Carbohydrate Polymers 93 (2013) 747–752

Contents lists available at SciVerse ScienceDirect

Carbohydrate Polymers

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

Formulation of a pectin gel that efficiently traps mycotoxin deoxynivalenol and reduces its bioavailability

Chikako Tamura[a], Makoto Nakauma[b], Hiroko Furusawa[a], Tomoyuki Kadota[c], Yoichi Kamata[a], Motohiro Nishijima[d], Seigo Itoh[e], Yoshiko Sugita-Konishi[a,*]

[a] Division of Microbiology, National Institute of Health Sciences, 1-18-1 Kamiyoga, Setagaya, Tokyo 158-8501, Japan
[b] San-Ei Gen F.F.I., Inc., 1-1-11, Sanwa-cho, Toyonaka, Osaka 103-0023, Japan
[c] Kirin Holdings Company, Limited, 3 Miyahara-cho, Takasaki, Gunma 330-9731, Japan
[d] Department of Food and Health Sciences, Jissen Women's University, 4-1-1 Osakaue, Hino, Tokyo 191-8510, Japan
[e] Department of Veterinary Science, Azabu University, 1-17-1 Fuchinobe, Sagamihara, Kanagawa pref. 229-8501, Japan

ARTICLE INFO

Article history:
Received 15 September 2011
Received in revised form 9 December 2012
Accepted 12 December 2012
Available online 5 January 2013

Keywords:
Low-methoxyl amidated pectin
Low-methoxyl non-amidated pectin
Mycotoxin
Deoxynivalenol
Bioavailability

ABSTRACT

We aimed to develop a new food-processing approach using pectin to reduce gastrointestinal absorption of mycotoxins. When Ca^{2+} is added to low-methoxyl pectin, a gel resembling an egg box-like structure forms that is able to trap certain molecules. We examined whether or not low-methoxyl amidated pectin (LMA) and low-methoxyl non-amidated pectin (LMNA) trapped the mycotoxin deoxynivalenol (DON) after being ingested. We first determined the trapping effects of LMA and LMNA on DON *in vitro* under conditions similar to those in the human stomach, with results showing that LMA gel trapped DON to a greater extent than the LMNA gel. We then performed *in vivo* experiments and demonstrated that the LMA gel containing DON reduced DON's absorption from the gastrointestinal tract. This new food-processing technique holds great promise for reducing the bioavailability of DON in contaminated food and may be useful in mitigating the effects of other mycotoxins.

1. Introduction

Raw materials used to produce foods and their final products may often be contaminated with fungi and their toxins, raising international concerns about food safety (Kuiper-Goodman, 1995). Mycotoxin concentrations in these raw materials can vary considerably depending on meteorological conditions (Battilani et al., 2006). Given their relative thermal stability, completely eliminating mycotoxins from raw materials and end products during processing is extremely difficult. Mycotoxins are known to cause cancer, nephritis, hepatitis, and gastrointestinal disease in both animals and humans (Bryden, 2007). In developing countries in particular, acute toxicity caused by mycotoxins remains a serious problem even in the 21st century. In contrast, in developed countries, chronic toxicity has emerged as a greater source of concern (Sherif, Salama, & Abdel-Wahhab, 2009). A worldwide movement has emerged calling for a reduction in mycotoxin exposure through acceptable agricultural practices and regulation of maximum acceptable levels for mycotoxin by international organizations such as the Codex Alimentarius Commission (2011).

Although implementation of these policies has been effective in several countries, eliminating mycotoxin contamination of raw materials worldwide has been difficult. As such, attempts to reduce mycotoxins in finished food products remain an urgent necessity. To date, a number of chemical, physical, and biological treatments for reducing mycotoxins have been made available, including ozone degradation (Karaca, Velioglu, & Nas, 2010), thermal inactivation, and biotechnological techniques (Bissessur, Permaul, & Odhav, 2001; Kimura et al., 2006), but in turn, produce unwanted side effects. For example, although roasting and extrusion processing do indeed decrease mycotoxin concentrations, the high temperatures required can produce unknown, possibly toxic by-products (Humpf & Voss, 2004). Similarly, biotechnological decontamination involving the use of bacteria is still associated with a number of safety issues that render it undesirable for use in mycotoxin reduction (Bata & Lásztity, 1999).

We developed a new processing method to reduce gastrointestinal absorption of mycotoxin deoxynivalenol (DON) using pectin. Generally accepted as a non-toxic food additive (Van Soest, Robertson, & Lewis, 1991), pectin is a component of plant cell walls and is an important ingredient required for the gelation of jams and other fruit products. Pectin is a dietary fiber (Asp, 1987; Evans & Shronts, 1992), indigestible by humans, consisting of a complex polysaccharide composed of α-D-(1,4)-galacturonic

* Corresponding author. Tel.: +81 3 3700 9048; fax: +81 3 3700 9049.
E-mail address: ykonishi@nihs.go.jp (Y. Sugita-Konishi).

