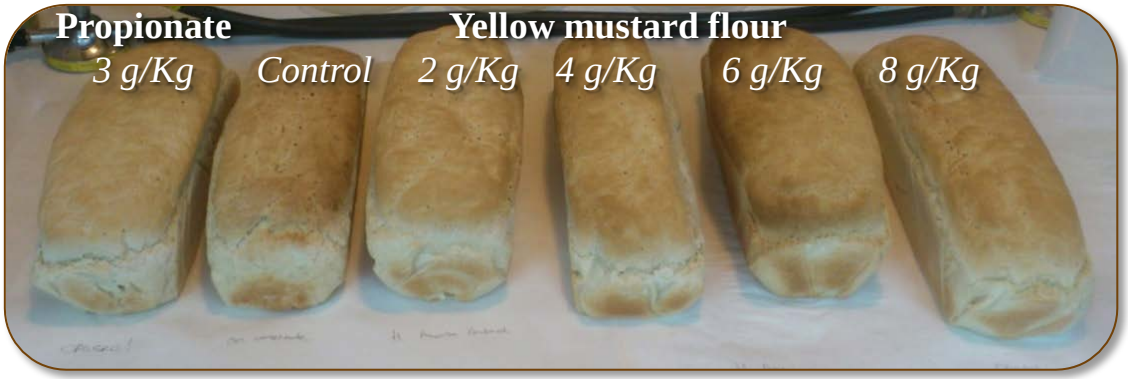
1. Ensayo en minisilo simulado ☑

2. Ensayo en silo pequeño simulado ☑

3. Ensayo en silo real ☐

Inhibición fúngica en pan empleando harina de mostaza amarilla

x 6

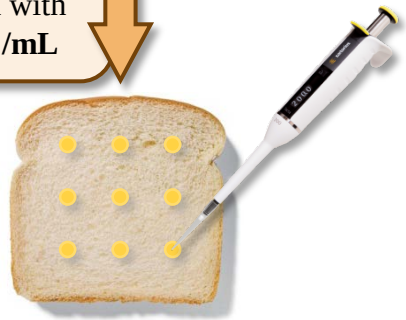


600 g of **flour, 250 mL** of **water, 40 g** of **yeast, 20 g** of **sugar** and **10 g** of **salt**

x 12

100 uL of **peptone water** suspension with **1x10^5 cfu /mL**

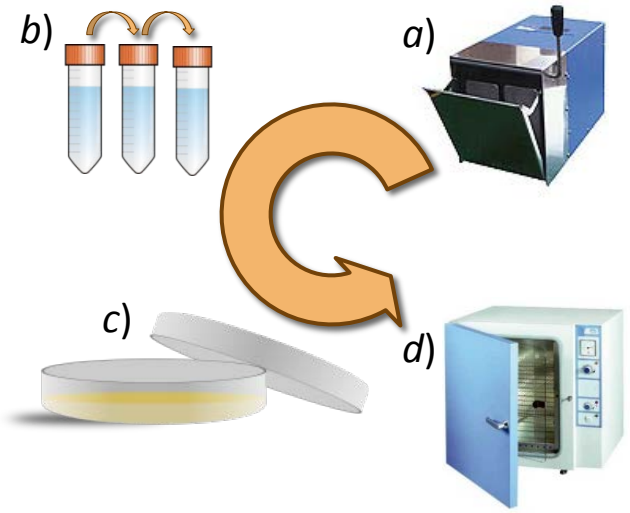
- ***A. flavus* 8111 ISPA**
- ***P. nordicum* 2320 CECT**



7 days, 25 ºC

ANALISIS

Determination of the fungal population (x9)



a. Homogenization by **stomacher**
b. Decimal serial dilutions in peptone water
c. Sowing in triplicate of 100 uL on plates with **PDA** medium
d. Count after **48 hours at 25 °C**

Determination of mycotoxins (x3)

(Saladino *et al*, 2017)

a. The samples were grounded
b. 5 g + 25 mL of **methanol** were homogenized by **ultraturrax**
c. Centrifuged 4000 rpm, 5 °C, 5 min
d. Evaporation by **Rotavapor** and **Turbovap**
e. Filter (**0.22 uM**), vialized and determination by **LC-MS/MS**

