Research Article

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Porcine *in vitro* digestion and matrix structure of undigested residue of xylanase- and cellulase-supplemented maize and wheat

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Abstract

Background: This study investigated the effects of supplementing maize and wheat with a combination of xylanase and cellulase on porcine *in vitro* digestibility, nonstarch polysaccharides (NSP) solubilization, and matrix structure. The latter was assessed using synchrotron-based micro-computed tomography (SR-μCT) and Fourier transform mid-infrared (FTMIR) spectroscopy after porcine *in vitro* degradation. Cereal grains were subjected to porcine *in vitro* digestion with or without enzyme supplementation (20 000 U kg^{-1} of each enzyme) in a 2 × 2 factorial design.

Results: *In vitro* dry matter digestibility (IVDDM) was lower for maize than for wheat ($P < 0.05$), and enzyme supplementation had no effect on IVDDM. Supplemental enzymes reduced arabinoxylan content in the undigested residue of wheat (53 vs 46 g kg^{-1}, $P < 0.05$) but not maize (42 vs 44 g kg^{-1}; $P > 0.05$). Synchrotron-based micro-computed tomography imaging revealed aleurone cells with intact content in the undigested residue of both maize and wheat, regardless of enzyme supplementation. Some endosperm cells in undigested maize residue retained their content, whereas nearly all endosperm cells in undigested enzyme-unsupplemented wheat residue were empty. No endosperm cells were detected in undigested enzyme-supplemented wheat residue. Fourier transform mid-infrared imaging indicated a higher presence of phenolic compounds in maize cell walls than in wheat.

Conclusion: Supplemental enzymes did not affect IVDDM for wheat, as they primarily degraded endosperm cell walls, the content of which was already released by pepsin and pancreatin digestion. Similarly, IVDDM for maize remained unaffected, which was probably due to the high phenolic content of its cell walls.

Keywords: maize; wheat; digestibility; non-starch polysaccharides-degrading enzymes; non-starch polysaccharides solubilization; matrix structure

INTRODUCTION

Arabinoxylan (AX) and cellulose are major nonstarch polysaccharides (NSPs) in maize and wheat, and hence the supplementation of maize and wheat-based diets for pigs with xylanase and cellulase, which target AX and cellulose,[1] respectively, is expected to improve nutrient utilization in pigs. However, adding xylanase and cellulase to maize- and wheat-based diets has shown variable effects on nutrient utilization in pigs.[2,3] Xylanase and cellulase supplementation has generally been associated with limited improvement in nutrient availability in the wheat-based diets, but not in maize-based diets.

The arrangement of the NSPs within the cell wall is such that the cellulose is surrounded by the AX.[4] Arabinoxylan consists of a linear xylose backbone, with some xylose units either remaining unmodified or with arabinofuranose substitutions.[5] Ferulic acid is esterified to arabinofuranosyl residues.[6] Ferulic acid acts as a cross-linking agent between polysaccharides or between polysaccharides and lignin.[7] Cross-linkages of AX to lignin and other cell wall components via arabinofuranose and ferulic acid may thus hinder the efficacy of xylanase and cellulase. However, recent observations have indicated limited effects of the addition of debranching enzymes (arabinofuranosidase and feruloyl esterase) to xylanase-supplemented wheat and maize on the porcine *in vitro* digestibility of these cereal grains.[8] Thus, there is still a need to discover what limits the efficacy of xylanase and cellulase with regard to the degradation of NSP in the maize and

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wheat. Determination of the matrix structure of these cereal grains before and after degradation with these two enzymes as well as an evaluation of their impact on NSP degradation, may help identify additional enzymes or combinations that could improve NSP digestibility in wheat and maize.

Microscopic techniques, including synchrotron-based micro-computed tomography (SR-μCT) and mid-infrared (MI) microscopy, can show the detailed matrix structure of plant tissue samples because: (1) synchrotron radiation-based microscopic equipment generates images that have higher resolution than those that are generated by ordinary microscopic equipment[9] and (2) the SR-μCT technique provides detailed three-dimensional (3D) microscopic structural information for the samples,[10] whereas the MI microscopy technique can be used to determine the quantity and spatial distribution of various components of plant tissues.[11] However, information is lacking regarding the effects of supplementing maize and wheat with xylanase and cellulase on the matrix structure, as determined by SR-μCT and MI microscopy, following digestion in the porcine gastrointestinal tract. The objectives of this study were to establish why a combination of xylanase and cellulase has limited effects on the porcine *in vitro* digestibility of nutrients and NSP solubilization in the maize and wheat, and to evaluate the effect of xylanase and cellulase on the matrix structure of these cereal grains using SR-μCT and MI microscopy after *in vitro* degradation.

MATERIALS AND METHODS

Feed and enzymes

Maize and wheat were sourced in Europe and were ground in a centrifugal mill (model ZM200; Retsch GmbH, Haan, Germany) using a 0.5 mm screen. The enzyme products (1000 U g^{-1} xylanase and 1000 U g^{-1} cellulase) used in this study were sourced from BIO-CAT (Troy, VA, USA). Cereal grains were supplemented with a combination of the two enzymes in a 2 × 2 factorial arrangement. The enzymes were added to the cereal grains to supply 20 000 U of xylanase and 20 000 U of cellulase per kilogram of cereal grain. The unsupplemented and supplemented cereal grains were subjected to porcine *in vitro* digestion.