0144-8617/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.carbpol.2012.12.046

acid with a small fraction of rhamnose and small side chains formed by other sugars (Mohnen, 2008). Depending on the degree of esterification (DE) of the carboxyl group, pectin may be classified as either high- or low-methoxyl pectin. Conversion of high-methoxyl pectin to a gel requires relatively large quantities of sugar (>50% (v/v)) and acidic conditions. In contrast, low-methoxyl pectin requires only Ca^{2+} to form a gel that possesses a stitch structure according to the so-called "egg box model" (Cabrera, Boland, Messiaen, Cambier, & Van Cutsem, 2008; Chambin, Dupuis, Champion, Voilley, & Pourcelot, 2006; Capel, Nicolai, Durand, 2005). To date, two types of low-methoxyl pectins have been identified: low-methoxyl non-amidated pectin (LMNA) and low-methoxyl amidated pectin (LMA).

Here, we examined the effects of gels made with LMNA or LMA on trapping DON in a gel *in vitro and in vivo* to provide a nutritional modality that might mitigate the toxic effects of DON-contaminated foods.

2. Experimental

2.1. Materials

Deoxynivalenol (DON) and Molish's reagent were purchased from Wako Pure Chemical Industries, Ltd. (Tokyo, Japan), and low-methoxyl non-amidated pectin (LMNA, DE: 35%) and low-methoxyl amidated pectin (LMA, DE: 35%, 15% amidated) were purchased from San-Ei Gen F. F. I., Inc. (Osaka, Japan). HPLC-grade ethanol was use for pectin purification.

2.2. Methods

2.2.1. LMNA and LMA purification

LMNA and LMA were purified *via* ethanol precipitation. Commercial pectin powder was dissolved in a 30-fold volume of 70% ethanol (v/v) and then heated at 80 °C for 30 min. After cooling at room temperature, the ethanol supernatant was assayed using Molish's reagent to confirm that all other sugars besides pectin had been removed from the crude pectin powder. This purification procedure was repeated until no other sugars were detected in the supernatant. The purified pectin precipitate was then dissolved in a 30-fold volume of 99.5% ethanol (v/v) and dried at 80 °C for 30 min, after which it was stored at room temperature.

2.2.2. Gelation protocol

The gelation buffer consisted of 3.94 g potassium citrate monohydrate, 1.0 g sodium benzoate, and 4.5 ml 50% citric acid solution (pH 2.7 ± 0.05) in 100 ml of Milli-Q water.

For the *in vitro* trapping experiment, after dissolving 500 μg DON in 1 ml of acetonitrile in 10-ml centrifuge tubes, the solvent was removed by evaporation using N_2 gas. Control tubes were then prepared as indicated above using acetonitrile only. Thereafter, 0.5, 1.0, and 1.5 g of purified LMNA or LMA powder, corresponding to respective final concentrations of 0.5%, 1.0%, and 1.5% (w/v), were added to the tubes. One half milliliter of Milli-Q water and 0.25 ml of gelation buffer was added at 1 drop/sec. After the solution was incubated at 70 °C for 30 min, 0.25 ml Ca^{2+} solution (to provide 0.48, 1.24, and 2.30 mg/ml equivalents in the respective final water solutions) was added to the tubes, and the gel was cooled to room temperature over 30–60 min. Fifty milliliters of gel prepared without DON was then used to measure gel strength.

2.2.3. Strength test of pectin gel

A gel strength test was carried out using a TA-TX 2i texture analyzer (Eko Instruments Co., Ltd., Japan) according to CP Kelco's (Denmark) instructions. The plunger speed was 0.5 mm/sec. Viscoelasticity was measured as gel strength and expressed as pressure (force [N]/3.14/0.0001[m^2]). The samples were compressed on the gel surfaces using a cylinder (20 mm height × 10 mm diameter).

2.2.4. Measurement of DON trapped in gels

To measure the ability of the LMA and LMNA pectin gels to trap DON under *in vivo*-like conditions, gels with DON were suspended in 10- or 50-fold Milli-Q water in a test tube and gently shaken overnight at 37 °C. In a preliminary test, the ability of a 1.0% pectin gel to trap DON was evaluated by separating the aqueous phase from the gel, which was then degraded in 1 M citric acid (pH 9.0). The amount of DON in each was determined by enzyme-linked immunosorbent assay (ELISA). Nine milliliters of 1 M citric acid was then added to 1 ml of the pectin gel. The suspension was agitated violently using a vortex mixer, maximum setting, at room temperature and then neutralized with 0.1 M HCl. In the preliminary trial, the total amount of DON recovered from the fluid plus that recovered from the gel was almost 100%. We calculated the trapping ratio of DON from the amount of DON released into the fluid using the following formula:

$$\text{DON concentration in the gel (trapped DON) (\%)} = \left[\frac{(\text{Spiked DON amount in gel} - \text{released DON amount in fluid})}{\text{Spiked DON amount in gel}}\right] \times 100$$