ESTUDIO DE LA VIDA ÚTIL (I)

Aspergillus flavus

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Propionate	-	-	+	+	+	+	+
Control	-	+	+	+	+	+	+
2 g/Kg	-	-	+	+	+	+	+
4 g/Kg	-	-	+	+	+	+	+
6 g/Kg	-	-	-	+	+	+	+
8 g/Kg	-	-	-	-	-	-	-

Propionate *Control* *2 g/Kg* *4 g/Kg* *6 g/Kg* *8 g/Kg*

ESTUDIO DE LA VIDA ÚTIL (I)

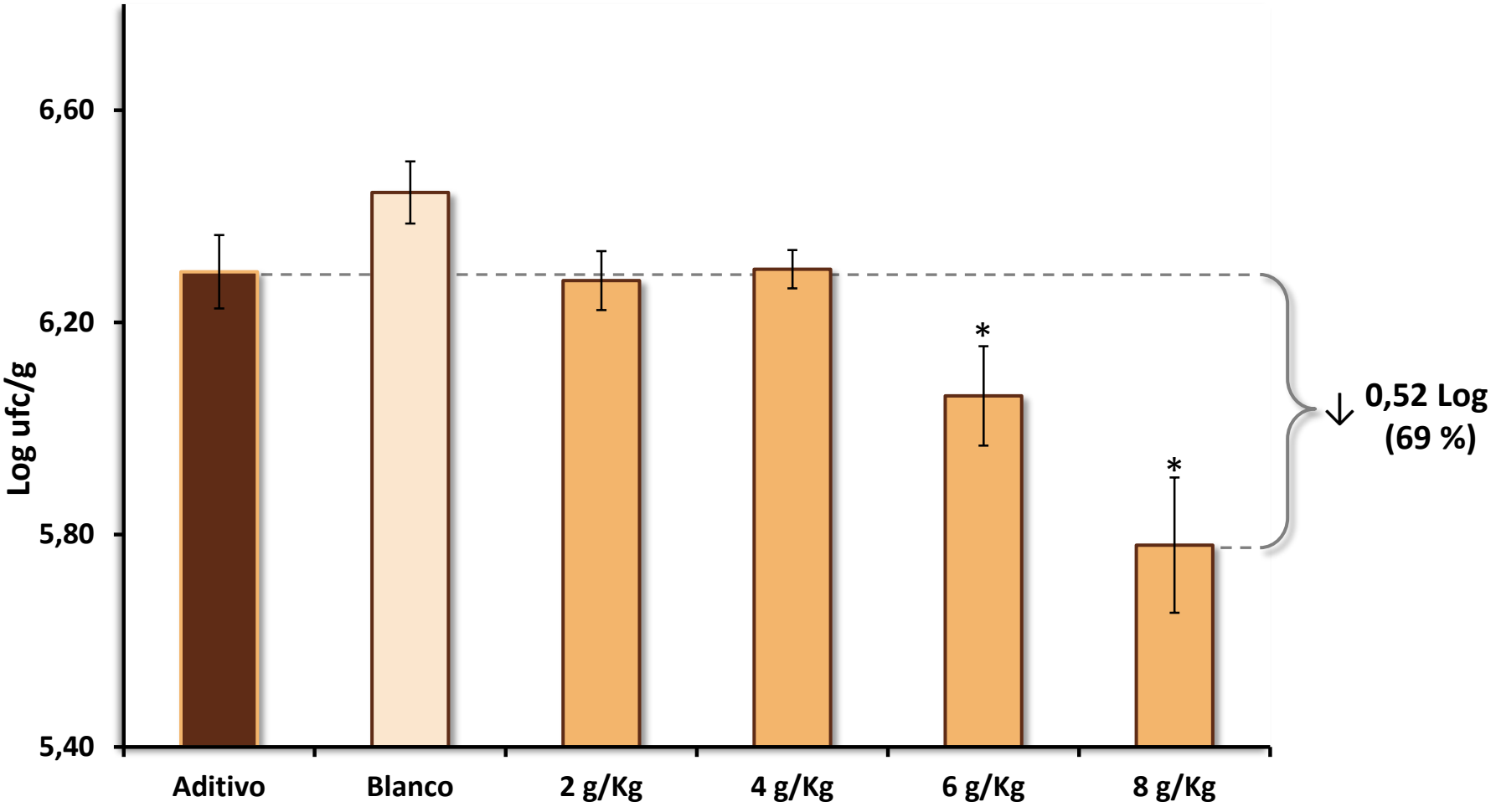
Penicillium nordicum

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Propionate	-	-	-	-	-	-	-
Control	-	+	+	+	+	+	+
2 g/Kg	-	+	+	+	+	+	+
4 g/Kg	-	-	-	+	+	+	+
6 g/Kg	-	-	-	-	-	-	-
8 g/Kg	-	-	-	-	-	-	-

