Chapter 5

Safety and Quality in the Agricultural Product Chain in Brazil

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Abstract

An agriculture-intensive country should be aware of natural toxins, including both mycotoxins and cyanotoxins, which are closely associated with the quality of raw materials, for food safety and industry. The major production chains – corn, wheat, beef, and broiler chicken – are the top components of agribusiness, and they should be tracked by reliable and practical tools. The corn chain is of particular concern in food production; intensive controls, multi-year mycotoxin monitoring, and improved harmless/sustainable management methods for uninterrupted farming in the tropic-subtropics are needed to achieve a long-lasting trend. The rapid control of natural toxins (mycotoxin and cyanotoxin) has focused on immunochemical methods developed with highly specific monoclonal antibodies (mAb) matched with chromatographic methods. In parallel, the promising widespread application of non-destructive analytical methods based on NIR (Near Infrared Reflectance)

spectroscopy, computer vision and hyperspectral imaging coupled with multivariate analyses have been introduced as an alternative for the prediction of quality and compositional parameters. Rapid quality control and product traceability are discussed, as well as accurate monitoring, which is essential for potentially launching an innovative system for food production in Brazil.

Keywords: Food quality and safety, rapid methods, immunoassay, natural toxins, sustainability

1. Introduction

Food matrices are organic materials with varied compositions, which also provide the nutritional components and perfect growing conditions for microorganisms, coupled with the simultaneous occurrence of several metabolic activities. Agricultural products, such as vegetables and fruits, are the basis of the food chain. Additional mechanical injuries during postharvest processing, storage and transportation may cause further points of contamination, leading to reduced quality, and compromising safety.

Preserving food products involves controlling external and internal conditions to avoid undesirable microbial growth and/or degradation processes, as well as the biosynthesis of unavoidable secondary metabolites, namely mycotoxins and phycotoxins.

Globalization demands high quality and competitiveness throughout the food chain. Quality and safety are typically achieved through a Hazard Analysis and Critical Control Points (HACCP) Risk Assessment. Providing raw materials of high quality and safe ingredients also includes the quality of water employed in food processes, which should be free of contaminants. Such strategies involve the detection of toxic secondary metabolites through continuous monitoring with reliable analytical methods, which should not only be restricted at the qualitative occurrence level but also the exact quantitative level compared with the maximal contamination limit proposed by guidelines.

The recommended techniques to detect ng and μg levels of toxic metabolites, and waste contaminants are based on High Performance Liquid Chromatography (HPLC) coupled with high sensitivity mass spectrometry (MS), which analyses residual contamination in a wide variety of products and materials.

These improvements are in contrast with the reality in raw material producing countries, highlighting the need for the innovative implementation of rapid methods combining simplicity, sensitivity and accuracy. Additionally, sequential processing and material resources in the food industry should be continuously monitored for safety and quality, which requires rapid monitoring *in loco*.

The rapid detection of natural toxins, such as mycotoxins and cyanotoxins, has been focused on immunochemical methods developed with highly specific monoclonal antibodies (mAb)

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matched with chromatographic methods. Such techniques arose based on antibodies, highlighting the immunoaffinity column (IAC) for the clean-up step, and enzyme linked immunosorbent assay (ELISA) with the advantage of eliminating toxic solvents (using buffer). The current commercial kits have been the practical tool of choice and have an important role in avoiding hazards for animals and humans. Immunoassays are advancing with developments in nano-engineering, resulting in compact, miniaturized electronic devices, such as biosensors, which combine high specificity and biological diversity with automation of diagnostics. The advantages of these developments are their specificity, speed and simplicity for the detection of dangerous levels of natural toxins.

Nevertheless, both chemical and biological analytical methods are destructive, i.e., the decision concerning the total batch is extrapolated based on data obtained with samples that were already destroyed for analysis. Non-invasive and non-destructive chemical-free techniques came as a welcome option in the industrial process, including optical methods (Fourier Transform coupled to Infrared Spectroscopy, FT-IR spectroscopy and transmittance in the near infrared, near-IR), as well as an "electronic nose" for volatile compounds. These technologies are able to integrate with online quality control monitoring systems in the food chain in real time and are able to detect imbalances caused by deteriorated quality, which can also indicate undesirable toxic metabolites.

Continuous tracking in the food chain should focus on safety and quality using practical and reliable analytical techniques. The combination of rapid biological assays, non-destructive physical technologies and primary chemical analysis are desirable procedures for extending the shelf life of a product.

We begin by presenting data on corn, a topic of concern in the food chain, as it is a universal ingredient with unavoidable mycotoxin hazards – even with extensive monitoring in the agro-industrial region of Southern Brazil. An ic-ELISA-based immunoassay was developed, established and optimized to analyse different food groups, using specific MAb produced by hybridomas (especially against non-immunogenic low molecular mass ochratoxin (OTA), aflatoxin (AF), deoxynivalenol (DON), zearalenone (ZEA), fumonisin B_1(FB_1), OTA and microcystin-LR (MCLR)). This has become important for rapid tracking, monitoring safety and quality, and providing guidance for the best conduct to establish a long-lasting trend focusing on harmless/sustainable management in uninterrupted tropical-subtropical farming, and in replacing chemical agrotoxicants. The control of natural toxins should begin at the field level through sustainable management, adequate water quality, predictive modelling, as well as in the food processing systems in agroindustry. Such an overall approach, could result in the production of healthy foods in potential food producing regions in Brazil.

2. Corn chain – Relevant aspects in a producing country

Brazil has a vast cultivated area of 53.20 million ha and continues to expand production, with 10.9 % in grain volume corresponding to 184.30 million from the 2012/2013 crop harvest, compared with 166.17 million ton in 2011/2012 [1]. Estimates indicate that grain production

will be approximately 200.08 million ton in 2014/2015, 3.4 % higher than the 2013/2014 production [2]. The total cultivated area of grain also showed growth (57.03 million to 57.39 million hectares), with the promising increase of second crops allowed by tropical climates.

Corn (*Zea mays* L.) is one of the major cereal crops in Brazil, with an annual production of 78.689 million (metric) ton, ranking the country as the third largest corn producer in the world. Paraná State was the second largest producer with 14.504 million ton in 2014 [3]. An abrupt increase in trading has occurred due to the large territorial extension associated with climatic diversity; i.e., the tropical climate in midwestern and northern regions, and subtropical-temperate climates in southern and southeastern Brazil, which enables production throughout the year. Therefore, the exportation of 3.37 million ton in February 2013 was 297 % higher than in January 2012. Corn is the second most produced crop (39.29 %), preceded only by soybean (47.19 %). From February 2014 to January 2015 – with 20.9 million ton, Brazilian exports were 400,000 ton higher than the projected capacity. The main corn importers were Iran, Vietnam, South Korea, Taiwan, Egypt, Indonesia, Malaysia, Japan, Saudi Arabia and Morocco [2].

Figure 1 shows the production of corn immediately following the soybean harvest, which allows up to three crop cycles per year in producing regions.

Figure 1. Harvesting of precocious soybean followed by simultaneous planting of corn as the second crop.

Approximately 70 % of Brazilian corn is intended for swine and broiler feeds, whereas processing for human consumption corresponds to 15 % [4]. Nevertheless, its high nutritional quality introduces risk for the growth of toxigenic fungi favoured by tropical and subtropical climates. Mycotoxins are natural thermostable metabolites responsible for substantial economic losses and their persistent residual levels can be detected even in post-processed meat, eggs, milk and dairy products. The Food and Agricultural Organization (FAO) estimated the worldwide mycotoxin contamination in crops at 25 %.

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The most important mycotoxins in tropical developing countries, such as Brazil, have been fumonisins produced mainly by *Fusarium verticillioides* and *F. proliferatum* [5], and aflatoxins produced by *Aspergillus flavus* and *A. parasiticus* [6].