2.2.5. ELISA

DON was assayed *via* competitive, indirect ELISA according to the methods described by Maragos, Busman, and Sugita-Konishi (2006) as follows: Briefly, a 96-well plate was coated (4 °C, overnight) with 100 μl DON-bovine serum albumin (BSA) conjugate in 0.05 M sodium phosphate buffer (pH 7.0). The coated plate was washed with 0.02% Tween 20 (v/v) in 0.01 M phosphate-buffered saline (PBS, pH 7.0) and blocked with 1.0% polyvinyl alcohol (v/v) in 0.01 M PBS at 4 °C overnight. In another plate, 75 μl of sample (or DON standard solution) and 75 μl of goat anti-mouse peroxidase conjugate were mixed, and then 100 μl of sample (standard)-peroxidase conjugate mixture was transferred to the BSA-coated plate. After incubating the mixture for 30 min at room temperature, 100 μl of TMB substrate reagent was added for 15 min at room temperature, and the reaction was stopped by addition of 100 ml of 2 M HCl. DON concentration was determined from its absorbance at 450 nm using a TriStar LB 941 micro plate reader (Berthold Technologies GmbH & Co., KG Ltd.). DON concentrations in the fluid (trapping experiments) and plasma (*in vivo* experiment) were quantified by ELISA.

2.2.6. In vivo bioavailability

All animal studies were conducted according to the Guidelines of the National Institutes of Health Sciences. Male B6C3F1 (C57B1/6J × C3H/HeJ) mice (aged 7 weeks, 20–22 g/animal) were obtained from SLC Inc. (Shizuoka, Japan) and maintained under controlled conditions (23 ± 2 °C; humidity, 55% ± 10%; 14-h light and 10-h dark cycles) and provided commercial chow pellets (Oriental Yeast Co., Tokyo, Japan) and water *ad libitum*. Mice were divided into four groups of eight mice each, and the four test samples were orally administered by gavage per 100 μg gel or 100 μl liquid/animal.

Test samples were as follows (gel-DON, pectin-DON, DON-PBS, and gel-cont), gel containing 100 μg of DON (gel-DON, equivalent to 5 mg/kg body weight (bw) DON), LMA liquid in the gelation-buffer containing 1.0 mg of DON/ml (pectin-DON), and 1.0 mg DON/ml in PBS (DON-PBS). The gel without DON (gel-cont) was used as a control. Food and water were restored immediately after treatment. At 15, 30, 90 min and 3, 6, 8, and 24 h after treatment, the

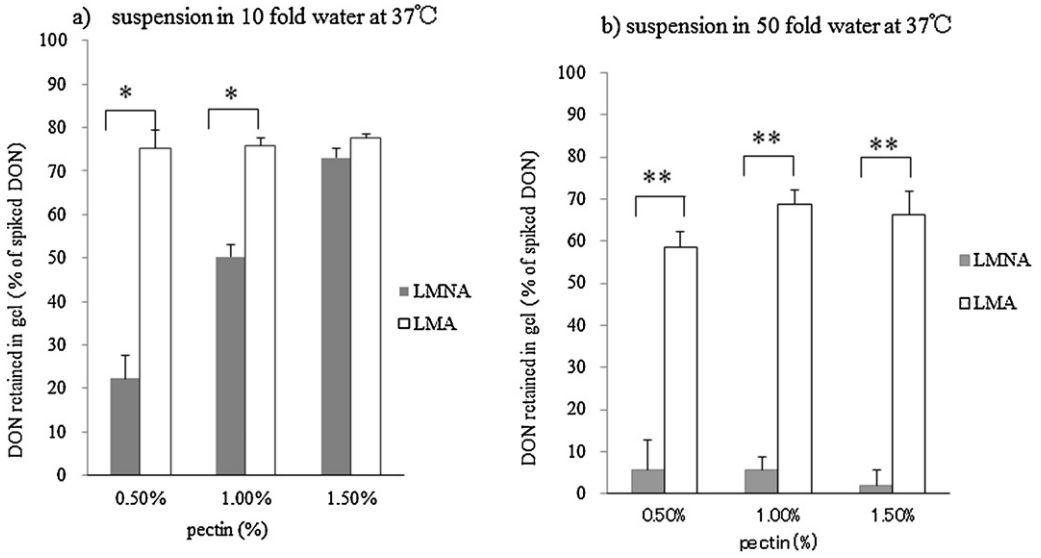

Fig. 1. Retention of DON by LMNA and LMA pectin gels at 37 °C. Gels containing varying concentrations of pectin along with 500 ng/kg DON were prepared as described in the text. The gels were suspended overnight in (a) 10- or (b) 50-fold volume of water. After collecting the supernatants, the amount of DON present was assayed *via* ELISA. The percentage of trapped DON was calculated using the formula described in the text. Data represent means ± standard deviation (SD) ($n = 4$). * Significantly different at $P < 0.05$; ** Significantly different at $P < 0.01$.