Propionate *Control* *2 g/Kg* *4 g/Kg* *6 g/Kg* *8 g/Kg*

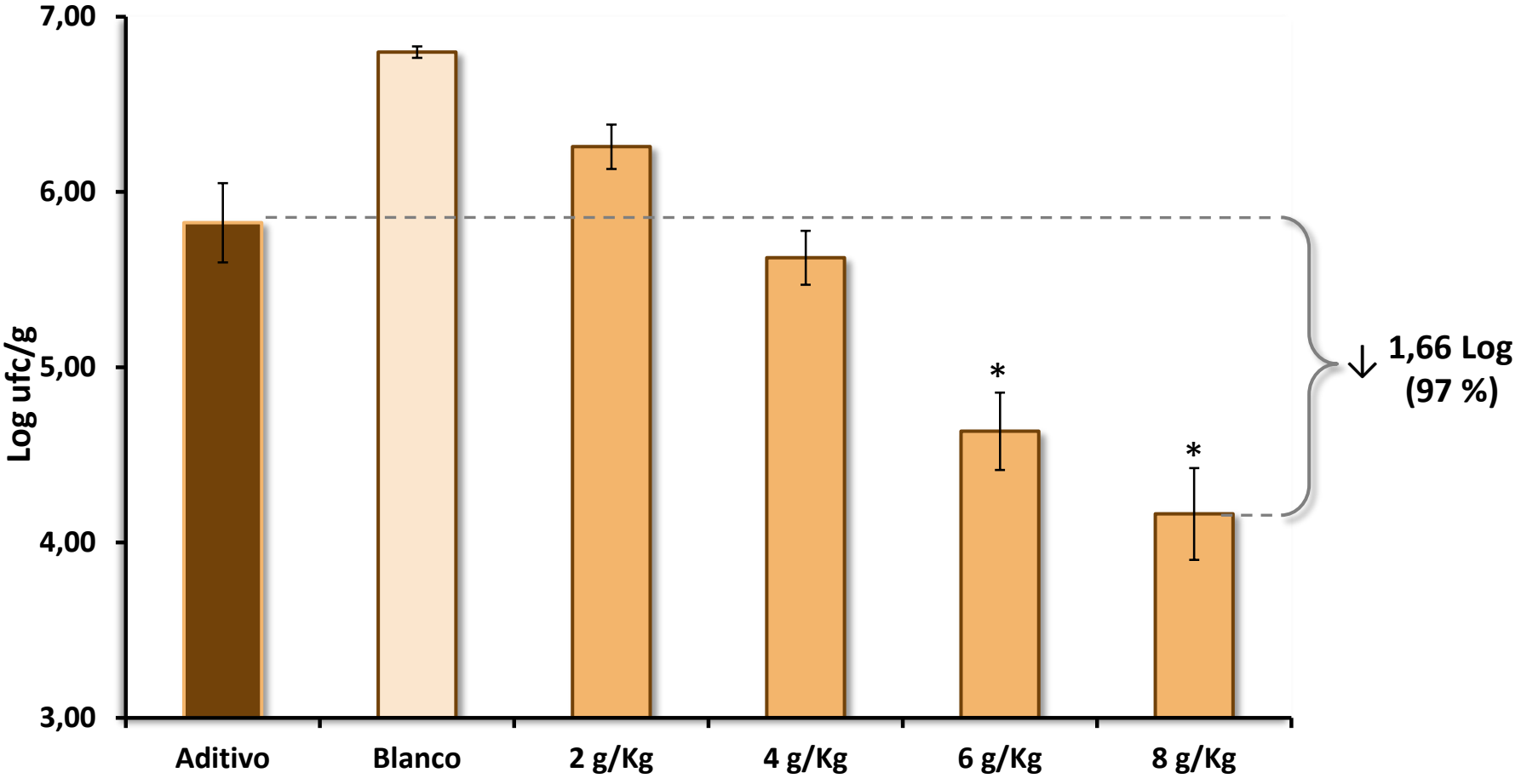
ANÁLISIS MICROBIOLÓGICO

Aspergillus flavus

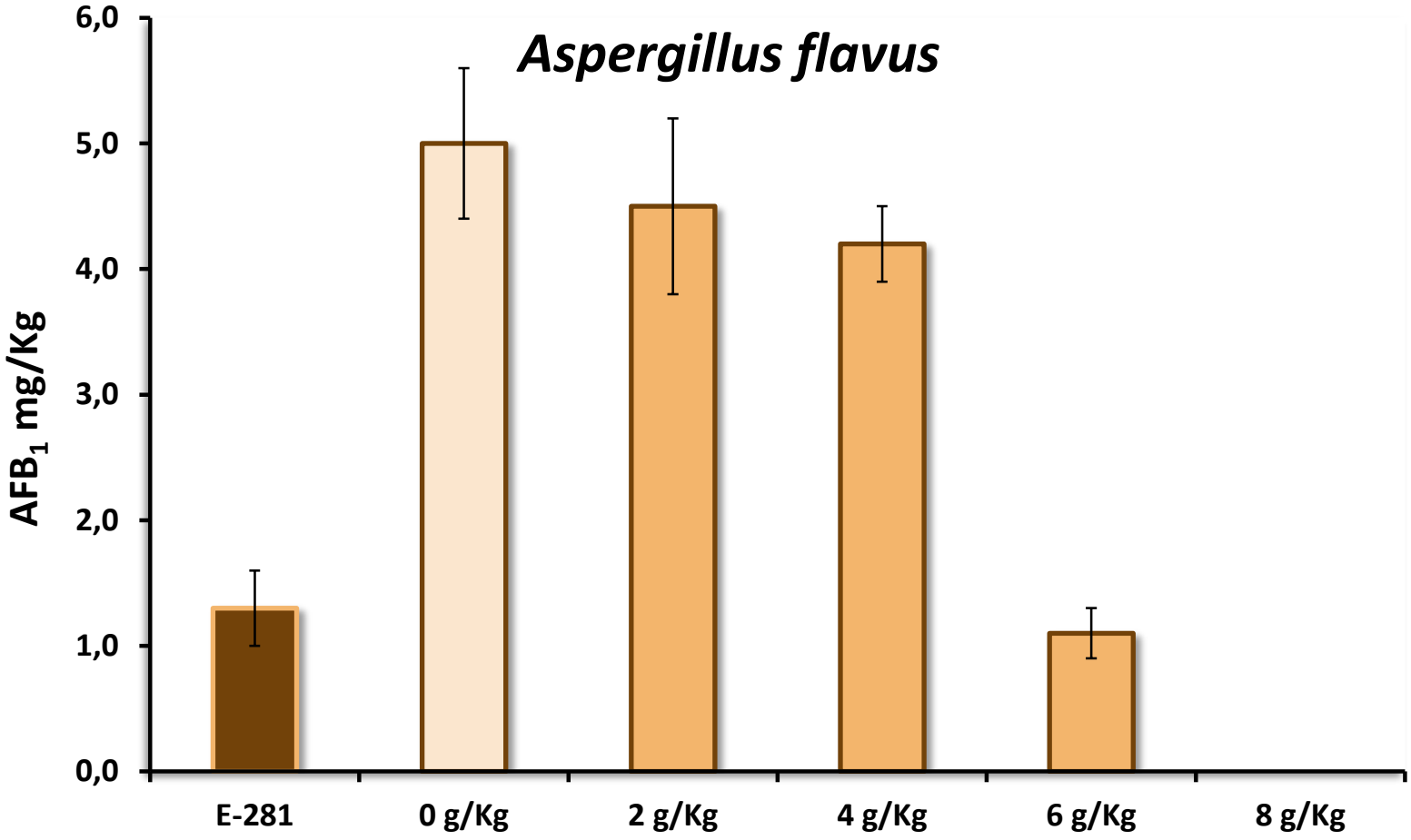


ANÁLISIS MICROBIOLÓGICO

Penicillium nordicum



ANÁLISIS DE MICOTOXINAS



Breads treated with **8 g/Kg** of **yellow mustard flour** showed a **100% reduction** in the formation of **AFB_1** compared to breads treated with commercial additives.