Corn quality associated with fungal and mycotoxin contamination in Paraná State, southern Brazil, has been studied since the 1980s, when fumonisin caused animal poisonings. The first report involving an animal outbreak detected fumonisin B_1 (FB_1) and B_2 (FB_2) in feed samples and determined that *F. verticillioides* isolates were acutely toxic to ducklings [7]. A survey of fumonisin in corn kernels also reported its occurrence in the states of Mato Grosso do Sul and Goiás [8], the most progressive region of grain production, where FB_1 and FB_2 were detected in 97.4 % and 94.8 % samples, respectively [2]. All corn from Northern Paraná (n = 39) was fumonisin positive (mean level of FB_1 = 4.79 μg g^{-1} and FB_2 = 3.95 μg g^{-1}). The samples from Mato Grosso and Goiás States (n = 9) showed mean FB_1 levels of 10.59 and 5.83 μg g^{-1} and FB_2 levels of 10.31 and 3.62 μg g^{-1}, respectively.

The co-occurrence of fumonisins and aflatoxins was investigated in 150 freshly harvested corn samples (1994/1995 crop) from the central-southern (n = 27 samples), central-western (n = 86) and northern (n = 37) regions of Parana State. Fumonisins and aflatoxins were detected in 98 % and 11.3 % samples, respectively. All the aflatoxin positive samples (mean, 191 ng g^{-1}) were from the central-western region and were co-contaminated with fumonisins. Higher fumonisin levels were detected in corn from the northern (9.85 μg g^{-1}) and central-western regions (5.08 μg g^{-1}) relative to the central-southern region (1.14 μg g^{-1}), suggesting an effect of climatic conditions in addition to the local predominance of toxigenic *Fusarium* biotypes [9].

Fumonisin monitoring in real time was established (2003-2004 crop) on critical steps (field, reception and pre-drying) of the corn chain [10]. Fumonisins were analysed in 490 samples of freshly harvested corn (2003-2004 crop) collected at three points of the production chain in Northern Paraná State, and correlated with the time interval between harvesting and the pre-drying step. The mean fumonisin level increased gradually from ≤ 5.0 μg g^{-1} to 19.0 μg g^{-1} when the time interval between harvesting and the pre-drying step increased from 3.22 to 8.89 hours. Fumonisin levels were correlated positively ($p \leq 0.05$) with time interval (ρ=0.96), indicating that a delay in the drying process could increase the levels of contamination.

A study [11] evaluated fumonisin in 870 freshly harvested corn samples (2003 and 2004 crops) used by processing industries in Northern Paraná State. Sampling was performed at two points of the corn chain, i.e., at reception and the pre-drying step in the processing industry. Fumonisins (FB_1 + FB_2) were detected in all samples from the two points in both crops. Fumonisin levels in reception (2.24 μg g^{-1}) and pre-drying samples (2.87 μg g^{-1}) of the 2003 and 2004 crops (1.46 and 1.52 μg g^{-1}, respectively) showed similar profiles, indicating that corn used by processing industries in this region showed lower fumonisin levels than in previous studies [8, 9, 12]. Years of monitoring have shown a decreasing trend of fumonisin contamination, which may be due to changing procedures at food and feed processing facilities.

Because determination of the degree of exposure is one of the most important parameters concerning the risk assessment of chemical compounds, a study [13] estimated the maximum probable daily intake (PDI_M) of fumonisins in a local population. This study was based on

fumonisin monitoring in 300 freshly harvested corn (2003 and 2004 crops) samples collected at two points of the production chain (reception and pre-drying) in Northern Paraná State. Based on the highest mean fumonisin levels being detected in the pre-drying samples (3.12 μg g^{-1}) and the average consumption of corn-based products, the maximum probable daily intake (PDI_M) of FB_1 estimated in the Brazilian population (0.95 μg kg^{-1} body weight day^{-1}) was below the tolerable daily intake (2.0 μg kg^{-1} body weight day^{-1}).

Such monitoring allowed the identification of fumonisin levels in different regions of the state, enabling it to gain a prominent position in corn exportation. Currently, the State of Paraná is responsible for 14.3 million tons/year, corresponding to 17.9 % of the national corn production [3].

3. Rapid immunoreagent monitoring in food safety

Advanced techniques in liquid chromatography using different detectors (UV-Vis-PDA, FLD, MS and LC-MS/MS) have been introduced for the analysis of chemicals in different matrices (food, microbial/plant metabolites, and water). Chromatographic techniques provide the most reliable data due to their precision and accuracy of analysis; therefore, they have also been recommended for use in evaluating alternative rapid techniques. Analytical methods should be appropriate and efficient for each matrix array, i.e., each modification introduced must be in accordance with validation criteria and the specific requests of regulatory organization.

Incomplete extraction and matrix effects of crude extract in the cleaning step can lead to a sub-estimation of real concentrations in analysis; thus, a minimum preparation is advantageous. The multi-toxin methods for HPLC and sequential mass spectrometry (LC-MS/MS) provides a high selectivity, lower limits of quantification and detection, the possibility of generating structural information of the analyte with minimal sample treatment, and the reduction of errors associated with pre-and post-column derivatization. Analyses by LC-MS/MS has gained much interest in analytics [14, 15].

Although there have been other advances in analytics, HPLC coupled with fluorescence and ultraviolet detectors remain the main detection method in Brazil [16, 17, 18]. In addition, the unavoidable occurrence of mycotoxins has obliged several countries to adopt regulatory guidelines, and maximum tolerated levels vary widely among countries [19].

Current regulations are increasingly based on international organizations, such as the FAO/WHO Joint Expert Committee on Food Additives of the United Nations (JECFA), and the European Commission. Strict guidelines on mycotoxins have been imposed by importing countries, demanding a rigorous and continuous monitoring of the food chain. The prevailing guidelines for mycotoxins require different protocols of extraction and analysis, and foods for infants and young children with more restrictive limits increases the number of analyses [20]. Such diversity in extraction procedures results in costly work.

Safe raw materials should be tracked by reliable analytical methods, and rapid methods are useful tools, especially in food-producing countries. Immunoassays based on ic-ELISA with

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highly specific monoclonal antibodies (MAb) against ochratoxin (OTA), fumonisin (FB), aflatoxin (AF), deoxynivalenol (DON), zearalenone (ZEA) and microcystin (MC) have been developed, previously tested for cross-reactivity with each analogue group, and correlated with HPLC as the primary method (Table 1, 2 and 3). A careful evaluation of ic-ELISA was conducted in the analysis of natural toxins in the food chain targeted to field/storage stage, beginning with the monitoring of fumonisins in corn [21]. The successful rapid technique motivated to use of this analysis for OTA in coffee and wine [22, 23], aflatoxin [24, 25], DON [26, 27, 28] and ZEA [29].

Hybridoma Cell line	Toxin	Cross reactivity (%)*	Hybridoma Cell line	Toxin	Cross reactivity (%)*
AF.2[30]	AFB_1	100	ZEN.2[34]	ZEA	100
	AFB_2	133		α-Zearalenol	60
	AFG_1	13.4		β-Zearalenol	5.7
	AFG_2	14.7		α-Zearalanol	7.1
	AFM_1	0.9		β-Zearalanol	0.9
DON.3[31]	DON	100	M8H5[35]	MCLR	100
	15-acetil DON	333		MCRR	106
	NIV	5		MCYR	44
	4-acetil NIV; Toxin T-2 tetraol	1.2		MCLA	26
	Others *	<0.5		3-desmethyl MCLR	51
				7-desmethyl MCLR	48
OTA.1 / OTA.7[32]	OTA	100 / 100		MCLR GSH conjugate	47
	OTC	63.1 / 79.4		MCLR methyl ester	30
	(4R)-4-HydroxyOTA	1.19 / 1.24		Nodularin	20
	OTB	0.63 / 1.07		6 (Z)-Adda -MCLR and - MCRR	<7
FB 1-2[33]	FB_1	100	MC. 5-3/ 8-3 / 2[36]	MCLR	100
	FB_2	224		MCRR	146 / 113 / 60
	FB_3	72		MCYR	88 / 65 / 113

AFB_1: Aflatoxin B_1; AFB_2: Aflatoxin B_2; AFG_1: Aflatoxin G_1; AFG_2: Aflatoxin G_2; AFM_1: Aflatoxin M_1; DON: Deoxynivalenol; NIV: Nivalenol; ZEA: Zearalenone; OTA: Ochratoxin A; OTC: Ochratoxin C; OTB: Ochratoxin B; FB_1: Fumonisin B_1; FB_2: Fumonisin B_2; FB_3: Fumonisin B_3; MCLR: Microcystin-LR; MCRR: Microcystin-RR; MCYR: Microcystin-YR; and MCLA: Microcystin-LA.