mice were anesthetized, and blood was collected from the tail vein. Plasma was prepared by centrifugation and stored at −30 °C until being analyzed for DON. The pharmacokinetic parameters, maximum concentration (C_{max}), maximum time concentration (T_{max}), and area under the curve (AUC) were calculated from the plasma concentration-time profile for each animal according to the equations described by Baggot (1970) and Klaassen (1986).

2.2.7. Treatment of LMA pectin gel with gastric and intestinal juices

Artificial gastric juice (pH 1.3) and intestinal juice (duodenal juice and bile juice plus HCO_3, pH 8.1) were prepared according to the methods described by Versantvoort, Oomen, Kamp, Rompelberg, and Sips (2005). At 1.0% LMA pectin gel was treated with a 10-fold volume of gastric juice at 37 °C for 0, 15 or 30 min or for 1 or 2 h. Treatment with intestinal juice (10-fold volume) was performed at 37 °C for 2 h. At each time point, after centrifuging the samples at 3,000 rpm × 5 min, the supernatant was harvested, adjusted to pH 7 and assayed by an ELISA.

2.2.8. Observation of scanning electron microscopy (SEM)

The cross section morphologies of the LMA pectin gel were observed using a scanning electron microscope (JSM 6610LA, JEOL, Japan). The gel was sectioned and then freeze-dried to observe its inner structure.

2.2.9. Statistical methods

Student's *t* test for independent samples was used to detect differences between means. When more than two groups were used, Scheffe's *F* test was used. *In vitro* experimental data was expressed as mean ± standard deviation, while *in vivo* experimental data was expressed as mean ± standard error.

3. Results

3.1. Gel strengths of LMA and LMNA

Table 1 shows the strength of gels formed under various concentrations of Ca^{2+} ion (0.48, 1.24 and 2.30 mg/ml). In LMNA, gel strength increased at all pectin concentrations in the presence of 1.24 and 2.30 mg/ml of Ca^{2+}, even though a gel was not formed at 0.48 mg/ml of Ca^{2+} (independent of the pectin concentration). In contrast, LMA did form a gel at 0.48 mg/ml Ca^{2+}. Strength of the LMA pectin gel increased in a Ca^{2+} concentration-dependent manner. Therefore, throughout this study, 2.30 mg/ml was used as the optimal Ca^{2+} concentration given its strength in forming a gel. Gel strength at this concentration was significantly higher with LMA than LMNA at any pectin concentration (Table 1). Taken together, results from this experiment demonstrate that the gel strengths of LMA and LMNA increased with increasing pectin concentration, although in every case, LMA was stronger than LMNA. Because an increased pectin concentration increased gel strength, amidation of pectin efficiently formed stronger gels.

3.2. Trapping effects of LMA and LMNA pectin gels

We examined the *in vivo* stability of the trapping effect of LMA and LMNA pectin gels at 37 °C (Fig. 1). When suspended in a 10-fold larger volume of water, the 0.5%, 1.0%, and 1.5% LMA pectin gels retained more than 75% of the spiked DON. In contrast, the DON retention abilities of LMNA pectin gels were lower than the LMA gels at all of the pectin concentrations. When suspended in a 50-fold larger volume of water, the LMA pectin gels still retained more than 50% of the spiked DON. In contrast, the LMNA pectin

Table 1
Gel strength of LMNA and LMA as a function of Ca^{2+} and pectin concentrations ($n = 4$).

Pectin (%)	Ca^{2+} (mg/ml)	Gel strength (pressure, N/m^2)	
		LMNA pectin gel	LMA pectin gel
0.5	0.48	n.d.	321.6 ± 8.4
	1.24	65.8 ± 1.8	363.1 ± 27.6
	2.30	104.0 ± 4.9	778.1 ± 38.4
1.0	0.48	n.d.	431.0 ± 13.3
	1.24	77.5 ± 1.8	713.4 ± 33.7
	2.30	151.8 ± 20.7	3225.1 ± 231.5
1.5	0.48	n.d.	380.0 ± 7.3
	1.24	78.6 ± 4.9	711.3 ± 13.3
	2.30	195.3 ± 3.7	8875.8 ± 368.0

Gel sample size was 50 ml, not including DON in the total volume.
n.d.: not determined