ESTUDIO DE LA VIDA ÚTIL (II)

Pan con harina de mostaza amarilla no contaminado (15 días)

Aditivo

Blanco

2 g/Kg

4 g/Kg

6 g/Kg

8 g/Kg

OTRAS ESTRATEGIAS DE CONSERVACIÓN: BIOCONSERVACIÓN

Empleo de microorganismos o sus productos metabólicos

Bacterias Ácido Lácticas

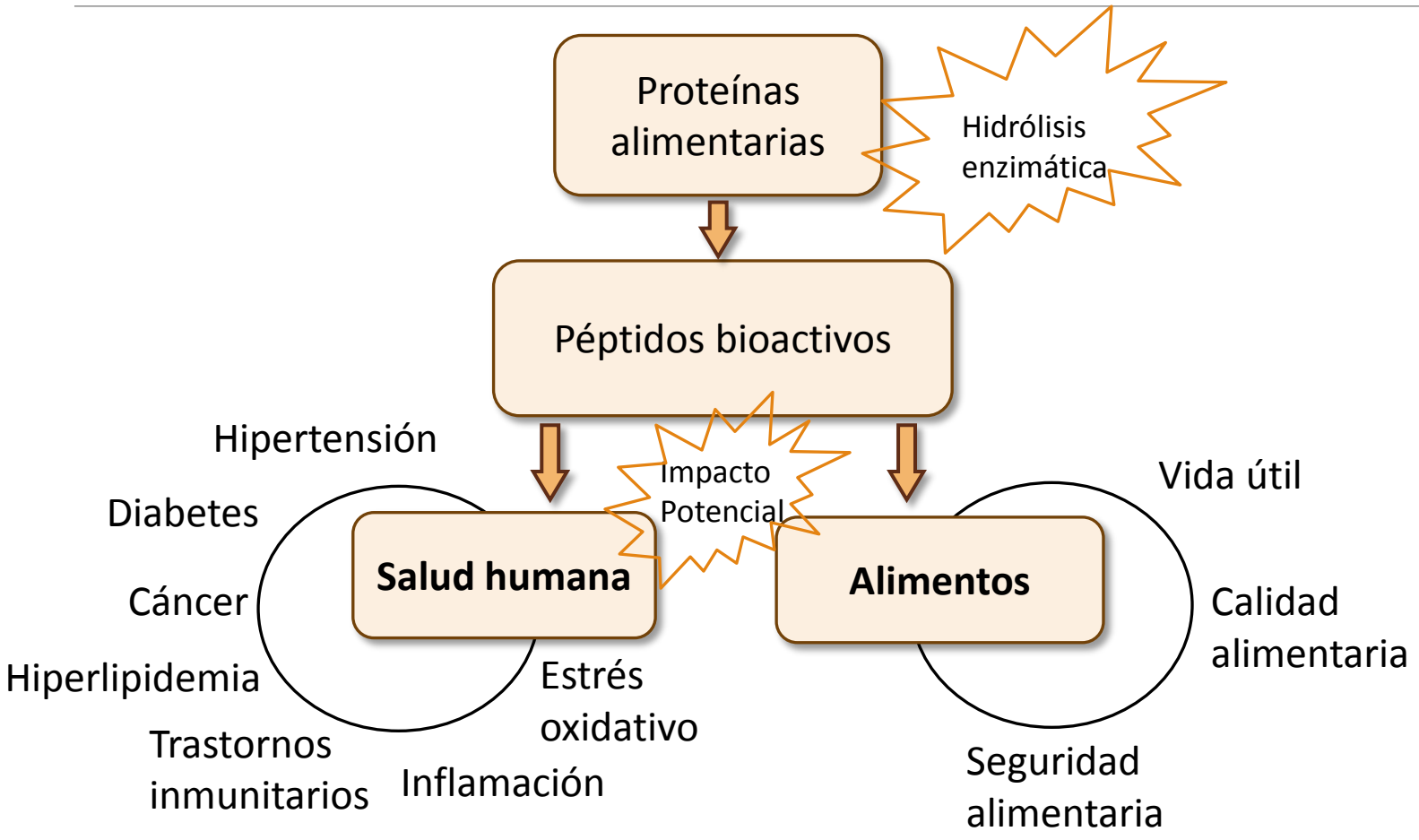
- Gram +
- No esporulados
- Fermentan carbohidratos
- **GRAS** (FDA)

