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Influence of different soluble dietary fibers on the bioaccessibility of the minor *Fusarium* mycotoxin beauvericin

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ABSTRACT

Beauvericin (BEA) is a bioactive compound produced by the secondary metabolism of several *Fusarium* strains and is known to have various biological activities.

This study investigated the bioaccessibility of the BEA tested in concentrations of 5 and 25 mg/L, in a model solution and in wheat crispy breads elaborated with different natural binding compounds as the soluble alimentary dietary fibers β-1,3 glucan, chitosan low molecular weight (L.M.W.), chitosan medium molecular weight (M.M.W.), fructooligosaccharides (FOS), galattomannan, inulin and pectin, added at concentrations of 1% and 5%. The bioaccessibility was determinated by employing a simulated gastrointestinal digestion that simulates the physiologic conditions of the digestive tract until the colonic compartment. The determination of BEA in the intestinal fluids was carried out by liquid chromatography–mass spectrometry detection (LC–MS). The mean BEA bioaccessibility data in the model solutions ranged from 31.8% of the samples treated with only the duodenal digestion until 54.0% of the samples processed including the colonic fermentation, whereas in the alimentary system composed by the wheat crispy breads produced with different fiber concentration the duodenal and the duodenal + colonic BEA bioaccessibility resulted in 1.9% and 27.0% respectively.

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1. Introduction

Beauvericin (BEA) is a cyclic hexadepsipeptide consisting of an alternating sequence of three D-a-hydroxy-isovaleryl and three *N*-methyl-L-phenylalanyl groups. It was originally isolated from *Beauveria bassiana* (Hamill et al., 1969) and has been detected since then in various fungal species, including *Fusarium* spp., a common contaminant of cereals and products composed by cereals (Jestoi, 2008).

The potential toxic role of BEA is exemplified by results from *in vitro* studies using cell lines. For instance, BEA induces significant cell deaths in insect, murine, and human tumor cell lines (Caló et al., 2004; Jow et al., 2004; Fornelli et al., 2004; Ferrer et al., 2009; Dornetshuber et al., 2009). Furthermore, BEA is a potent and specific cholesterol acyltransferase inhibitor in rat liver microsomes (Tomoda et al., 1992). In mammalian cell lines, cell deaths caused by BEA have been suggested to involve a Ca^{2+} dependent pathway, in which BEA induces a significant increase in intracellular Ca^{2+} concentration that leads to cell death as a result of a combination of both apoptosis and necrosis (Jow et al., 2004; Lin et al., 2005).

BEA has also been found as a natural contaminant of maize from Poland, Italy, USA, South Africa, Switzerland and Slovakia; feed samples from USA; rye from Finland; and oats, wheat and barley from Norway and Finland (Jestoi, 2008). Logrieco et al. (1993) reported high levels of BEA up to 60 mg/kg in maize from Poland, while Ritieni et al. (1997) reported high levels of BEA up to 520 mg/kg in maize from Italy. Recently, Meca et al. (2010b) have reported the contamination of cereals available in the Spanish market with BEA and levels ranged from 0.51 to 11.78 mg/kg.

In the analysis of the risk evaluation related to the human health, food ingestion is considered to be one of the important routes of exposure of many contaminants (Carolien et al., 2005).

To achieve any effect in a specific tissue or organ, the mycotoxins must be available, which refers to the compound's tendency to be extracted from the food matrix, and they must then be absorbed from the gut via the intestinal cells (Fernández-García et al., 2009).

The term bioaccessibility has been defined as the fraction of a bioactive compound present in a food matrix that passes unmodified complex of the biochemical reactions relationated to the gastrointestinal digestion and thus becomes available for intestinal absorption (Fernández-García et al., 2009).

Studies on animals and humans show that oral bioaccessibility of some bioactive compounds present in food can be significatively modified depending on the food source. Nowadays, there is no data in the literature regarding the bioaccessibility of the minor *Fusarium* mycotoxin BEA, but the study of the bioaccessibility of other mycotoxins has been evaluated by many authors. In particular,

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Avantaggiato et al. (2003, 2004) studied the bioaccessibility of zearalenone (ZEA), fumonisin B_1 (FB_1), fumonisin B_2 (FB_2), ochratoxin A (OTA), deoxynivalenol (DON) and aflatoxin B_1 (AFB_1) present in feed enriched with adsorbent materials, as activated carbons and others, that have the properties to adsorb the mycotoxins, and so reduce the presence of these compounds in the gastrointestinal tract, utilizing a laboratory system that simulated the metabolic processes of the gastrointestinal tract of healthy pigs.

In addition, Avantaggiato et al. (2007) evaluated the influence of the carbon/aluminosilicate based product added in the production of feeds, on the reduction of the bioaccessibility of FB_1, FB_2, OTA, DON and ZEA, demonstrating that the employment of the adsorbent materials can prevent the individual and combined adverse effects of some *Fusarium* mycotoxins in animals.