* Percentage of relative cross-reactivity was calculated as the amount of toxin required for 50 % binding inhibition/amount of other toxins requiring 50 % binding inhibition × 100.

** Others: 3-acetyl DON, 3,4-diacetyl NIV, tetraacetyl NIV, Toxin T-2, Toxin T-2 acetyl, and diacetoxyscirpenol.

[30] Kawamura et al., 1988; [31] Kawamura, 2005; [32] Kawamura et al., 1989; [33] Iijima et al., 1996; [34] Kawamura e Emoto, 2006; [35] Nagata et al., 1995; [36] Tabuchi et al., 2015.

Table 1. Cross-reactivity of monoclonal antibodies (anti-mycotoxins & microcystins) applied in a monitoring study in Brazil.

Table 1 shows the cross-reactivity of MAb (anti-mycotoxins & microcystins). It confirmed the high specificity of selected hybridomas, which were adequate for application in rapid surveys. Cross-reaction in immunoassays would be expected due to the biosynthesis of natural toxins in a sequential cluster of closely related structural substances. Nevertheless, the cross-reactivity within analogues can be advantageous in screening surveys of natural toxins compared with strongly specific individual analogue detection by HPLC.

Table 2 shows how ic-ELISA became established as reliable rapid technique to analyse mycotoxins and microcystins. Such local set-ups can allow safe supervision in one of the major food producing regions in Brazil, which was made possible due to joint research involving cell culture technologies, adaptation and proliferation of MAb producing hybridomas, and the development of immunoassays *in loco*. The standardized immunoassay was obtained through enhancing its sensitivity and adjusting to local conditions for the reagents, dilutions in ic-ELISA steps (upgrading crude extract preparation, antigen-protein conjugates for microplate coating, and dilutions of both the first and second antibody), and the analogue group for detection. The safety of the food, derived products, and water for analysis were amplified by awareness. These assays should be conducted for local consumption safety, the balance of agribusiness and exportation demand and importation independence.

Item	Hybridoma	Toxins	Reagents: ic-ELISA steps			LOD / LOQ ($\mu g\ kg^{-1}$)	ELISA/ HPLC (r)	Cereal & products
	Cell line	Mycotoxin/ Cyanotoxin	Coating	First MAb	Second Ab: IgG-enzyme			
1	DON.3	DON	DON-HG-OVA 2 $\mu g\ mL^{-1}$	1200 $\mu g\ mL^{-1}$	1:2000	177.1 / -	0.93	Wheat grain[26]; wheat flour[27]
2			DON-HS-OVA 2 $\mu g\ mL^{-1}$	19.2 $\mu g\ mL^{-1}$	1:1000	113.5 / 445.3		Wheat grain[28]
3			DON-HS-OVA 2 $\mu g\ mL^{-1}$	10.9 $\mu g\ mL^{-1}$	1:2000	159.3 / 370	-	Biscuit[a]
4	ZEN.2	ZEA	ZEN-OVA 2.5 $\mu g\ mL^{-1}$	10.3 $\mu g\ mL^{-1}$	1:2000	33.7 / 87	-	Wheat grain[a]
5			ZEN-OVA 2.5 $\mu g\ mL^{-1}$	10.3 $\mu g\ mL^{-1}$	1:2000	9.7 / 23.7	-	Biscuit[a]
6	FB 1-2	FB_1	FB1- OVA 0.77 $\mu g\ mL^{-1}$	1:50	1:5000	93 / -	0.94	Corn grain[a,21]
7	AF.2	AF	AFB_1-BSA 0.25 $\mu g\ mL^{-1}$	0.094 $\mu g\ mL^{-1}$	1:2000	2.0 / 4.6	-	
8	DON.3	DON	DON-HS-OVA 2 $\mu g\ mL^{-1}$	10.9 $\mu g\ mL^{-1}$	1:2000	302.8 / 589.3	-	
9	ZEN.2	ZEA	ZEN-OVA	**10.3 $\mu g\ mL^{-1}$**	**1:2000**	**51.7 / 93.2**	**0.91**	

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Item	Hybridoma	Toxins	Reagents: ic-ELISA steps			LOD / LOQ (μg kg^{-1})	ELISA/ HPLC (r)	Cereal & products
	Cell line	Mycotoxin/ Cyanotoxin	Coating	First MAb	Second Ab: IgG-enzyme			
			2.5μg mL^{-1}					
10	AF.2	AF	AFB_1-BSA 0.25 μg mL^{-1}	0.094 μg mL^{-1}	1:2000	1.25 / 1.43	0.97	Broiler feed[24]
11			AFB_1-BSA 0.25 μg mL^{-1}	0.094 μg mL^{-1}	1:2000	1.41 / 1.75	0.98	Laying hen feed[25]
12	OTA.1	OTA	OTA-BSA 0.077 μg mL^{-1}	0.043 μg mL^{-1}	1:1000	0.17 / 0.32	0.97	Red wine[a]
13						0.14 / 0.23		White wine[a]
14						0.17 / 0.32		Table wine[a]
15	OTA.7	OTA	OTA-BSA 4.76 μg mL^{-1}	1:2000	1:1000	3.75/ -	0.98	Green cofee[22]
16	M8H5	MCLR	MCLR-BSA 1:20000	1:20000	1:5000	- / 0.05	-	Fresh Water[37]

ic-ELISA: Indirect competitive enzyme linked immunosorbent assay.

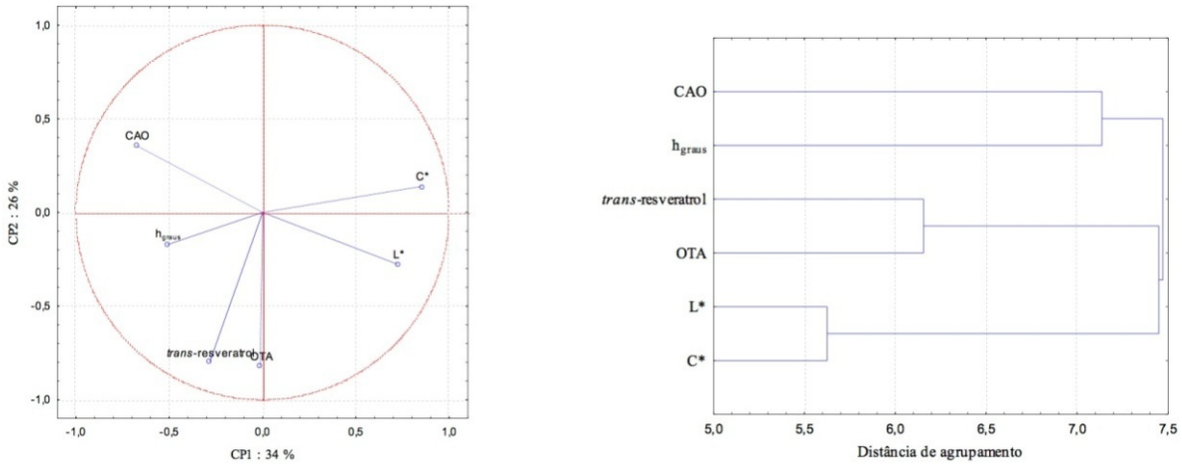
DON: Deoxynivalenol; ZEA: Zearalenone; FB_1: Fumonisin B_1; AF: Aflatoxin; OTA: Ochratoxin A; MCLR: Microcystin-LR; DON-HG-OVA: Deoxynivalenol-hemiglutarate-ovalbumin; DON-HS-OVA: Deoxynivalenol-hemisuccinate-ovalbumin; ZEN-OVA: Zearalenone-ovalbumin; AFB_1-BSA: Aflatoxin B_1- Bovine Serum Albumin; OTA-BSA: ochratoxin A- Bovine Serum Albumin; and MCLR-BSA: Microcystin-LR - Bovine Serum Albumin.