FERMENTACIÓN

Compuestos antimicrobianos

- Ácidos Orgánicos
- Ácidos fenólicos
- Péptidos
- Bacteriocinas

OTRAS ESTRATEGIAS DE CONSERVACIÓN: BIOCONSERVACIÓN



Inhibición fúngica en pan empleando suero de leche fermentado por BAL

1 Preparación de las muestras de pan:

Ingredientes

Mezclado y amasado

Fermentación 1h

Horneado 200ºC/45´

Pan elaborado

Liofilizado de **suero de leche** fermentado por ***L. plantarum*** al **3**, **6** y **9%**

Inoculación de las muestras de pan con *P. expansum*:

2

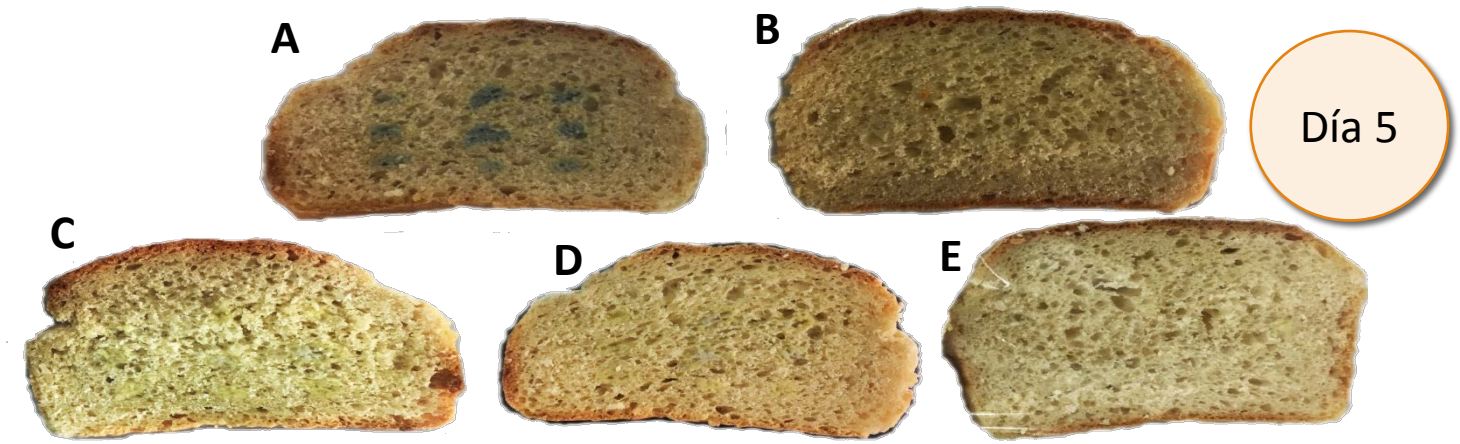
3

Conservación a Tª ambiente

ESTUDIO DE LA VIDA ÚTIL (III)