Motta and Scott (2009) studied the percentage of total bound fumonisin B_1 (TB-FB_1), formed for the reaction of the FB_1 with some components present in food as amino acids or sugars, in corn flakes applying an *in vitro* gastro-digestion model.

As demonstrated by the studies previously mentioned, the employment of the adsorbent materials in the technology of the production of feeds has the property to reduce the exposure risk to the *Fusarium* mycotoxins in animals fed with these bioactive feeds. These typologies of capturing mycotoxin materials are thus applicable to the animal nutrition, but, in nature, there are products that are capable of capturing some toxic components present in food, like the alimentary fiber.

According to the American Association of Cereal Chemists (AACC), dietary fiber is defined as the edible part of plants or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine (Nair et al., 2010).

Dietary fibers may be classified as water-soluble fibers, that are represented principally by pectins, β-glucans, glucomannans, fructooligosaccharides (FOS), galactooligosaccharides (GOT), and inulin, and water insoluble fibers represented mainly by cellulose, lignin, and hemicelluloses present mainly in wheat, most grain products, and vegetables (Nair et al., 2010). In particular, the soluble fibers are important for several biological and technological activities such as: prebiotic, glycemic index reducers, fat, protein and carbohydrate replacers, reducer in cholesterol and triglycerides absorption, etc. (Min et al., 2010; Cugnet-Anceau et al., 2010;Pereira et al., 2010; Nair et al., 2010; Rodrigues et al., 2011). There are only two publications relating mycotoxins to the dietary fiber, which are the studies by Meissonnier et al. (2009) and Rabassa et al. (2010). The first one demonstrated that the supplementation of an animal diet with glucomannan protects the same against immunotoxicity caused by AFB_1, and T-2 toxin during a vaccinal protocol. Rabassa et al. (2010) reported that the dietary fiber glucomannan added as sorbent material in a ruminant's diet, reduced possible liver aggression caused by AFB_1.

Considering all these aspects, the aim of the study was to evaluate different soluble fibers such as β-1,3 glucan, chitosan low M.W., chitosan medium M.W., fructooligosaccharides (FOS), galattomannan, inulin and pectin used as sorbent material, that influence the duodenal and colonic bioaccessibility *in vitro* of the minor *Fusarium* mycotoxin BEA: (a) in model solutions composed by aqueous solutions of each fiber (1% and 5% (w/v)) contaminated with 5 and 25 mg/L of BEA, (b) in cooked crispy breads prepared with 1% and 5% (w/w) of each fiber and contaminated with 5 and 25 mg/kg of BEA.

2. Materials and methods

2.1. Materials

Potassium chloride (KCl), potassium thiocyanate (KSCN), monosodium phosphate (NaH_2PO_4), sodium sulfate ($NaSO_4$), sodium chloride (NaCl), sodium bicarbonate ($NaHCO_3$), urea, α-amylase, hydrochloric acid (HCl), sodium hydroxide (NaOH), formic acid, pepsin, pancreatin, bile salts, phosphate buffer saline (PBS, pH 7.5), β-1,3 glucan, chitosan low M.W., chitosan medium M.W., fructooligosaccharides (FOS), galattomannan, inulin and pectin were obtained from Sigma–Aldrich (Madrid, Spain). Acetonitrile, methanol and ethyl acetate were purchased from Fisher Scientific (Madrid, Spain). Deionized water (<18 MΩ cm resistivity) was obtained from a Milli-Q water purification system (Millipore, Bedford, MA, USA). Chromatographic solvents and water were degassed for 20 min using a Branson 5200 (Branson Ultrasonic Corp., CT, USA) ultrasonic bath.

The BEA employed/utilized in this study were produced and purified according to the method of Meca et al. (2010a).

2.2. Model solution preparation

The model solutions were prepared in 100 mL Erlenmeyers, suspending 1 and 5 g of each soluble alimentary fiber (β-1,3 glucan, chitosan low M.W., chitosan medium M.W., (FOS, galattomannan, inulin and pectin) in 100 mL of distilled water, to obtain solutions at 1% and 5% (w/v) of each fiber. The solutions were mixed using ultrasound bath (Lab Police, Barcelona, Spain) operating at a temperature of 30 °C, and then 10 mL from each solution was contaminated with 5 mg BEA/L, and other 10 mL with 25 mg BEA/L. The contamination of the solutions was carried out using a stock methanolic solution (1000 mg/L) of BEA.

The solutions were digested with a simulated gastrointestinal digestion to assess the bioaccessibility of the BEA.

2.3. Wheat crispy breads production

For the production of the wheat crispy breads with different fiber concentrations, 300 g of wheat flour, 3 g of sucrose, and 6 g of NaCl, were mixed with 3.0 and 15.4 g of each dietary fiber (β-1,3 glucan, chitosan low M.W., chitosan medium M.W., (FOS, galattomannan, inulin and pectin) to obtain dough with 1% and 5% (w/w) of each prebiotic compound employed. These mixtures were then mixed with 180 mL of water during 5 min. No fermentation was done. The dough, divided in the shape of small round breads, was treated at 220 °C during 20 min.