[26]Santos et al. 2011; [27] Santos et al. 2013; [28] Souza et al. 2014; [21] Ono et al. 2001; [24] Rossi et al. 2013a; [25] Rossi et al. 2013b; [22] Fujii et al, 2006; [37] Kamogae et al. 2006; [a]Data not published.

Table 2. Development of ic-ELISA: standardized immunoassay for mycotoxins and microcystins analysis.

The optimized ic-ELISA showed a correlation coefficient of >0.9 with HPLC (Table 2). The result obtained with anti-OTA MAb produced by hybridoma OTA.1 was adequate to analyse wine using 1:10,000 anti-OTA MAb and 1:30,000 OTA-BSA. However, the matrix interference in the OTA analysis in wine by ic-ELISA should be considered. In analysing 60 wine samples, only one was OTA positive by HPLC (0.12 ± 0.01 ng mL^{-1}), whereas 11 false-positives were observed by ic-ELISA (range from 0.32 ± 0.02 to 0.47 ± 0.14 ng mL^{-1}). False-positive data in red wine may be attributed to the interference of anthocyanins and other pigments on OTA-binding to the antibody [38, 39]. The influence of matrix interference in OTA detection by ic-ELISA could be explained using a principal component analysis through the relationship of higher *trans*-resveratrol and OTA levels in the positive samples (Figure 2). In contrast, the addition of condensed tannins can inhibit the binding activity of antibodies in ELISA [40].

The undesired matrix effect and be minimized by diluting the crude extract prior to ic-ELISA; a 1:100 dilution of coffee extract minimized the matrix effect on OTA detection, regardless of the maturity stage [22]. Additionally, a dilution factor of 1:80 minimized the matrix effect when anti-DON MAb produced by Hybridoma DON.3 was used in ic-ELISA for wheat grain.



(a) Projection of variables. **(b)** Cluster analysis of parameters

Figure 2. Principal Component Analysis in the evaluation of wine samples, Paraná State: OTA (ochratoxin A), CAO (antioxidant capacity), *trans*-resveratrol and chromatic (h_{graus}, L*, C*). a) graphical representation in two dimensions (Principal Component 1- CP1 and Principal Component 2- CP2) and b) cluster analysis of parameters.

Table 3 shows the monitoring of natural toxins (mycotoxins & microcystins) by ic-ELISA developed for different food specimens, as well as in the freshwater since the 1990s. Corn, coffee, wheat, grain-derived products, wine, broiler and laying hen feeds, and fresh water in agricultural regions were analysed (Table 3). The table also shows some maximum limits established by Brazilian guidelines, the European Commission, and the World Health Organization.

Item	SAMPLING / MONITORING				Ic-ELISA		
	Cereal & products, Feed, Wine[41, 42]		Locality	Crop Year	Toxins Mycotoxin[a]	+/total (n)	Mean (μg kg^{-1})
1	Wheat	Grain	North-West, North-East, South-West /RS	2006 – 2008		15 / 15	2918.1
			North, Central, South-West /PR	2006 - 2008		7 / 23	1578.6
			North/PR	2009	DON	36 / 50	2379.4
		Flour	North/ PR	2009		21 / 23	2455.9
2		Grain	Central-South /PR	2010 - 2011		84 / 84	1879.3
			North /PR			159 / 160	848.9
3		Biscuits	North /PR (Retail Market)	2013		29 / 56	742.4
4		Grain	North, Central-South	2010 - 2011	ZEA	11 / 125	161.4

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Item	SAMPLING / MONITORING				Ic-ELISA		
	Cereal & products, Feed, Wine[41, 42]		Locality	Crop Year	Toxins Mycotoxin[a]	+/total (n)	Mean (μg kg^{-1})
5		Biscuits	North (Retail Market)	2013		17 / 56	56.1
6	Corn	Grain	Central-South, Central-West, North / PR	1995 - 1996	FB_1	147 / 150	5610.0
7					AF	12 / 75	8.1
8			Central-South / PR	2010 - 2012	DON	6 / 75	2142.3
9					ZEA	36 / 75	522.3
10	Feed[44]	Broiler	North / PR	2010	AF	114 / 158	2.2 -6.4
11		Laying hen	North / PR	2010	AF	66 / 95	9.61
12	Wine	Red	North / PR (Retail Market)	2006 - 2011		8 / 47	0.42
13		White		2006 - 2009	OTA	4 / 23	0.68
14		Table wine	South-West, West	2010 - 2011		10 / 34	0.37
15	Coffee	Green coffee	North / PR	2003	OTA	15/68	5.28
					Cyanotoxin	+/total (n)	Mean (μg L^{-1})
16	Fresh Water[43]	Tibagi River	North / PR	1999 - 2000		13 / 24	0.28
		Itaipu Lake	West /PR		MCLR	22 / 24	18.35
*		Tibagi River	North /PR	2014		1 / 6	0.67
		Itaipu Lake	West / PR			3 / 6	0.65

ic-ELISA: Indirect competitive enzyme linked immunosorbent assay.

* These analysis were carried out using a commercial Kit (Beacon Microcystin Plate Kit, USA).

PR: Paraná State; RS: Rio Grande do Sul State.

[41] The Brazilian Health Surveillance Agency (ANVISA, 2011) established the maximum levels of DON in flour and biscuits (1,750.0 μg kg^{-1}), ZEA for biscuits (200.0 μg kg^{-1}), AF for corn (20.0 μg kg^{-1}), and OTA for wine (2.0 μg kg^{-1}). The deadlines were established in RDC 07/2011 and will be extended to January 1, 2017 for DON in wheat and corn (3,000.0 μg kg^{-1}), ZEA in wheat and corn (400.0 μg kg^{-1}), and $FB_1 + FB_2$ in corn (5,000.0 μg kg^{-1}) [42].

[43] The World Health Organization (WHO, 1998) established maximum levels of 1 μg of MCLR L^{-1} for drinking water and a Tolerable Daily Intake of 0.04 μg of MCLR kg^{-1} body weight.

[c] Mycotoxin / Cyanotoxin: DON, Deoxynivalenol; ZEA, Zearalenone; FB_1, Fumonisin B_1; AF, Aflatoxin; OTA, Ochratoxin A; and MCLR, Microcystin-LR.

[44] European Commission (2003). The maximum limit allowed by the European Commission is 0.02 mg aflatoxin B_1 kg^{-1}.

Table 3. Monitoring of natural toxins (mycotoxins & microcystins) by the rapid ic-ELISA method.

The application of ic-ELISA to monitoring freshly harvested corn from Paraná State (1991 to 2004 crops) indicated the widespread occurrence of fumonisins but a low occurrence of aflatoxins. In a recent study conducted in Paraná State, 74 corn samples were contaminated with an average of 1,840 μg of fumonisin kg^{-1}, 36 of poultry feeds with 239 μg of fumonisin kg^{-1}, and 9 corn factory residues with 23,676 μg of fumonisin kg^{-1}, whereas the aflatoxin and trichothecene levels were approximately at the LOD values [45]. Ic-ELISAs, using monoclonal mAb produced by hybridoma cells (AF.2, ZEN.2 and DON.3), were developed and optimized for AFs, ZEA and DON detection (Table 2). In corn samples from an experimental farm in central-southern Paraná State, 12 samples were found to be positive for AF (mean of 8.1 μg kg^{-1}), 36 samples for ZEA (mean of 522.3 μg kg^{-1}) and 6 samples for DON (mean of 2142.3 μg kg^{-1}) (Table 3).

