available at www.sciencedirect.com

ScienceDirect

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

Potential of near infrared spectroscopy for the analysis of mycotoxins applied to naturally contaminated red paprika found in the Spanish market

J.M. Hernández-Hierro [a,b], *R.J. García-Villanova* [b], *I. González-Martín* [a,*]

[a] *Departamento de Química Analítica, Nutrición y Bromatología, Facultad de Ciencias Químicas, Universidad de Salamanca, C/Plaza de la Merced s/n, 37008 Salamanca, Spain*
[b] *Departamento de Química Analítica, Nutrición y Bromatología, Facultad de Farmacia, Universidad de Salamanca, Campus "Miguel de Unamuno", 37007 Salamanca, Spain*

ARTICLE INFO

Article history:
Received 20 March 2008
Received in revised form
20 May 2008
Accepted 21 May 2008
Published on line 28 May 2008

Keywords:
Aflatoxins
Ochratoxin A
Spanish paprika
Near infrared spectroscopy
Fibre-optic probe

ABSTRACT

The potential of the near infrared spectroscopy (NIRS) technique for the analysis of red paprika for aflatoxin B_1, ochratoxin A and total aflatoxins is explored. As a reference, the results from a chromatographic method with fluorescence detection (HPLC-FD) following an immunoaffinity cleanup (IAC) were employed. For the NIRS measurement, a remote reflectance fibre-optic probe was applied directly onto the samples of paprika. There was no need for pre-treatment or manipulation of the sample. The modified partial least squares (MPLS) algorithm was employed as a regression method. The multiple correlation coefficients (RSQ) and the prediction corrected standard errors (SEP(C)) were respectively 0.955 and 0.2 $\mu g\,kg^{-1}$, 0.853 and 2.3 $\mu g\,kg^{-1}$, 0.938 and 0.3 $\mu g\,kg^{-1}$ for aflatoxin B_1, ochratoxin A and total aflatoxins, respectively. The capacity for prediction of the developed model measured as ratio performance deviation (RPD) for aflatoxin B_1 (5.2), ochratoxin A (2.8) and total aflatoxins (4.4) indicate that NIRS technique using a fibre-optic probe offers an alternative for the determination of these three parameters in paprika, with an advantageously lower cost and higher speed as compared with the chemical method. Content of aflatoxin B_1 and total aflatoxins are the parameters currently employed by the food regulations to limit the levels of the four aflatoxins in many foodstuffs. In addition, aflatoxin B_1 itself is an excellent indicator for aflatoxins' contamination since it is always the most abundant and toxic.

1. Introduction

Aflatoxins are mycotoxins produced under certain conditions by the *Aspergillus* species, namely *A. flavus*, *A. parasiticus* and *A. nominus*, whilst ochratoxin A is produced by *Penicillium verrucosum* and *Aspergillus ochraceus*. All four aflatoxins are considered to play a role in liver cancer aetiology (*carcinogenic to humans*, Group 1, IARC) [1] and aflatoxin B_1, the most abundantly found one, is, in addition, immunosuppressive [2]. In the year 2002 the European Commission (EC) set a legal limit in spices [3] for the sum of four aflatoxins, plus an individual one for aflatoxin B_1 owing to its higher toxicity, but no other mycotoxin was regulated. The next most sought mycotoxin in foods is ochratoxin A. It is a potent renal toxin in all animal species tested, but there is still no adequate evidence for its renal carcinogenicity (*possibly carcinogenic to humans*, Group 2B, IARC)

* *Corresponding author. Present address*: Departamento de Química Analítica, Nutrición y Bromatología, Facultad de Ciencias Químicas, C/Plaza de la Merced s/n, 37008 Salamanca, Spain. Tel.: +34 23 294483; fax: +34 23 294483.
E-mail address: inmaglez@usal.es (I. González-Martín).
0003-2670/$ – see front matter
doi:10.1016/j.aca.2008.05.049

Table 1 – Mycotoxins in samples of ground red paprika

	Calibration set (53 samples)			Validation set (10 samples)		
	Aflatoxin B_1 ($\mu g\,kg^{-1}$)	Ochratoxin A ($\mu g\,kg^{-1}$)	Total aflatoxins ($\mu g\,kg^{-1}$)	Aflatoxin B_1 ($\mu g\,kg^{-1}$)	Ochratoxin A ($\mu g\,kg^{-1}$)	Total aflatoxins ($\mu g\,kg^{-1}$)
Minimum	0.0	0.0	0.0	0.7	0.0	0.7
Maximum	3.8	73.8	4.5	1.5	13.1	3.7
Mean	1.0	8.4	1.5	0.9	5.3	1.6
S.D.	0.8	17.3	1.2	0.3	4.4	1.0