2.4. Bacterial strains and growth conditions

Thirteen commercial probiotic strains were obtained for the *in vitro* system that simulates the physiologic condition of the colonic intestinal compartment. In particular *Lactobacillus animalis* CECT 4060T, *L. casei* CECT 4180, *L. casei rhamnosus* CECT 278T, *L. plantarum* CECT 220, *L. rhuminis* CECT 4061T, *L. casei casei* CECT 277, *Bifidobacterium breve* CECT 4839T, *B. adolescentes* CECT 5781T and *B. bifidum* CECT 870T, *Corynebacterium vitaeruminis* CECT 537, *Streptococcus faecalis* CECT 407, *Eubacterium crispatus* CECT 4840, *Saccharomyces cerevisiae* CECT 1324 were obtained at the Spanish Type Culture Collection (CECT Valencia, Spain), in sterile 18% glycerol.

For longer survival and higher quantitative retrieval of the cultures, they were stored at −80 °C. When needed, recovery of strains was undertaken by two consecutive subcultures in appropriate media prior to use (Laparra and Sanz, 2009; Meca et al., 2012a).

2.5. In vitro digestion model

The procedure was adapted from the method outlined by Gil-Izquierdo et al. (2002), with slight modifications. The method consists of three sequential steps; an initial saliva/pepsin/HCl digestion for 2 h at 37 °C, to simulate the mouth and the gastric conditions, followed by a digestion with bile salts/pancreatin for 2 h at 37 °C to simulate duodenal digestion (Fig. 1). The colonic conditions were simulated adding to the duodenal simulated fluid some bacteria representative of the gastrointestinal tract.

For the saliva/pepsin/HCl digestion, 10 mL of the model solution or 10 g of the crispy bread contaminated with 5 and 25 mg/kg of BEA, were mixed with 6 mL of artificial saliva composed by: KCl 89.6 g/L, KSCN 20 g/L, NaH_2PO_4 88.8 g/L, $NaSO_4$ 57 g/L, NaCl 175.3 g/L, $NaHCO_3$ 84.7 g/L, urea 25 g/L, 290 mg of α-amylase. The pH of this solution was corrected at 6.8 with NaOH 0.1 N.

These mixtures composed by model solutions and by the artificial saliva were placed in plastic bags, containing 40 mL of water and homogenized by Stomacher IUL Instruments (Barcelona, Spain) during 30 s.

To this mixture, 0.5 g of pepsin (14,800 U) dissolved in 25 mL of HCl 0.1 N was added. The pH of the mixture was corrected to a value of 2 with HCl 6 N, and then incubated in a 37 °C orbital shaker (250 rpm) (Infors AG CH-4103, Bottmingen, Switzerland) for 2 h.

After the gastric digestion, the pancreatic digestion was simulated. The pH was increased to 6.5 with $NaHCO_3$ (0.5 N) and then 5 mL of (1:1; v/v) pancreatin (8 mg/mL):bile salts (50 mg/mL), dissolved in 20 mL of water, was added and incubated in a 37 °C orbital shaker (250 rpm) for 2 h. An aliquot of 5 mL of the duodenal simulated fluid was sampled for the extraction of the BEA and the determination of the duodenal bioaccessibility.

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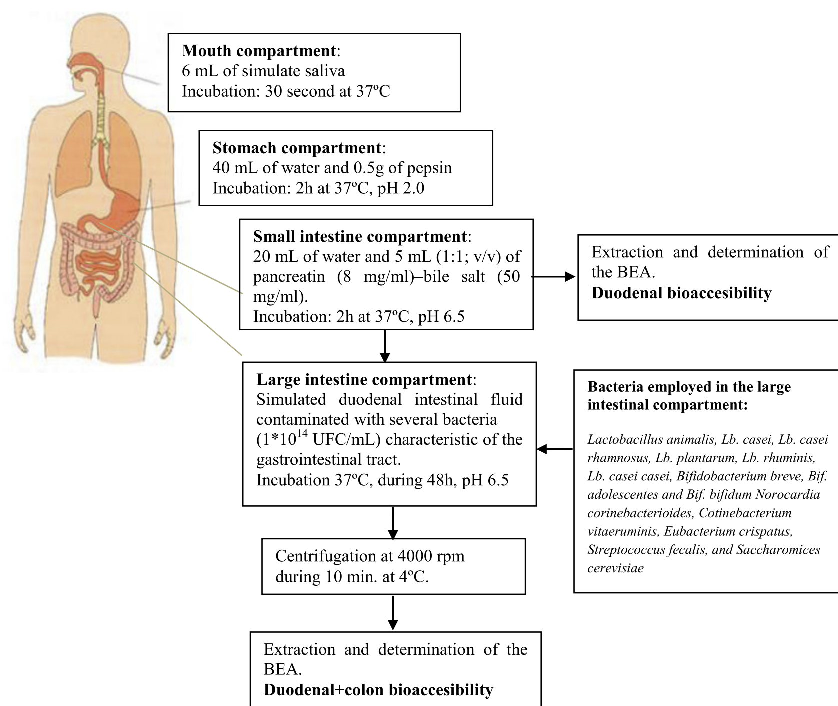

Fig. 1. Schematic representation of the *in vitro* digestion model. The *in vitro* digestion model describes a four-step procedure simulating the digestive processes, considering the mouth, stomach, small and large intestines. In each compartment, the matrix was incubated at 37 °C and the pH was modified according to the physiologic conditions. This procedure permits to simulate *in vitro* the physiologic condition of the human gastrointestinal tract and to study how some components present in the diet can influence the bioaccessibility of the bioactive compounds.