An emphasis was placed on DON evaluations by ic-ELISA in wheat from 2006 to 2011 (Table 3). Paraná and Rio Grande do Sul States in southern Brazil produce 90 % of the national wheat [1]. This country depends on the importation of 5 to 6 million ton per year to provide for an annual domestic consumption of approx. 11 million ton, mainly used in bakery (55 %), pasta (17 %) and biscuit (13 %) processing [2, 46, 47]. Brazil is the world's second-largest biscuit producer, but the current low exportation (54,083 tons) results in nearly all production earmarked for domestic consumption, despite its ranking [48, 49]. In the wheat samples from experimental farms of north and central-southern of Parana State analysed by ic-ELISA, DON was detected in almost all of samples (243 positive samples of 244) and ZEA was detected in 10 of 125 samples (Table 3). In, wheat-based biscuits acquired from a local retail market in Londrina, Paraná State (56 samples) DON was detected in 29 samples (mean of 742.4 μg kg^{-1}) and ZEA in 17 samples (mean of 56.1 μg kg^{-1}) (Table 3). A study [50] analysed 23 cracker biscuit samples produced in Southern Brazil and group A trichothecene was non-detectable, but 18 samples were contaminated with DON (378 – 5295 μg kg^{-1}), with 22 % of the samples at level over the Brazilian guideline limit (1,750 μg kg^{-1}). When zearalenone was analysed in corn-based products (51 samples of popcorn and 50 corn grits) and cracked wheat (n = 109) commercialized in 18 counties of the Paraná state, ZEA was non-detected in cracked wheat samples, but one cracked corn sample contaminated 64 μg of ZEA kg^{-1} [50]. Fusariotoxin monitoring in wheat should be conducted in both domestic production and in imported wheat, which represents 50 % of the category.

Due to the possible carry-over of mycotoxins to tissues, the degree of exposure of broiler chicken and laying hens to fumonisins and aflatoxins through naturally contaminated feeds has been assessed (Table 3). Occurrence of fumonisins and aflatoxins were evaluated in four feed types intended for broilers (n=158), collected from a poultry breeding farm in Northern Paraná State [24]. Fumonisins were detected in 94.9 % of the feed samples at mean levels ranging from 0.52 μg g^{-1} (finisher) to 0.68 μg g^{-1} (pre-starter and grower), and aflatoxins were detected in 72.1 % of the feed samples at mean levels ranging from 2.22 ng g^{-1} (pre-starter) to 6.41 ng g^{-1}(grower). The maximum estimated daily intake of FB_1 for broilers (0.057 mg/kg body weight/day) was below the Lowest Observed Adverse Effect Level (2 mg kg^{-1} body weight day^{-1}). Most of the aflatoxin positive samples (97 %) showed levels below the maximum limit allowed by the European Commission (0.02 mg aflatoxin B_1 kg^{-1}). To estimate the degree of

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exposure of laying hens to mycotoxins, a total of 95 mash feed samples were collected from January to December 2010 from the Experimental Farm at the University, Northern Paraná State, Brazil. Aflatoxins and fumonisins were detected in 69.7 % and 89.5 % of the feed (n=95) intended for laying hens at mean levels of 9.61 ng g^{-1} and 1.28 μg g^{-1}, respectively. The estimated daily intake of FB_1 for laying hens (0.038 mg kg^{-1} body weight day^{-1}) was below the Lowest Observed Adverse Effect Level (2 mg kg^{-1} body weight day^{-1}). Aflatoxin levels were below the maximum allowed limit by the European Commission in the majority of the positive samples (85.1 %), which indicated that some of the feed samples could have a negative effect on animal health and performance, but the risk would be very low.

Intensive agricultural activity has become an increasing concern due to the eutrophication of aquatic environments. Microcystins (MCs) were monitored in Itaipu Lake and Tibagi River in the north and west of Paraná State, respectively (1999 to 2000 and 2014). The reduction of microcystin levels in Itaipu Lake was likely a consequence of ecological programs encouraging the recovery of riparian forests, in addition to a change in planting management (Table 3). Such a reliable MAb-based rapid immunoassay has been a good choice for tracking mycotoxins and cyanotoxins and determining actions to be performed in a control strategy.

4. Monitoring strategies: The importance of reliable analysis as controlling guidance

Monitoring of the corn chain in northern Paraná showed that 81 % (n = 435, crop 2003) and 98.8 % (n = 435, crop 2004) of corn was safe for human consumption, in regard to fumonisin. The decreasing trend in fumonisin contamination, when compared to previous studies [8, 9, 12], could suggest a conscious monitoring procedure at the quality control level, in accordance with the strict guidelines imposed by importing countries.

The main approaches in corn phytosanitary control involve pesticides and agricultural practices with an emphasis on tillage and crop rotation. Efforts have been focused on novel fungicides for *Fusarium* sp. control to maximize grain yield [51, 52]; however, several studies have shown that fungicide application can increase mycotoxin levels [53, 54]. The recommended dose of fludioxonil + metalaxyl-M (2.5 + 1.0 %) was insufficient to inhibit *F. verticillioides* growth *in vitro*, but it increased FB_1 production from 3.5-fold to 12.5-fold (2.58 μg mL^{-1} when compared with 0.72 μg mL^{-1} in control), with an alteration in mycelial morphology [55, 56]. A scanning electron microscopy analysis showed that the fungicide caused the inhibition of hyphal growth and defects of hyphae, such as excessive septation, cell wall disruption, and withered hyphae, and extracellular material around the hyphae was rarely observed (Figure 3) [57].

Therefore, efforts to reduce mycotoxin levels should be focused on sustainable production. In uninterrupted planting in tropical regions, non-drastic management of cropping systems using culture rotation in no-tillage areas under different fertilizations emphasizing nitrogen rate, and low cost organic waste remain concerns in the protection of grain and soil conservation [58, 59, 60, 28].

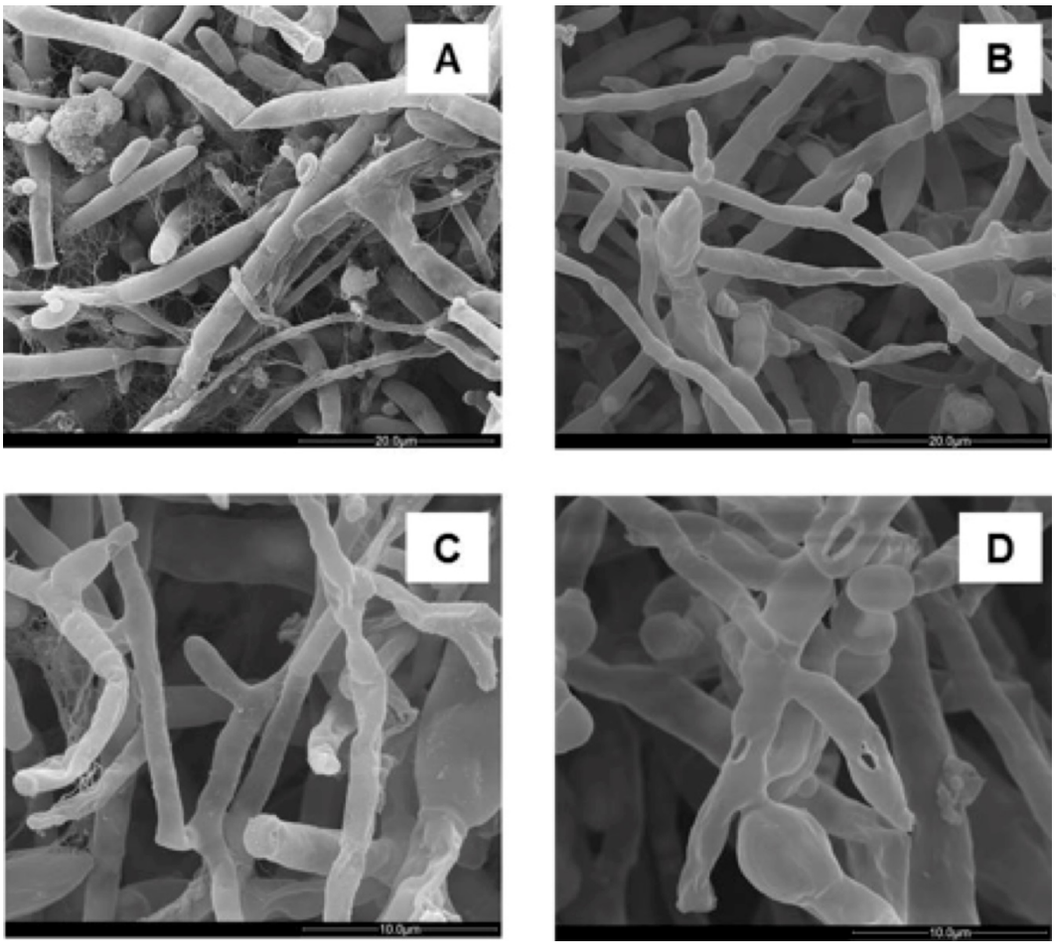

Figure 3. Electron micrographs of *F. verticillioides* 103 F mycelia cultured in defined liquid media in the absence (control) and presence (treatment) of fludioxonil + metalaxyl - M at the dose recommended by the manufacturer (1.5 μL mL^{-1}) showing the fibrillar extracellular material present in the control cultures (A and C) and withered hyphae and disruption of cell walls in the treatments (B and D).