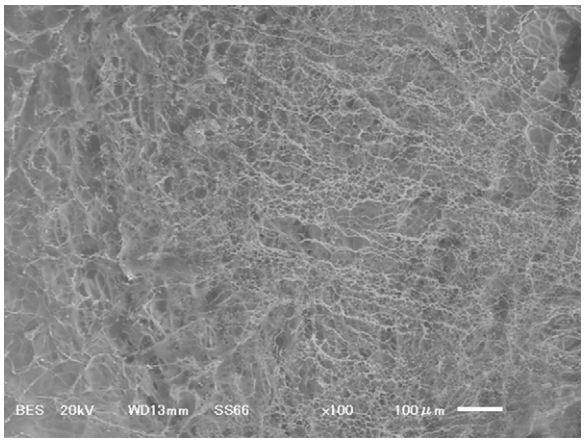

Fig. 2. Scanning electron micrographs of a LMA pectin gel cross section. A 1.0% LMA pectin gel was prepared according to the method described in the text. The gel was sectioned, freeze-dried and observed by SEM.

gels did not retain any substantial amount of DON in the gel. Fig. 2 shows the morphology as observed by SEM. The formation of intermolecular junction zones was noted, and the stitch structure took on an elaborate and uniform structure. When the gel is suspended in water, the micro network structure was observed to form.

3.3. Effect of gelation on the bioavailability of DON

Given our findings in the *in vitro* experiment that the trapping effect of LMA on DON was superior to that of LMNA, the *in vivo* experiment employed a 1% LMA pectin gel. To examine the effectiveness of the LMA pectin gel to reduce the absorption of DON, a single oral administration test was performed. The DON concentration in serum was measured by ELISA at various time intervals after its administration. In all groups except for the gel-cont group, DON was detected in plasma from 15 min post administration but was thereafter quickly eliminated, as shown in Table 2. In the pectin-DON group, two peaks were observed at 15 min and 8 h post administration, whereas in the gel-cont group, no DON was observed throughout the experimental period.

Parameters such as C_{max}, T_{max}, and AUC were compared among the gel-DON, pectin-DON, DON-PBS, and gel-cont groups. C_{max} and AUC values in the gel-DON group were approximately 25% of those in the pectin-DON and DON-PBS groups, and T_{max} in the gel-DON group was greater than in other groups. These results suggest that gelation with LMA suppresses and delays absorption of DON in the gastrointestinal tract. Further, C_{max}, T_{max}, and AUC values in the pectin-DON group were similar to those in the DON-PBS group (Table 2), indicating that liquid pectin does not suppress DON absorption.

Although the *in vivo* experiment revealed that LMA pectin gel has the ability to reduce the absorption of DON, the behaviors of the gel in the stomach and intestine were not clear. To examine the changes in retention abilities, the pectin gel was treated with gastric or intestinal juices.

Table 2
Bioavailability parameters of gel-DON, pectin-DON, DON-PBS, and gel-cont.

	C_{max} (ng/ml)[a]	T_{max} (min)[b]	AUC (ng h/ml)[c]
Gel-DON	460.7 ± 73.5*	30.0	1608.2
Pectin-DON	2070.3 ± 152.4	15.0	5540.2
DON-PBS	2532.0 ± 280.0	15.0	5509.6
Gel-cont	n.d	n.d	n.d

[a] C_{max}, maximum concentration.
[b] T_{max}, maximum time concentration.
[c] AUC, area under curve, which was calculated from a representative sample from each group. Values are expressed as average ± SE. $n = 5–7$. n.d: not detected. Pectin-DON, gel-DON, and DON-PBS were compared with Student's *t*-test.
* $P < 0.01$.

Table 3
The retention of DON in the LMA pectin gel under acidic conditions.

Treatment	Duration of treatment	DON retention (%)
Gastric juice (pH 1.3)[a]	0 min	100.0
	15 min	89.3 ± 2.4
	30 min	81.8 ± 5.0
	1 h	74.0 ± 4.6
	2 h	67.8 ± 3.7

[a] Artificial gastric juice (pH 1.3) was prepared according to the method described in the text.

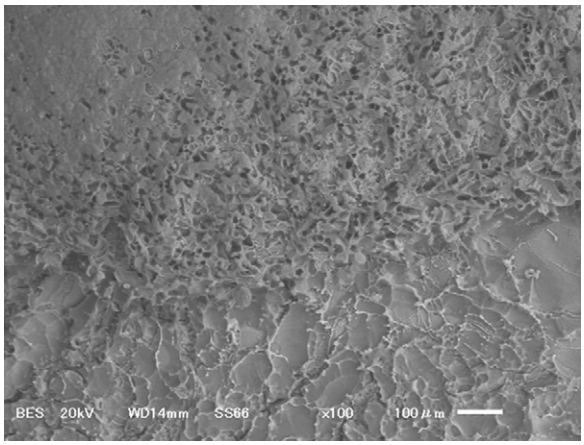

Fig. 3. Scanning electron micrographs of the cross section of the LMA pectin gel treated with gastric juice (pH 1.3). A 1.0% LMA pectin gel was treated with gastric juice for 1 h according to method described in the text. The gel was sectioned, freeze-dried and observed by SEM.