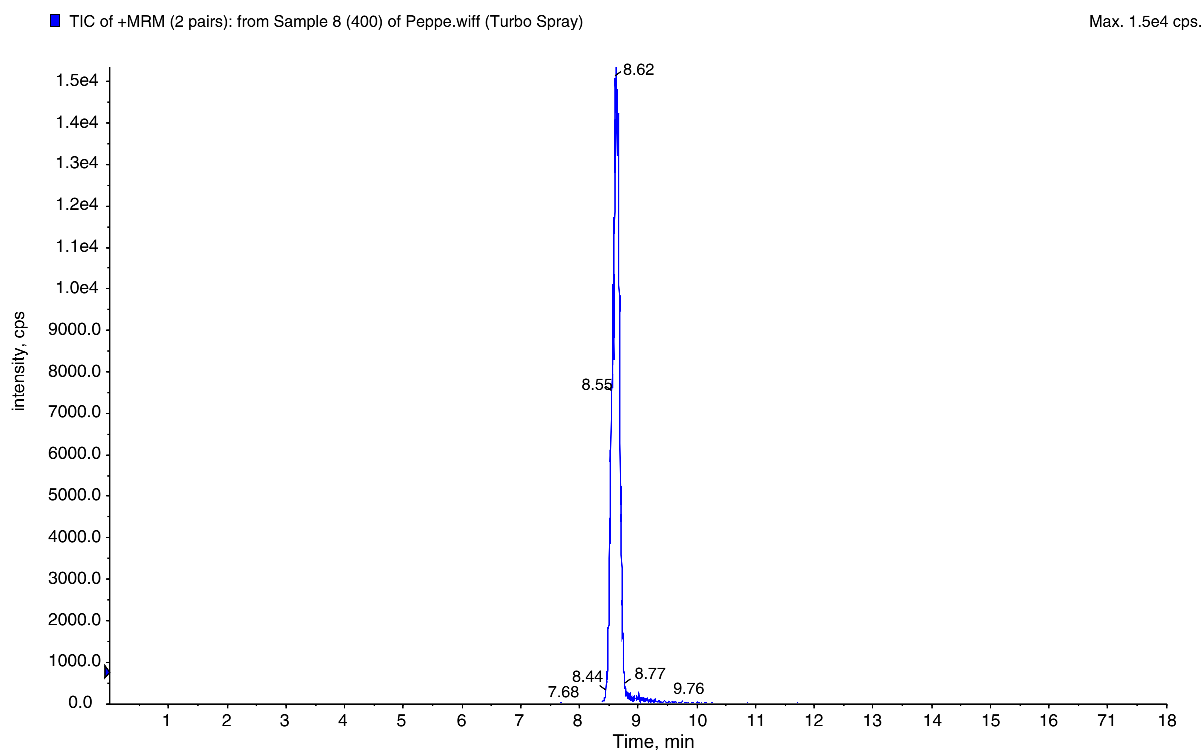

Fig. 2. LC–MS/MS chromatogram of the minor *Fusarium* mycotoxins BEA present in the bioaccessible fraction were obtained applying a simulated gastrointestinal digestion model to a crispy bread sample produced with 5% of the dietary fiber inulin and contaminated with 5 mg/kg of the bioactive compound.

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To simulate the colonic compartment *Bacterial* strains (previously described) were grown in a sterile plastic centrifuge tube overnight at 37 °C in MRS broth (Oxoid, Madrid, Spain) under anaerobic conditions (5% CO_2/95% air). Then, the tubes were centrifuged at 4000 rpm during 5 min. at 23 °C and the bacteria were resuspended in sterile PBS. A 500 µL of a mixture of the bacterial suspensions at concentrations of 10^{14} CFU/mL was added to duodenal simulate intestinal fluid and incubated at 37 °C in 5% CO_2/95% air 48 (Laparra and Sanz, 2009).

After this last digestion, 5 mL of the mixture was centrifugated at 4000 rpm during 10 min at 4 °C, and extracted for BEA determination and for the estimation of the duodenal + colonic bioavailability.