The effect of conventional and no-tillage cropping systems in corn cultivated in summer following either oats or fallow in winter on natural fumonisin levels (2006 and 2007 growing seasons) has been assessed [60]. No-till corn following oats showed stronger fumonisin contamination patterns than the other treatments (2006 season, $P < 0.05$). Although no-till could be advantageous from a soil conservation standpoint, it may enhance fumonisin contamination in the tropics, contrasting another report [61] that there was no significant difference between conventional and no-till in fumonisin in monoculture corn in Northern Italy. When the nitrogen fertilizer rates (0 to 90.0 kg ha^{-1} N) on fumonisin contamination was evaluated, higher fumonisin levels were detected in plots with lower N (≤ 22.5 kg ha^{-1}) than ≥ 45.0 kg ha^{-1} N, indicating a negative correlation between fumonisin and N rates [60]. Both N stress due to deficiency and excessive rates can increase the FB_1 level in corn [62].

The nitrogen-fixing potential of *Azospirillum* sp. in the rhizosphere can increase yields, reduce costs and improve the nutritional quality of corn kernels. An experiment was conducted matching the inoculums of the *Azospirillum brasilense* Ab-V5 and Ab-V6 strains in corn seeds

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with N doses in Northern and Central-Southern Paraná in the 2010/2011 and 2011/2012 seasons [63]. Although the seed inoculation associated with N doses showed non-significant effects on fungal count, the inoculum treated plots showed lower fumonisin levels ($p < 0.05$) than the non-treated plots, indicating favourable trend towards agricultural practices with inoculants. Fumonisins were detected in 90 % of samples in 2010/2011 (mean, 0.62 μg g^{-1}) and 97.5 % in 2011/2012 (mean, 4.34 μg g^{-1}) in the northern region, whereas its occurrence was 45 % in 2010/2011 (mean, 0.14 μg g^{-1}) and 100 % in 2011/2012 (mean, 2.67 μg g^{-1}) in central-southern Paraná.

The use of landfill leachate in agricultural soils as fertilizers has been suggested as an alternative for the disposal of this effluent; however, heavy metals may be a limiting factor [63]. The application of increasing doses of leachate (0 to 130.8 m^3 ha^{-1}) increased the yield, protein content, lipid and ash in corn grain, but no effect was observed on fumonisin reduction, which occurred in all samples, with 31.2 of samples with levels over the maximum tolerable limit in Brazilian guidelines (5.0 μg g^{-1}). An increasing trend in lead content was also observed in the 2009/2010 seasons, and in sodium (2011/2012 seasons) when the leachate rate was increased [63].

The management of plant density (60 to 105 thousand plants/ha) with N doses (0 to 240 kg ha^{-1}) showed no effect on corn fungal count, but there was an increasing trend in fumonisin levels when plant density was increased. Total fumonisins (FB_1 + FB_2) were detected in corn grain at levels ranging from non-detectable to 7.80 μg g^{-1} (mean, 1.50 μg g^{-1}) in the 2009/2010 season, while it was non-detectable to 23.36 μg g^{-1} (mean, 1.72 μg g^{-1}) in the 2010/2011 season [63].

Efforts also should be focused on the safety and quality of the wheat chain, one of major universal components in food. Although 90 % of the national crop is centred in southern Brazil, domestic consumption still depends on importation [1, 2]. Table 3 shows that natural contamination of DON in wheat was non-equally distributed among different crops and was dependent on local and climatic conditions (the impact of agricultural management practices was evaluated in 2010 and 2011 seasons). Environmental conditions can shift the metabolic route of *Fusarium graminearum*, changing the fusariotoxin profile in grains, ex., leading to an increase of acetylated analogues of DON [64, 65]. Although such acetylated trichothecenes are considered less toxic than DON, a rapid deacetylation can take place in the digestive tract of mammals, turning into it to DON [66]. DON of the group B has been regarded as a unique trichothecene in wheat products under current Brazilian guidelines [41], although studies have indicated that group B trichothecenes, such as nivalenol (NIV) and acetylated analogues (3-acetyl-DON and 15-acetyl-DON), should be included [17].

In addition, lactic bacteria of the *Lactobacillus plantarum* group have shown versatile profiles concerning genes that can code for special functions such as biodegradation, absorption, and adherence to different surfaces in the vast natural microbiota in food niches [69, 68]. Lactic acid bacteria strains can be isolated from multiple wheat sources (grains, germ, bran, and flour) and have been tested against *F. graminearum* strain IAPAR 2218 (Figure 4). All tested strains demonstrated some DON-reducing potential, and the non-viable autoclaved *L. plantarum* cells (71.19 %) showed a higher effectiveness in DON reduction than viable cells (16.41 %). The

probable mechanism of reduction would be the adsorption by cell walls [69, 70]. Lactic acid bacteria can also degrade a range of low molecular weight compounds, carrier compound families, and influx and efflux facilitators and mycotoxin degrading enzymes have been detected [71].

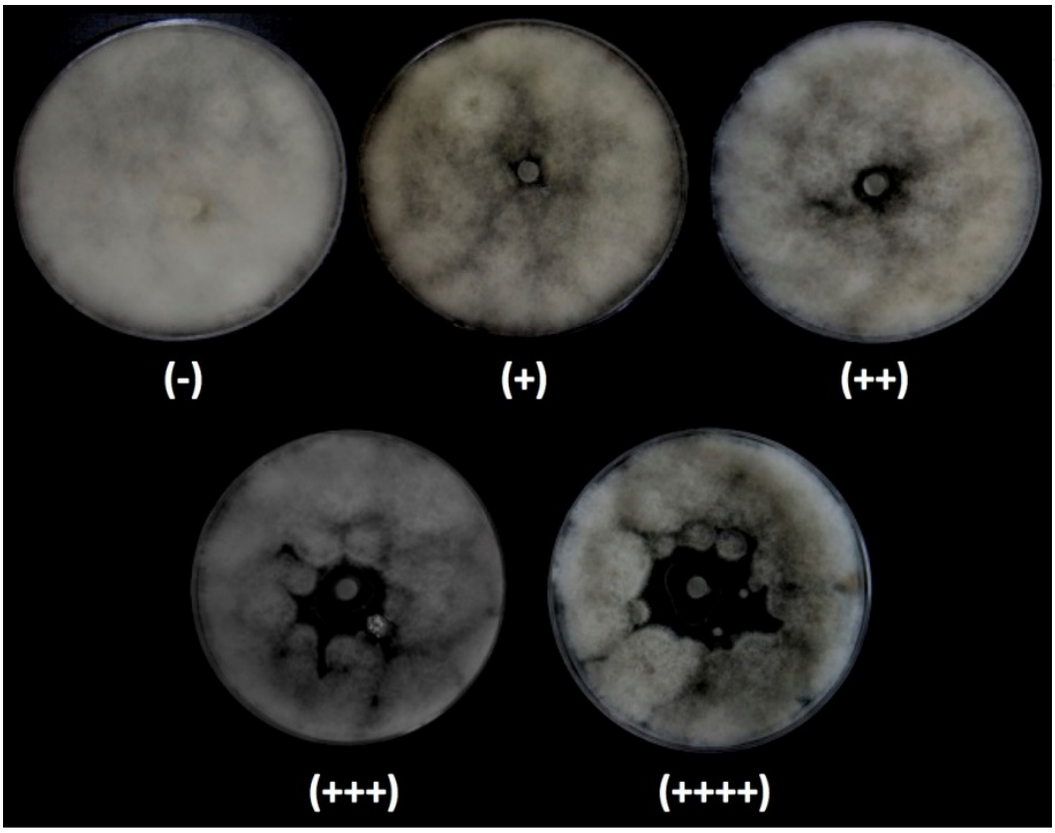

Figure 4. Effect of lactic acid bacteria isolated from different wheat sources (grains, germ, bran, flour) against *F. graminearum* strain IAPAR 2218. Inhibition Halo scale: (-): no inhibition; (+):1 to 5 mm; (++): 6 to 10 mm; (+++): 11 to 15 mm; and (++++): >15 mm.