Table 3 shows the retention of DON in the LMA pectin gel under acidic conditions. Treatment with gastric juice under pH 1.3 led to DON leakage from the gel in a time-dependent manner. However, 2 h later, 67.8% of the DON was retained in the gel. A scanning electron micrograph of the gel treated with gastric juice for 1 h (Fig. 3), demonstrated that the acidic condition (pH 1.3) induced the breakage or aggregation of some of the egg box structure, suggesting that DON should leak from the interspace of the broken egg box structure. The breakdown of the micro network structure would occur following the replacement of Ca^{2+} to H^+, which bound the carboxylate groups of the galacturonic acid residues under acidic conditions. In contrast, under the alkaline condition (pH 8.1), the gel turned into "sol" immediately. All of the DON spiked in the gel was subsequently detected in the sol by ELISA (data not shown).

4. Discussion

Dietary fiber such as pectin is believed to enhance excretion (Garcia-Diez, Garcia-Mediavilla, Bayon, & Gonzalez-Gallego, 1996), and given its roles in reducing cholesterol, preventing colon cancer, and regulating blood glucose in humans, pectin represents a functional food as opposed to simply a nutrient (Brown, Rosner, Willet, & Sacks, 1999; Brennan, 2005; Truswell & Judo, 1985). At present, low-methoxyl pectin, in which calcium ions interact with the pectin to form an "egg box" is utilized as a material for trapping drugs in medical applications such as the colonic drug delivery system (Wakerly, Fell, Attwood, & Parkins, 1997; Ashford, Fell, Attwood, Sharma, & Woodhead, 1993; Wakerly, Fell, Attwood, & Parkins, 1996).

In the present study, we demonstrated that low-methoxyl pectin gel trapped mycotoxin DON and significantly reduced its

bioavailability. We initially examined the effects of low-methoxyl pectin gel on trapping of DON *in vitro* using LMA and LMNA. While increasing calcium ion and pectin concentrations increased gel strength of both LMA and LMNA pectin gels, the LMA pectin gel was the stronger of the two overall (Table 1). Although the LMA used in the present study has the same DE as LMNA, it additionally undergoes partial amidation of the galacturonic residues. The observed difference in gel strength between LMA and LMNA seems to therefore be due to the degree of amidation. The gelation model of non-amidated LM pectin has been reported to have ionic interactions between galacturonic acid residues in a calcium-dependent manner. The model of LMA pectin also has galacturonic acid residues and hydrogen bonding between amidated galacturonic acid residues, along with same interactions as LMNA (Voragen & Pilnik, 1995). Therefore, amidation plays an important role in increasing the gel strength.

In this experiment, the volume of a pectin gel product, such as a dessert jelly, was assumed to be 20–50 ml. The volume of the human stomach is reported to be 182 ml during fasting and about 700 ml postprandial (Burton et al., 2005; Weishaupt et al., 2007). Based on this, the effect of these pectin gels in trapping DON was measured by suspending them in 10- or 50-fold volumes of water. Our comparisons showed that the LMA gel trapped DON more efficiently than LMNA and we propose that this trapping effect was related to its greater gel strength. The LMA gel maintained its solute trapping ability in 10- and 50-fold water volumes. We noted no detectable difference in trapping when the pectin concentration was varied (Fig. 3). The experiment carried out at 37 °C revealed that the LMA pectin gel was stable in the gastrointestinal tract, therefore suggesting the potential applicability of LMA pectin gels for processing food and animal feed.

To investigate the bioavailability of DON trapped in the LMA pectin gel, we examined the plasma profile of DON after orally administering the gel to mice. On comparing the three groups containing DON (gel-DON, pectin-DON and DON-PBS), gelatin was the most effective at trapping and significantly suppressed the absorption of DON in the gastrointestinal tract (Table 2). The T_{max} value of the gel-DON group showed that the release of DON from the gel would be slower than from the pectin or PBS mixtures. Taken together, these results demonstrate that LMA pectin gel was a useful additive for reducing DON absorption.