2.6. BEA extraction from the simulated intestinal fluids

BEA contained in the duodenal and duodenal + colonic fluids were extracted as follows. Five milliliters of each mixture previously described were placed in a 20 mL test tube, and extracted three times with 5 mL of ethyl acetate using a vortex VWR international (Barcelona, Spain) for 1 min. Then, the mixtures were centrifugated (Centrifuge 5810R, Eppendorf, Germany) at 4000 rpm and at 4 °C for 10 min. The organic phases were completely evaporated by a rotary evaporator (Buchi, Switzerland) operating at 30 °C and 30 mbar pressure, resuspended in 1 mL of methanol and filtered with a 0.22 µM filter (Phenomenex, Madrid, Spain) before being analyzed by LC–MS/MS.

2.7. BEA analysis

The separation of BEA was achieved by an Agilent 1100 (Agilent Technologies, Santa Clara, California) LC coupled to an Applied Biosystems/MDS SCIEX Q TRAP TM linear ion trap mass spectrometer (Concord, Ontario, Canada). A Gemini (150 × 2.0 mm, 5 µm) Phenomenex (Torrance, California) column was used. LC conditions were set up using a constant flow at 0.2 mL/min and acetonitrile:water (70:30, (v/v) with 0.1% of HCOOH) as mobile phase in isocratic condition was used. The instrument was configured in the positive ion electrospray mode using the following parameters: cone voltage 40 V, capillary voltage 3.80 kV, source temperature 350 °C, desolvation temperature 270 °C and collision gas energy 5 eV. Multiple reaction monitoring (MRM) technique was used for identification and quantification, in which protonated molecule $[M+H]^+$ of the BEA m/z 784.50, was fragmented in the collision cell to the product-ion m/z 244.20. For quantification, the product-ion m/z 244.20 was used (Jestoi et al., 2009).

2.8. Calculations and statistical analysis

All experiments were performed by triplicate. Statistical analysis was carried out using the analysis of variance (ANOVA), followed by Dunnet's multiple comparison tests. Differences were considered significant if $p < 0.05$.

3. Results and discussion

It is now well established that the colonic microflora has a profound influence on health (Steer et al., 2000). Consequently, there is currently a great deal of interest in the use of prebiotics as functional food ingredients to manipulate the composition of colonic microflora in order to improve health (Wang, 2009).

Thus, prebiotics, such as oligosaccharides, are defined as "non-digestible food ingredient(s) that beneficially affect host health by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon" (Gibson and Roberfroid, 1995). This definition was updated in 2004 and probiotics are now defined as "selectively fermented ingredients that allow specific changes, both in the composition and/or activity in the gastro-intestinal microbiota that confers benefits upon host well-being and health" (Gibson et al., 2004).

Prebiotics show both important technological characteristics and interesting nutritional properties (Huebner et al., 2007). Several of them are found in vegetables and fruits and can be industrially processed from renewable materials. In food formulations, they can significantly improve organoleptic characteristics, upgrading both taste and mouth feel. In order to be used as functional food ingredients, prebiotics must be chemically stable to food processing treatments, such as heat, low pH, and Maillard reaction conditions.

Some probiotic compounds have also the property to capture some bioactive compounds present in food as polyphenols, and after the hydrolysis of their structure by the enzymes complex of the bacteria present in the intestinal colonic compartment, they can interact with the intestinal epithelium, be absorbed and act on the different organs and tissues of the human body.

The object of this study was to evaluate if some soluble alimentary fibers can interact with the minor *Fusarium* mycotoxin BEA, reducing the bioaccessibility of this bioactive compound, in a model solution and in a food model composed by a wheat crispy bread produced with two percentages of the prebiotics (1% and 5%), and spiked with 5 and 25 mg BEA/L.

As is possible to observe in the Fig. 3a, the duodenal bioaccessibility (%) of the BEA present in the model solution produced without the fibers (control) at the two concentrations tested was of 92.6 ± 1.2 and 90 ± 1.3, respectively for 5 and 25 mg BEA/L. However, when the soluble alimentary fibers are added to the model solutions a great reduction of BEA bioaccessibility was obtained at the two concentrations employed. In particular, at the concentration of 5 mg/L, the mean bioaccessibility datum was 13.2%. The lowest reduction of BEA bioaccessibility was evidenced by the fiber FOS (5%), with 21.5 ± 1.4%. At the concentration of 25 mg/L, the mean bioaccessibility of the bioactive compound employed was 50.4%. The highest bioaccessibility reduction of BEA was evidenced by the dietary fiber galattomannan that in the model solution evidenced only the 10.8 ± 0.9%, whereas the lowest reduction was evidenced by the fiber FOS (1%) with 85.0 ± 2.3%. Another fiber that considerably reduced BEA bioaccessibility is inulin, which, used in the concentration of 5%, showed a BEA bioaccessibility of only 2.6 ± 0.5%.