Another use of naturally occurring microorganisms in the biocontrol/biodegradation of undesired natural toxins has been assessed for the reduction of cyanobacteria in drinking water. The potential of microcystin (MC) biodegradation has been tested in the following microorganisms: *Sphingosinicella microcystinivorans* (B9) isolated of the Lago Tsukui, Kanagawa-Japan; water kefir (mixture of lactic and acetic bacteria and yeast) (P4); *L. acidophilus* La-5 (P5); and yeast isolated of sugarcane (L5). The strain B9 degraded 99 % of MCs, while the strains P4, P5 and L5 degraded 44, 43 and 54 % of total MCs, respectively, after 96 h (Figure 5).

Strain B9 (*S. microcystinivorans*) showed the highest MCs degradation capacity and has been evaluated for its anti-cyanobacterial activity against 5 cyanobacteria strains, *Microcystis* sp. (C1), *Microcystis* sp. (C2), *Anabaena ucrainica* (C3), *Phormidium tenue* (C4), and *Synechocystis* (C5). After 96 h, the inhibition percentages (cellular counts) against cyanobacteria strains ranged from 41.4 to 79.3 %, while the inhibition percentages (concerning chlorophyll-a) ranged from 34.4 to 68.9 %.

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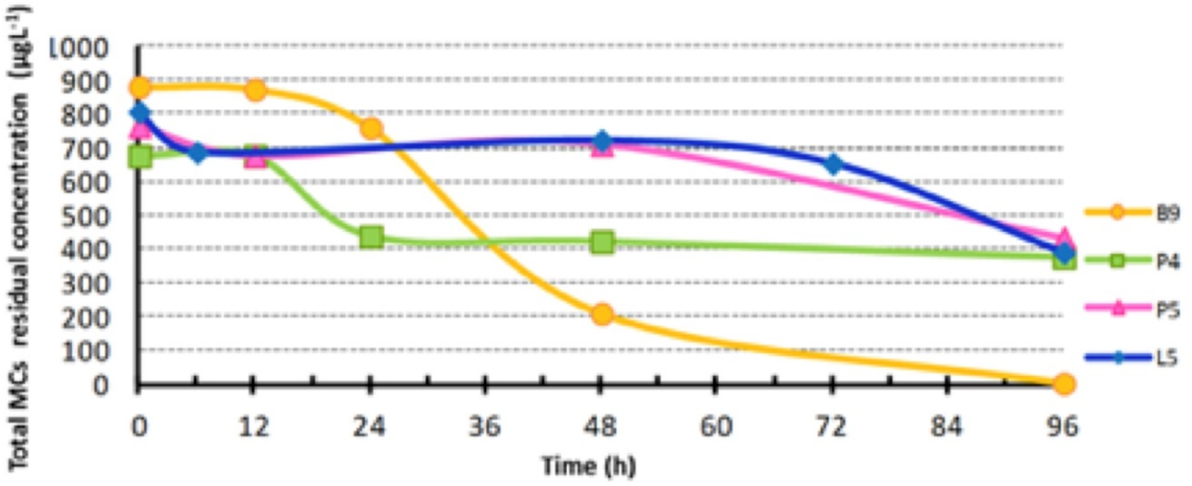

Figure 5. Biodegradation of microcystin by *Sphingosinicella microcystinivorans* (strain B9), water kefir (P4), *Lactobacillus acidophilus* La-5 (P5) and yeast (L5).

In summary, adequate agricultural practices based on crop rotation, fertilization, soil biodiversity, resistant crops, and post-harvest management could reduce mycotoxin contamination in field. Further long-term strategies encouraging no-tillage cultivation, and the maintenance of riparian forests in extensive agricultural land would be goals to maintain water quality; and the sustainable production of nutrient-rich high-quality products would still be possible.

5. Novel emerging tools for quality & safety: non-destructive technology in the food chain

Traditional analytical techniques for food and feed quality inspection and compositional assessment are typically invasive and time-consuming, requiring extensive sample preparation, thus being unsuitable for applications in the highly demanding, fast-paced food processing segment. Recently, novel techniques have been investigated for fast, reliable and chemical-free food quality assessments. Near-infrared (NIR) hyperspectral imaging has emerged as an efficient and advanced tool, combining both computer vision techniques and NIR spectroscopy, which can be used for continuous monitoring, process control and quality assessments of agricultural products, food and feed materials. Because most food quality features are related either to the external appearance of the product or its chemical composition, either computer vision or NIR spectroscopy alone is adequate for monitoring organic samples in a fast, reliable manner. However, such techniques are still strongly dependent on other reference methods. Prediction of physical characteristics and chemical composition using NIR spectroscopy and/or computer vision methods has been reported on meat (chicken, pork, beef, lamb), cereals and grains (corn, wheat, soy, rye, coffee, cocoa), and fruits and vegetables (apple, citrus, berries). More specifically, there has been major interest in this technique in quality control, food safety and security, i.e., detection and prediction of contamination in agricultural products.

A study [72] compared NIR calibration methods for predicting protein, oil and starch contents in both whole and ground maize samples in the spectral range of 1100–2500 nm for reflectance and 680–1235 nm in transmittance modes. While the best models were obtained for the reflectance spectra of the ground samples, it was suggested that the transmittance mode for whole grains might be more useful due to its greater speed of analysis. Another study [73] developed a rapid single kernel NIR sorting instrument for maize and soybean. Prediction models for moisture of both seed types, and protein contents for soybeans were developed utilizing a spectrometric range from 906 to 1683 nm.

NIR reflectance and transmittance technologies have been investigated for contamination assessments of a range of cereal grain physical quality and chemical traits, and detecting and predicting levels of mycotoxins. Numerous applications have been developed, and cover almost all cereals in the globally important food grains, i.e., corn, wheat, rice and barley. An additional application has been to demonstrate the value in sorting grains infected with fungus or mycotoxins, such as deoxynivalenol, fumonisins and aflatoxins [74].

A shortwave infrared (SWIR) hyperspectral imaging system in the wavelength range between 1000 and 2500 nm was used to assess the potential AFB_1 contaminants on the surfaces of healthy corn kernels. Key wavelengths that can indicate AFB_1 and are used to differentiate levels of AFB_1 were identified. A minimum classification accuracy of 88 % was achieved for the validation set and verification set, indicating that hyperspectral imaging technology could be used to detect AFB_1 at levels as low as 10 ng/g, when applied directly on the corn surface [75]. Another study assessed the applicability of NIR for the rapid identification of mycotoxigenic fungi and their toxic metabolites produced in naturally and artificially contaminated products. Two hundred and eighty corn samples were collected in north-central Italy and analysed for fungal infection, ergosterol, and FB_1 content. The results indicated that NIR could predict the incidence of kernels infected by *F. verticillioides* and also the quantity of ergosterol and fumonisin B_1 in the meal. The best predictive ability for the percentage of global fungal infection and *F. verticillioides* was obtained using a calibration model utilizing corn kernels (r 2 0.75 and SECV 7.43) and maize meals (r 2 0.79 and SECV) 10.95), respectively [76].

A recent study on the quality assessments of meat products [77] reported the application of NIR reflectance as a potential method to predict quality attributes of chicken breast (*Pectoralis major*). Spectra in the wavelengths between 400 and 2500 nm were analysed, presenting clear differences between different quality grades of chicken (Figure 6). PCA performed on the NIR dataset revealed the influence of muscle reflectance (L*) influencing the spectra. PCA was not successful to completely discriminate between pale, soft and exudative (PSE) and pale-only muscles. High-quality PLSR were obtained for L* and pH models predicted individually (R2CV of 0.91 and 0.81, and SECV of 1.99 and 0.07, respectively). Sample mincing and different spectra pre-treatments were not necessary to maximize the predictive performance of the models. The results suggest that NIR spectroscopy may represent a useful tool for the quality assessment of chicken meat.