As no suppressed absorption was observed in the group administered liquid LMA mixed with DON, gelation is obviously important for preventing absorption of DON. These results suggested that a gel containing DON would maintain its structure in the stomach, although the generally accepted theory is that the structure of the pectin gel is unstable in alkaline conditions and consequently degrades in the human large intestine (Holloway, Tasman-Jones, & Maher, 1983). Our results using artificial gastric and intestinal juices supported the notion that, under acidic conditions, the LMA pectin gel trapped DON in the gel even though there was a slow leak. However, under alkaline conditions, degradation of the gel occurred, and the DON trapped in the gel was released. We believe that the broad peak of DON released 8 h after the administration of pectin-DON may have been due to the degradation of the gel in the intestine. Since DON is absorbed quickly from stomach and the upper intestine (Danicke, Valenta, & Doll, 2004; Pestka & Amuzie, 2008), the stability of the pectin gel in the stomach would then suppress its release and absorption. Regarding the DON present in the gel, the results of DON ELISA performed on the gel after treatment with intestinal juice suggested that DON seemed to present as a free style in the egg box, because if DON interacted with LM pectin on the molecular level and formed a complex, it would not have been detected by the ELISA.

Taken together, our findings from the present study indicate that the low-methoxyl pectin gel trapped DON physically, and significantly suppressed its absorption in the gastrointestinal tract. And the suppression by the gelation would not affect the absorption of other essential nutrients contained in the gel by the intestine because the gel degraded in intestine. While further studies will be required to formulate applications for cereal or corn-based foods contaminated with DON, our findings reported here demonstrate the development of a new processing approach for reducing the gastro-intestinal absorption of DON on consumption of food and animal feed.

5. Conclusion

We successfully devised a method of trapping a mycotoxin using a low-methyl pectin gel and were able to demonstrate reduced toxin absorption by the gastrointestinal tract. Specifically, the LMA gel more efficiently trapped DON than did the LMNA gel under *in vivo*-like conditions. This finding was further supported by our experiments in mice, which demonstrated that trapping DON suppressed absorption in the gut. Our results here offer a new approach for food and feed processing to reduction absorption of DON from contaminated food and feed, and to our knowledge, this study is the first to report that pectin can be used effectively to reduce mycotoxin exposure.

Acknowledgments

We gratefully acknowledge the financial support provided by the Iijima Memorial Foundation for the Promotion of Food Science and Technology, the Japan Food Chemical Research Foundation and a grant from the Ministry of Education, Culture, Sports, Science and Technology, Japan. The authors thank Professor Shigeru Sawamura of Tokyo University of Agriculture for his excellent advice.

References

Ashford, M., Fell, J. T., Attwood, D., Sharma, H., & Woodhead, P. (1993). An evaluation of pectin as a carrier for drug targeting to the colon. *Journal of Controlled Release*, *26*, 213–220.

Asp, N. G. (1987). Dietary fibre – definition, chemistry and analytical determination. *Molecular Aspects of Medicine*, *9*, 17–29.

Baggot, J. D. (1970). Principle of drug disposition in domestic animals. In J. D. Baggot (Ed.), *The basis of veterinary clinical pharmacology* (pp. 22–189). Philadelphia: Saunders.

Bata, Á., & Lásztity, R. (1999). Detoxification of mycotoxin-contaminated food and feed by microorganisms. *Trends in Food Science & Technology*, *10*, 223–228.

Battilani, P., Barbano, C., Marin, S., Sanchis, V., Kozakiewicz, Z., & Magan, N. (2006). Mapping of *Aspergillus* section Nigri in Southern Europe and Israel based on geostatistical analysis. *International Journal of Food Microbiology*, *111*(Suppl. 1), S72–S82.

Bissessur, J. L., Permaul, K., & Odhav, B. (2001). Reduction of patulin during apple juice clarification. *Journal of Food Protection*, *64*, 1216–1219.

Brennan, C. S. (2005). Dietary fibre, glycaemic response, and diabetes. *Molecular Nutrition & Food Research*, *49*, 560–570.

Brown, L., Rosner, B., Willet, W. W., & Sacks, F. M. (1999). Cholesterol-lowering effects of dietary fiber: A meta-analysis. *American Journal of Clinical Nutrition*, *69*, 30–42.

Bryden, W. L. (2007). Mycotoxins in the food chain: Human health implications. *Asia Pacific Journal of Clinical Nutrition*, *16*(Suppl. 1), 95–101.

Burton, D. D., Kim, H. J., Camilleri, M., Stephens, D. A., Mullan, B. P., O'Connor, M. K., et al. (2005). Relationship of gastric emptying and volume changes after a solid meal in humans. *American Journal of Physiology – Gastrointestinal Liver Physiology*, *289*, G261–G266.

Cabrera, J. C., Boland, A., Messiaen, J., Cambier, P., & Van Cutsem, P. (2008). Egg box conformation of oligogalacturonides: The time-dependent stabilization of the elicitor-active conformation increases its biological activity. *Glycobiology*, *18*, 473–482.

Capel, F., Nicolai, T., & Durand, D. (2005). Influence of chain length and polymer concentration on the gelation of (amidated) low-methoxyl pectin induced by calcium. *Biomacromolecules*, *6*, 2954–2960.