When the model solutions were digested with the duodenal and colonic digestions, the data obtained are completely different. In particular, as it is shown in Fig. 3b, the BEA bioaccessibility increases in all the assays carried out. The mean values reported at 5 and 25 mg/L were 36.6 and 69.8%, respectively. The same fibers,

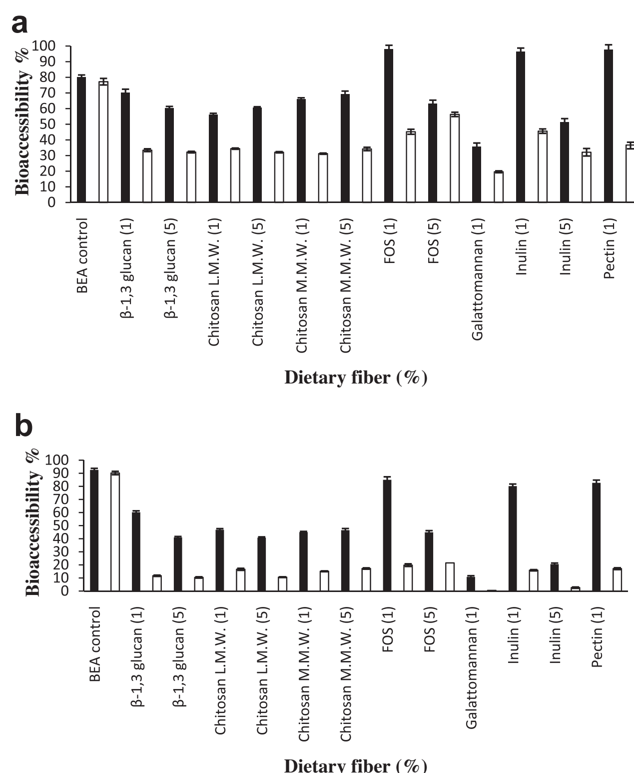

Fig. 3. Bioaccessibility evidenced by the BEA in the model solutions constituted of water suspensions containing different soluble dietary fibers contaminated with 5 (black square) and 25 mg/kg (white square) of the minor *Fusarium* mycotoxin (a) until duodenal digestion and (b) duodenal + colonic digestion.

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that evidenced a good BEA capture action in the model solution digested only with the duodenal compartment, can complex effectively the mycotoxin using sequentially the duodenal and the colonic simulated gastrointestinal digestions as well.

BEA bioaccessibility ranged from 1.3 to 38-fold higher than the data reported using only the duodenal digestion. This difference can be related to the fermentation of the fibers carried out by the bacteria employed.

Regarding the experiments performed with the food system composed by wheat crispy bread produced with the prebiotic compounds and contaminated with 5 and 25 mg/L of BEA, the results are reported in the Fig. 4a and b. The mean bioaccessibility data of the bioactive compounds tested at the two concentrations (5 and 25 mg BEA/L) were of 2.4% and 1.5% respectively. All the prebiotic compounds tested reduced the bioaccessibility of the BEA, and in particular the reduction evidenced ranged from 96% to 99% considering the two concentrations employed. These data demonstrated that, considering only the duodenal intestinal compartment, the alimentary matrix enriched with fibers has the property to capture BEA, thus reducing its bioaccessibility. A LC–MS/MS chromatogram of BEA present in the bioaccessible fraction obtained applying the simulated gastrointestinal digestion model to a crispy bread sample produced with 5% of the dietary fiber inulin and contaminated with 5 mg/kg of the bioactive compound is plotted in the Fig. 2.

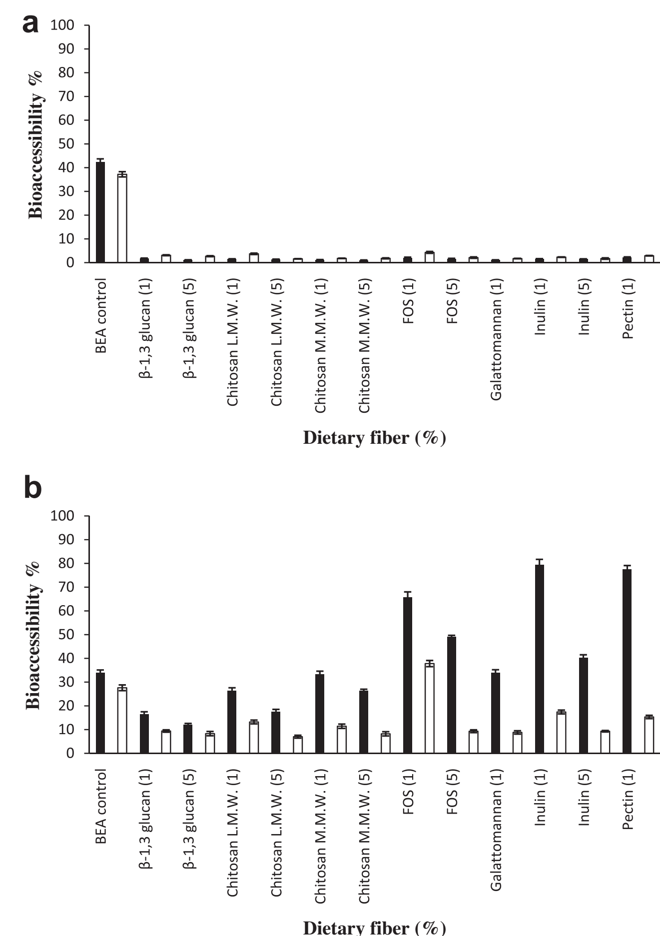

Fig. 4. Bioaccessibility evidenced by BEA in the alimentary system constituted of wheat crispy breads produced with 1 and 5% of each soluble alimentary fibers and contaminated with 5 (black square) and 25 mg/kg (white square) of the minor *Fusarium* mycotoxin (a) until duodenal digestion and (b) duodenal + colonic digestion.