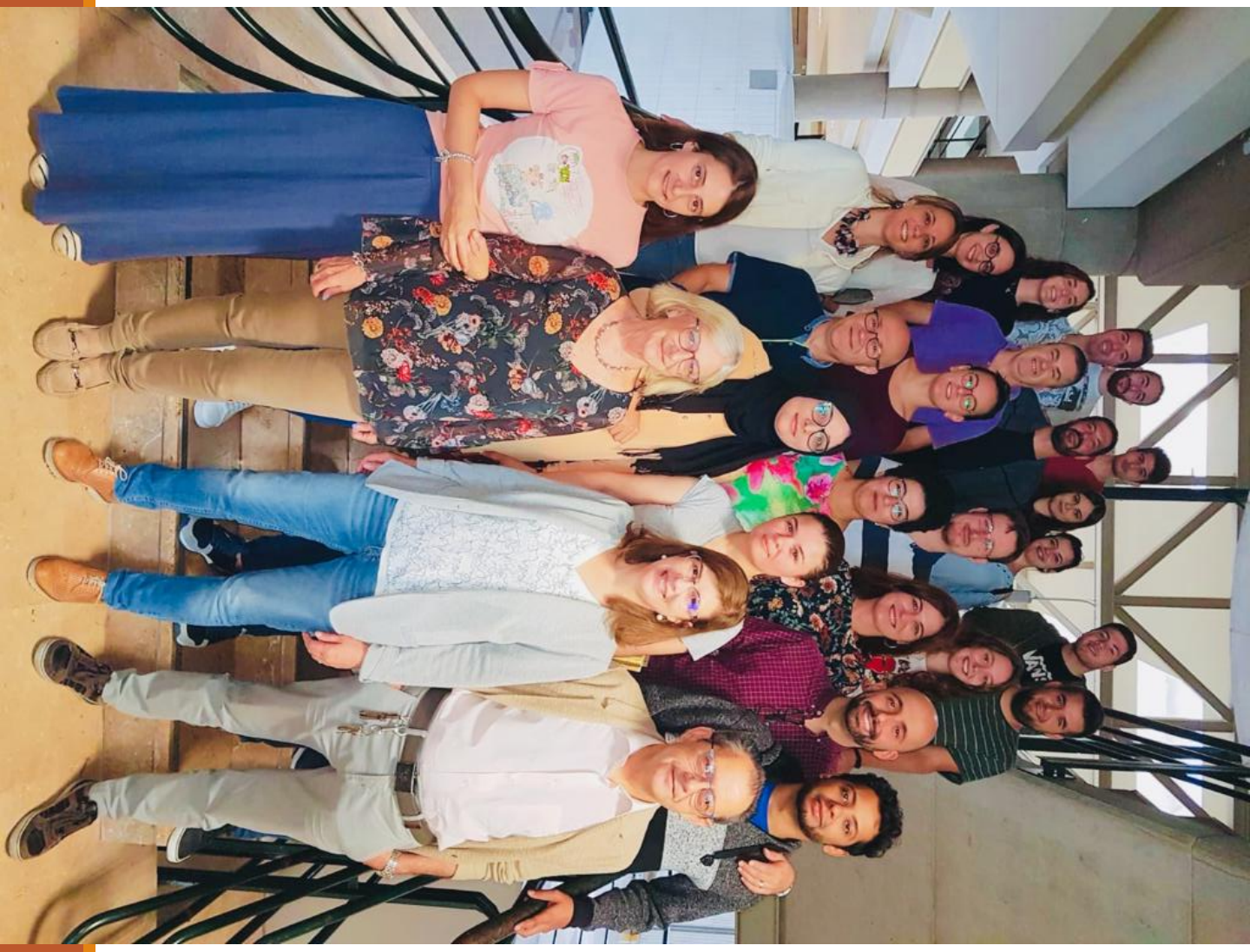
Penicillium expansum

		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
A	Blanco	-	-	-	+	+	+	+
B	Aditivo	-	-	-	-	-	+	+
C	3 %	-	-	-	+	+	+	+
D	6 %	-	-	-	+	+	+	+
E	9 %	-	-	-	-	-	+	+



Grupo de Investigación CoaL
CONTAMINACIÓN DE ALIMENTOS

30 AETC 2018
Jornadas Técnicas



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ALIMENTOS A BASE DE CEREALES Y MICOTOXINAS

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VNIVERSITAT DE VALÈNCIA Facultat de Farmàcia

Grupo de Investigación CoaL CONTAMINACIÓN DE ALIMENTOS

30 AETC 2018 Jornadas Técnicas