Chambin, O., Dupuis, G., Champion, D., Voilley, A., & Pourcelot, Y. (2006). Colon-specific drug delivery: Influence of solution reticulation properties upon pectin beads performance. *International Journal of Pharmaceutics*, *321*, 86–93.

Codex Alimentarius Commission. (2011). www.codexalimentarius. net/download/standards/56/CXS_171e.pdf

Danicke, S., Valenta, H., & Doll, S. (2004). On the toxicokinetics and the metabolism of deoxynivalenol (DON) in the pig. *Archives of Animal Nutrition*, *58*, 169–180.

Evans, M. A., & Shronts, E. P. (1992). Intestinal fuels: Glutamine, short-chain fatty acids and dietary fiber. *Journal of the American Dietetic Association*, *92*, 1239–1249.

Garcia-Diez, F., Garcia-Mediavilla, V., Bayon, J. E., & Gonzalez-Gallego, J. (1996). Pectin feeding influences fecal bile acid excretion, hepatic bile acid and cholesterol synthesis and serum cholesterol in rats. *Journal of Nutrition*, *126*, 1766–1771.

Humpf, H. U., & Voss, K. A. (2004). Effects of thermal food processing on the chemical structure and toxicity of fumonisin mycotoxins. *Molecular Nutrition and Food Research*, *48*, 255–269.

Holloway, W. D., Tasman-Jones, C., & Maher, K. (1983). Pectin digestion in humans. *The American Journal of Clinical Nutrition*, *37*, 253–255.

Karaca, H., Velioglu, Y., & Nas, S. (2010). Mycotoxins: Contamination of dried fruits and degradation by ozone. *Toxin Reviews*, *29*, 51–59.

Kimura, M., Takahashi-Ando, N., Nishiuchi, T., Ohsato, S., Tokai, T., Ochiai, N., et al. (2006). Molecular biology and biotechnology for reduction of Fusarium mycotoxin contamination. *Pesticide Biochemistry and Physiology*, *86*, 117–123.

Klaassen, C. D. (1986). Distribution, excretion, and absorption of toxicants. In C. C. Klaassen, M. O. Amdur, & J. Doull (Eds.), *The basic science of poisons* (3rd ed., pp. 33–66). New York: Macmillan.

Kuiper-Goodman, T. (1995). Mycotoxins: Risk assessment and legislation. *Toxicology Letters*, *82-83*, 853–859.

Maragos, C., Busman, M., & Sugita-Konishi, Y. (2006). Production and characterization of a monoclonal antibody that cross-reacts with the mycotoxins nivalenol and 4-deoxynivalenol. *Food Additives and Contaminants*, *23*, 816–825.

Mohnen, D. (2008). Pectin structure and biosynthesis. *Current Opinion in Plant Biology*, *11*, 266–277.

Pestka, J. J., & Amuzie, C. J. (2008). Tissue distribution and proinflammatory cytokine gene expression following acute oral exposure to deoxynivalenol: Comparison of weanling and adult mice. *Food and Chemical Toxicology*, *46*, 2826–2831.

Sherif, S. O., Salama, E. E., & Abdel-Wahhab, M. A. (2009). Mycotoxins and child health: The need for health risk assessment. *International Journal of Hygiene and Environmental Health*, *212*, 347–368.

Truswell, A. S., & Judo, P. A. (1985). The hypocholesterolaemic effects of pectins in rats. *British Journal of Nutrition*, *53*, 409–425.

Van Soest, P. J., Robertson, J. B., & Lewis, B. A. (1991). Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *Journal of Dairy Science*, *74*, 3583–3597.

Versantvoort, C. H. M., Oomen, A. G., Kamp, E. V., Rompelberg, C. J. M., & Sips, A. J. A. M. (2005). Applicability of an in vitro digestion model in assessing the bioaccessibility of mycotoxins from food. *Food and Chemical Toxicology*, *43*, 31–40.

Voragen, A. G. J., & Pilnik, W. (1995). Pectin. In A. M. Atephen (Ed.), *Food Polysaccharides and Their applications* (pp. 287–339). New York: Marcel Dekker, Inc.

Wakerly, Z., Fell, J. T., Attwood, D., & Parkins, A. (1996). In vitro evaluation of pectin-based colonic drug delivery systems. *International Journal of Pharmaceutics*, *129*, 73–77.

Wakerly, Z., Fell, J., Attwood, D., & Parkins, D. (1997). Studies on amidated pectins as potential carriers in colonic drug delivery. *Journal of Pharmacy and Pharmacology*, *49*, 622–625.

Weishaupt, M., Thumshirn, M., Fried, W., Schwizer, O., Goetze, A., Steingoetter, D., et al. (2007). The effect of macronutrients on gastric volume responses and gastric emptying in humans: A magnetic resonance imaging study. *American Journal of Physiology – Gastrointestinal and Liver Physiology*, *292*, G11–G17.