The results obtained when the crispy breads were digested using the complete gastrointestinal model (included the colon compartment) are very different. In these experiments, due to the action of the fermentation operated by the probiotic strains, the fibers were hydrolyzed, increasing the BEA concentration in the colonic simulated intestinal fluid and then incrementing its bioaccessibility.

The mean bioaccessibility values of the mycotoxin obtained at the two concentrations used were of 12.9% and 42.4% respectively, 5.3 and 28.2-fold higher than the data evidenced with the sample treated only with the duodenal digestion. The highest bioaccessibility data at 5 mg/L, was evidenced by the dietary fiber FOS, whereas at 25 mg/L by inulin, with 37.9 ± 1.3% and 79.5 ± 2.2%, respectively. This phenomenon is probably due to the high capacity of the microbial enzymes to degrade the fructooligosaccharides as FOS and inulin, with respect to other alimentary fibers. The highest bioaccessibility reductions were obtained by the chitosan low M.W., with 7.0%, and by the β-1,3 glucan with 16.6%, at the BEA concentrations of 5 and 25 mg/L respectively. These two alimentary fibers, used nowadays in nutraceutical products, have the capacity to resist the fermentation operated by bacteria, reducing the bioaccessibility of the bioactive compounds captured.

The data evidenced in this study demonstrate that some prebiotic compounds help to reduce the risk associated with the intake of the minor *Fusarium* mycotoxin BEA, with a mechanism of retention for this compound similar to the phenomenon already evidenced for the polyphenols.

In addition, this study demonstrates that the binding capacity of the bioactive compound BEA to the soluble fibers employed is higher when BEA was used in the lowest concentration (5 mg/L). This phenomenon can probably be related to the presence of restricted active binding sites in the fibers that prevent the binding of toxic compounds to the highest concentration as previously demonstrated by Ta et al. (1999) and Zhang et al. (2011) for other types of contaminants.

Comparing the reduction of BEA bioaccessibility in the model solution *vs* the crispy bread, the results obtained were clearly different. Considering only the duodenal bioaccessibility at 5 and 25 ppm concentrations, the bioaccessibility evidenced in the alimentary system composed by wheat crispy bread was 5.3 and 33.3-fold lower than the one in the model solutions. Considering the duodenal + colonic bioaccessibility, no significant differences were detected at 5 ppm concentration whereas at 25 ppm, the bioaccessibility detected in the food system resulted 5.4-fold lower than the data obtained in the model solution. In the experiments carried out using the alimentary system, there is a considerable decrease of BEA bioaccessibility compared to the model solution. This phenomenon can be related to the food composition. In particular, the mycotoxins that are spiked in food or that naturally contaminate the food are complexed to the food matrix. The formation of this complex is dependent on the amount of the micro and macronutrients contained in the food, such as fibers, sugars, proteins, minerals, etc. (Kabak et al., 2009). Different bioavailability values were also reported by several authors (Carolien et al., 2005), comparing the bioavailability of the same compounds contained in liquid or solid food (Meca et al., 2012b).

In the scientific literature there is no data regarding the reduction of the bioaccessibility of the minor *Fusarium* mycotoxin BEA in food by employing prebiotics as soluble alimentary fiber compounds as adsorbent materials, and few studies have been published on the employment of inulin in feed in order to reduce the toxic effects of the trichothecenes on different organs of several animals.

In particular Meissonnier et al. (2009) investigated how the dietary supplements with yeast-derived glucomannan protected pigs against the deleterious effects by the exposure to AFB_1 or T-2 toxin.

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Three doses of pure mycotoxin (AFB_1 trial: 482, 968 and 1912 μg/kg feed; T-2 toxin trial: 593, 1155 and 2067 μg/kg feed) with or without dietary glucomannan supplementation (2 g/kg feed) were tested in weaned pigs for 28 days. In the AFB_1 trial glucomannan decreased the severity of liver lesions in animals exposed to 968 μg/kg feed. Exposure to both AFB_1 and T-2 toxin were associated with impaired phase I liver enzyme activities, but glucomannan demonstrated a limited protective effect on these enzymes. Glucomannan supplementation restored the ovalbumin-specific lymphocyte proliferation that was delayed in pigs exposed to AFB_1, regardless of dose. In the T-2 toxin trial glucomannan supplementation restored anti-ovalbumin immunoglobulin G production, which was significantly reduced in pigs exposed to both medium and high doses of the toxin. The authors demonstrated that glucomannan dietary supplementation demonstrated no deleterious effects in control animals and protective effects against AFB_1 and T-2 toxin immunotoxicity during a vaccinal protocol.