[2,4]. Several attempts to establish a maximum level for it have failed, so that the last Commission Regulation [5], in spite of setting limits for many other foodstuffs, again postponed a figure for spices. Since a legal limit of $10\,\mu g\,kg^{-1}$ is currently being considered for approval [6], maximum levels of $10–20\,\mu g\,kg^{-1}$ are generally agreed in the commercial specifications for most transactions in the international spice trade [7]. So far, no other mycotoxin is regulated or being considered for regulation in spices within the EU. Regarding both groups of mycotoxins, there is a rising concern among the health authorities, since contamination involves many products not produced within the EU, but more and more imported: peanuts, pistachios, dried figs, certain spices and grains, in the case of aflatoxins; dried vine fruit, grape juice, wine, liqueur wines, both green and roasted coffee, liquorice and, to a lesser extent, meat products in the case of ochratoxin A.

A few varieties of pepper fruit (*Capsicum annuum*) are extensively employed as spices after drying and grinding. In most Spanish-speaking countries this is called "pimentón" and normally refers to the red varieties, although the name commonly used worldwide is paprika, which includes even yellow colour varieties. Traditionally, the most important producing countries in the world have been China, Mexico and Turkey [8], and Spain and Hungary within the EU.

Contamination of red pepper spice by mycotoxins may occur at any stage of production, from pre-harvest to drying and storage. It is influenced by many factors: climate of the region, genotype of the crop planted and soil type [9]. It is promoted by stress or damage to the crop due to drought, insect activity, heavy rains at and after harvest and inadequate drying of the fruit before storage [2,10].

As a result of the high costs for manufacturing paprika, an increasing export market is noticeable in developing countries, many of them with climatic conditions or manufacturing practices that easily promote fungal proliferation and production of these mycotoxins. Regarding aflatoxins, both scientific references and official controls do not report frequently high levels in paprika within the EU market, but for ochratoxin A this information is, as yet, limited. A recent review on foodstuffs [11] states that paprika was found among the most contaminated products, together with nutmeg, chilli, coriander and pepper, whereas other authors report of a direct correlation with the geographical zone of production [7,12]. Another recent study on paprika for sale in Spain, has reported that aflatoxins where absent or found at very low levels, whilst ochratoxin A was much more frequently found and in quite varying amounts [13].

The increasing need for multimycotoxin analysis has promoted research on simultaneous methods with the employment of adequate extraction and cleanup procedures. Immunoaffinity column (IAC) or solid-phase dispersion (SPD) cleanup methods have been extensively developed for the liquid chromatographic determination [14–18], in spite of its tediousness and cost. Techniques based on Fourier-transformed and attenuation (FT-IR/ATR) [19] and near-infrared (NIRS) spectroscopy [20,21] have also been reported, but in a much lower number and for a few mycotoxins, such as deoxynivalenol and ochratoxin A in cereals. Owing

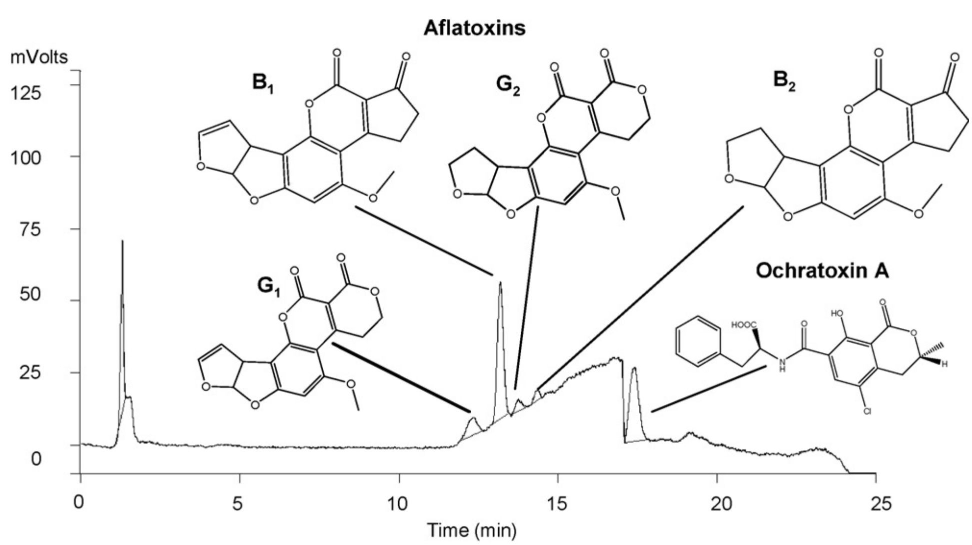

Fig. 1 – HPLC-FD chromatogram from a real sample of paprika.