The contamination of meat products has also been investigated. NIR transflectance and Fourier transform-infrared (FT-IR) attenuated total reflectance spectra of intact chicken breast muscle were collected and investigated for their potential use in the rapid, non-destructive detection

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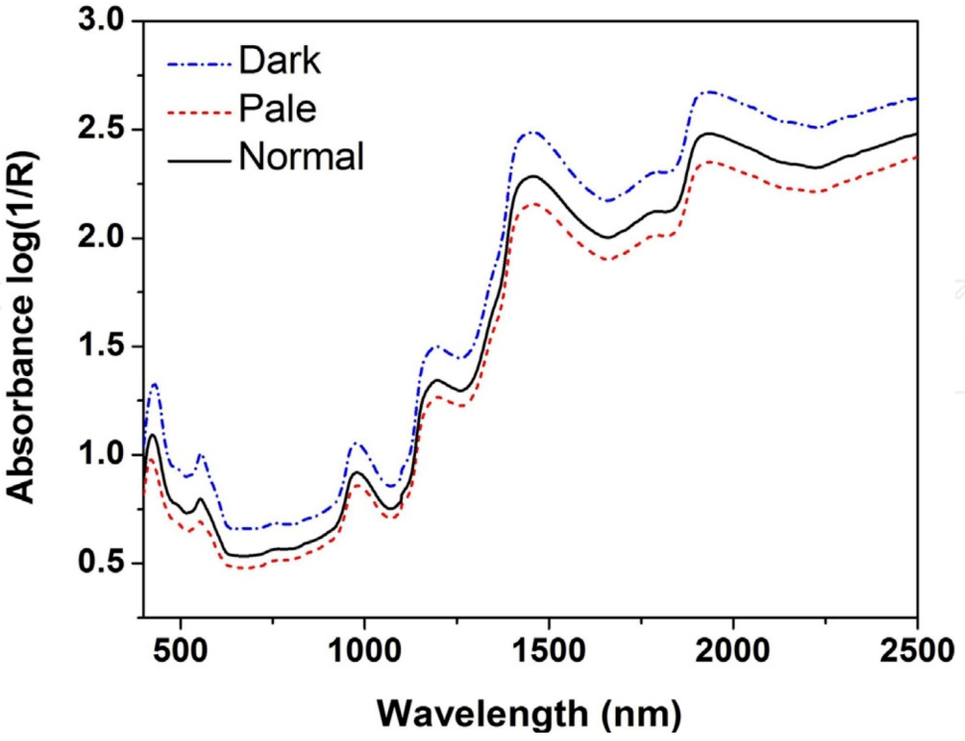

Figure 6. Average spectra for dark, normal and pale chicken samples (see [76] - "Reprinted from Food Chemistry, 168, Douglas Fernandes Barbin, Cintia Midori Kaminishikawahara, Adriana Lourenco Soares, Ivone Yurika Mizubuti, Moises Grespan, Massami Shimokomaki, Elisa Yoko Hirooka, Prediction of chicken quality attributes by near infrared spectroscopy, 554-560, 2015, with permission from Elsevier").

of spoilage. PCA and PLS2-DA regression correctly classified 8 and 14 day samples (TVC days 8 and 14= 9.61 and 10.37 log10 CFU g^{-1}) with several correlations that highlight the effect of proteolysis influencing the spectra. These correlations indicate that an increase in free amino acids and peptides could be the main factor in the discrimination of intact chicken breast muscle.

These studies have demonstrated that NIR methodology can be applied to monitor bacterial and fungal contamination in postharvest grains and fresh meats and to distinguish contaminated from clean batches to avoid cross-contamination with other materials during storage. However, there is still a demand for the development of cost-effective technologies for high-speed sorting. In the area of food safety, it is important to create robust prediction models based on reference methods by including a wide range of samples from different regions. For instance, it is well known that a major drawback of this technology is the application of ready-to-use prediction models from one country into samples from another region of the world. Prediction models are usually built in developed countries, but they are not useful for samples originating in developing countries, mostly due to inherent differences in sample composition, cultivation methods, climate and soil characteristics, etc. Once researchers overcome these obstacles, this technology will benefit farmers, the industry and consumers if it enables contaminated grain and other food samples to be identified and removed from the food chain.

6. Trends in globalized agribusiness

Globalization demands quality and competitiveness throughout the food chain, as well as safe raw materials without deterioration. Agribusiness exports in Brazil reached US$ 5.64 billion in January 2015, according to the foreign trade statistics system of Brazilian agribusiness [78]. The five main exporter states in Brazil were São Paulo, Paraná, Mato Grosso, Minas Gerais and Rio Grande do Sul, whose participation represented approximately 69 % of total Brazilian exports, involving soy, sugar-alcohol complex, beef, chicken meat, soybean oil, cereal sales, and coffee.

Brazil is currently the third-leading country in chicken production, behind the USA and China, and the leading exporter of chicken meat [79]. The long-term trade projection estimates a production of up to 20.576 million tons by 2023, corresponding to an increase of 46.4 % between the years 2013 and 2023. In addition, the exportation of meat has been forecasted at 4.675 million tons in 2023 [80]. Further, poultry (broilers and turkeys) trade long-term projections of the USDA (2015) indicate that exportation could reach up to 4.982 million tons in 2024.

In this scenario of globalized agribusiness, corn stands out as the major component of animal feed production [4]. Figure 7 shows a scenario concerning corn purchased as a raw material in a potential importer country. The trend for importing of Brazilian grains continues to depend on the decrease or delay of the American harvest of agriproducts due to the unmatched infrastructural facilities and the storage, transport and port system established in the United States.

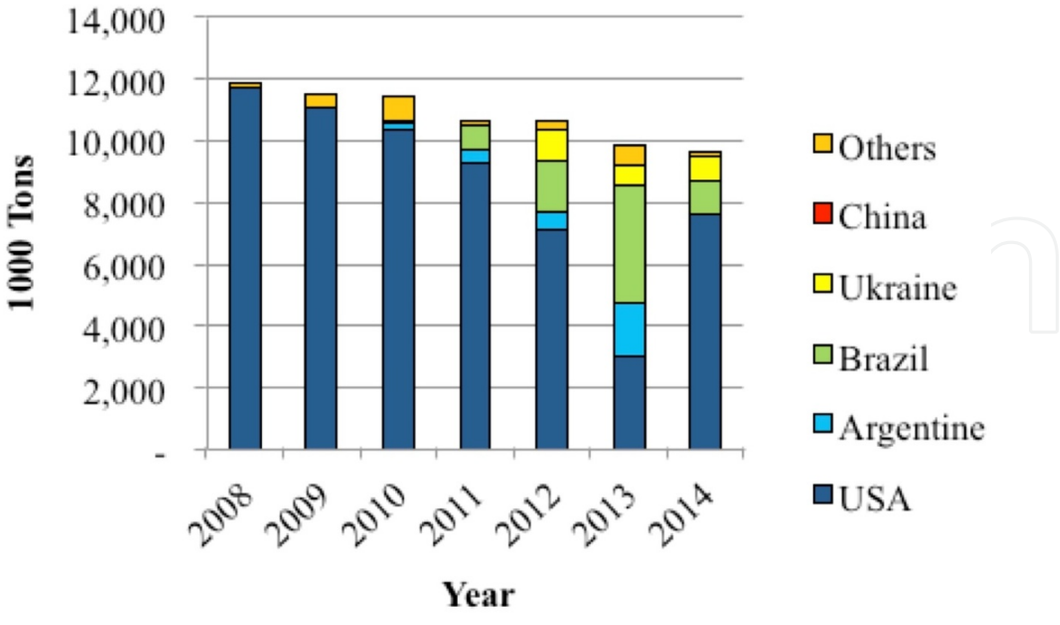

Figure 7. Corn importation in Japan, 2008 to 2014 [81].

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Even with infrastructural problems, the bar chart shows that the decision to import Brazilian raw materials could unexpectedly increase and reached nearly five times the quota between the year 2012 and 2013.

The current effort on aggregation of value in agriproducts should be combined with continuous work on safety in highly productive regions, ex. broiler chickens for export, which depends on practical and reliable analytics facilitating quality and safety. Such an overall approach could result in promising healthy foods from potential producer regions in Brazil.

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