Awad et al. (2009) studied the influence in feed for chicken nutrition, of a probiotic strain as *Eubacterium* sp. and of a prebiotic as the inulin, in the reduction of the injuries of the gastrointestinal tract induced by the mycotoxin DON. The authors demonstrated that the alteration caused by DON was reduced by supplementing the Don containing diets with probiotic feed additive. In the presence of the acute toxicity of DON, the dietary inulin supplementation may be useful in reducing the toxic effects of DON on intestinal glucose transport. This indicates that in the case of DON contamination of feedstuffs the addition of feed additive would be a proper way to counteract the possible impacts due to this mycotoxin.

Rabassa et al. (2010) studied the influence of the sorbent modified glucomannan on metabolic parameters of sheep submitted to diets containing AFB_1 and ZEA. For this, 34 females were used and they were divided into six groups, receiving 1.0 mg/kg of ZEA, 1.5 mg/kg of AFB_1 and/or 2 kg/ton of sorbent. From the analysis of the results it resulted that ZEA caused metabolic alterations in sheep, and the modified glucomannan was effective in reducing the possible liver aggression caused by this mycotoxin, shown by the decrease in the levels of AST.

Nowadays, the study of the bioaccessibility of other mycotoxins in the presence or not of binding material used in animal nutrition has been evaluated by many authors.

In particular, Avantaggiato et al. (2003) studied the intestinal absorption of ZEA by using a laboratory model that simulated the physiology of the gastrointestinal tract of healthy pigs. Approximately 32% of ZEA was released from the food matrix to the bioaccessible fraction during 6 h of digestion and was rapidly absorbed at the intestinal level.

Avantaggiato et al. (2004), studied an *in vitro* screening of 14 adsorbent materials, including some commercial products used to detoxify *Fusarium*-mycotoxins, were tested in the pH range of 3–8 for DON and nivalenol (NIV)-binding ability. A dynamic laboratory model simulating the gastrointestinal (GI) tract of healthy pigs (TIM system) was used to evaluate the small-intestinal absorption of DON and NIV and the efficacy of activated carbon in reducing the relevant absorption. The invitro intestinal absorptions of DON and NIV were 51% and 21%, respectively, as referred to 170 mg DON and 230 mg NIV ingested through contaminated (spiked) wheat. Most absorption occurred in the jejunal compartment for both mycotoxins. The inclusion of activated carbon produced a significant reduction in the intestinal mycotoxin absorption. The binding activity of activated carbon for these trichothecenes was lower than that observed for zearalenone, a mycotoxin frequently co-occurring with them in naturally contaminated cereals.

In 2005, Carolien et al. described the applicability of an *in vitro* digestion model to study the bioaccessibility of the AFB_1 from peanut slurry and of the OTA from buckwheat, evidencing bioaccessibility data of 94% and 100%, respectively.

Recently, Kabak et al. (2009), studied the bioaccessibility of the AFB_1 in pistachio nuts, buckwheat, and in infant foods using an *in vitro* model under fed condition. The average bioaccessibility of AFB_1 and OTA was about 90%, and 30%, respectively, depending on several factors, such as food product, contamination level, compound and type of contamination (spiked vs. naturally contaminated).

Marroquın-Cardona et al. (2009) evaluated twelve different additives distributed in Mexico as mycotoxin (AFB_1) binders in feed utilizing: Ca-montmorillonite, Na-montmorillonite, aluminosilicates, organo-aluminosilicates, glucomannan/β-D-glucan containing yeast product and a synergistic mixture of minerals, biological constituents, including enzymes and BBSH microbe, plant-derived extracts such as flavonolignans, saponins and terpenoids, and some algae materials, as mycotoxin binders. The experiment adsorptions were conducted at pH 2 and pH 6.5, mimicking pH conditions in the stomach and small intestine. The results obtained by the authors demonstrated that adding the binder to the feed preparation the reaction of the AFB_1 in the alimentary preparation digested was reduced from 50% to the 95%, depending on the additive utilized.

4. Conclusion

This is the first time that the bioaccessibility of the *Fusarium* mycotoxin BEA in the presence of some alimentary soluble fibers was investigated using a system that simulates the physiologic conditions of the gastrointestinal tract. In the experiments carried out employing the model solutions contaminated with 5 and 25 mg/L of BEA and containing 1% and 5% of each dietary fiber the highest reduction of BEA bioaccessibility was evidenced in the experiments carried out with the fibers galattomannan obtaining a mean BEA bioaccessibility data of 5.2%. In the experiments carried out simulating an alimentary system composed of wheat crispy bread, the more efficient fibers that reduced BEA bioaccessibility were chitosan MMW and the galattomannan.

In conclusion the fibers used resulted in binding the bioactive compound tested until the treatment with the colonic bacteria that hydrolyze the prebiotic compounds increasing partially the bioaccessibility of the mycotoxin. The results help to explain as some natural components present in the food can help to modulate the intake of some toxic compounds.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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