Porcine *in vitro* digestion

The *in vitro* digestion method used in this study was originally developed by Boisen and Fernández[12] and modified by Jaworski *et al.*[13] and Vangsøe *et al.*[14] (Fig. 1). The experimental scheme was four treatments × three replicates per treatment. The experimental design was completely randomized with a 60 mL flat-bottomed tube used as an experimental unit. Based on the *in vitro* digestibility of dry matter (IVDDM) data from a previous study in our laboratory,[15] the number of replicates per treatment chosen for this study was three.

Total carbohydrate analysis

All samples were analyzed for dry matter (AOAC method 930.15[16]), starch,[17] crude protein (AOAC method 990.03[16]) and NSP.[17] Cereal grains were also analyzed for Klason lignin.[17] The insoluble residue and soluble flow through were analyzed for total carbohydrates (poly-, oligo-, and monosaccharides) and neutral sugar residues of NSP as described by Bach Knudsen.[17]

Microscopic analysis

Synchrotron based micro-computed tomography

X-ray imaging of the cereal grains and their undigested residues was conducted at the Biomedical Imaging and Therapy Bending Magnet (BMIT-BM 05B1-1) beamline of the Canadian Light Source Synchrotron (Saskatoon, SK, Canada). Samples were loaded into a

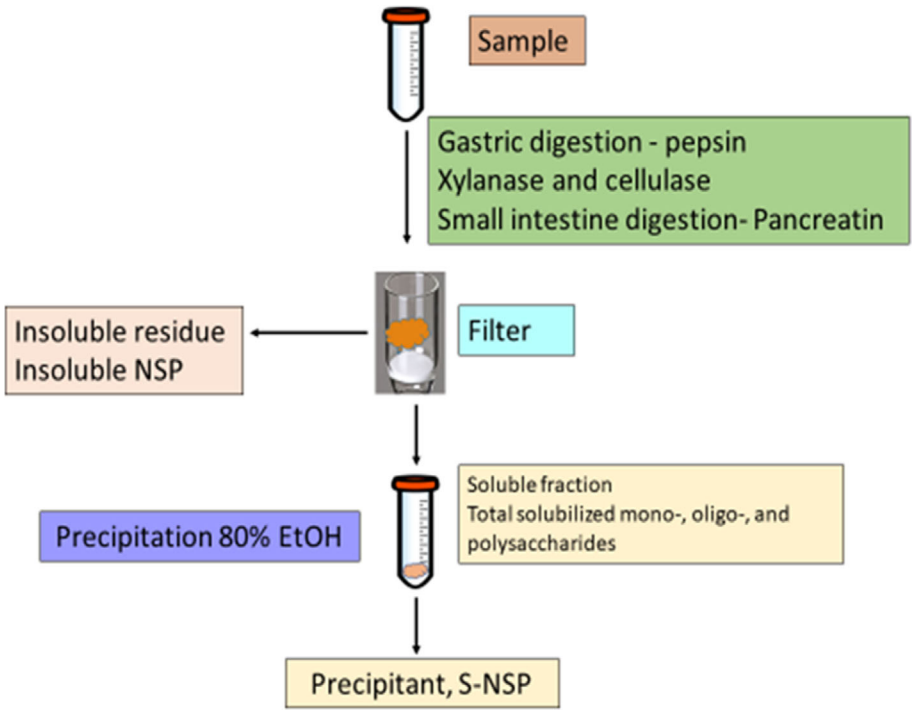

Figure 1. Schematic presentation of the *in vitro* procedure for the determination of *in vitro* digestible dry matter and carbohydrates in insoluble and soluble fractions.

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1.5 mL centrifuge tube and mounted on a manual goniometer head (model 1002; Huber GmbH, Rimsting, Germany) with dental wax. Twenty flat images (no sample in beam path) and 20 dark images (no beam on the detector) were acquired in order to remove fixed-pattern noise and normalize the intensity of the X-ray images. Sets of 2000 projection images (sample in beam path) per sample were collected through a 180° rotation of the sample. All images were collected at an exposure time of 2 ms. Image processing and 3D reconstructions were performed as described by Willick *et al.*[18] Data were phase retrieved, filtered, and reconstructed using EZ-TOFU software, following the methods of Faragó *et al.*[19] and Vogelgesang *et al.*,[20] with the following parameters: large spot removal (threshold = 1000, sigma = 2); phase retrieval (energy = 25.5 keV, pixel size = 0.72 µm, sample-detector distance = 0.01 m, delta/beta ratio = 200); ring removal (2D stripe filter, sigma horizontal = 3, sigma vertical = 2); and histogram clipping (16-bit, minimum value in 32-bit histogram = 3.489e-15, maximum value = 1.5e-15). Reconstructed images were opened, volume-rendered to generate 3D images from 2D stacks, segmented, and analyzed using Avizo 2020.2 (Thermo Scientific Amira-Avizo, Thermo Fisher Scientific, Waltham, MA, USA). Simple thresholding was used to segment the solid material from the air. Using this segmentation as a mask, voids within the material were identified and analyzed to determine porosity. Undigested maize residue was selected for the generation of 3D volume rendering images.

Fourier transform mid-infrared spectroscopy

Fourier transform mid-infrared (FTMIR) spectroscopy of the cereal grains and undigested residue after *in vitro* digestion with or without the supplemental enzyme was carried out at the MI beamline provided by the Canadian Light Source Inc. Samples were compacted into embedding capsule (BEEM #1001, Ted Tella, Redding, CA, USA) and mixed with Millipore water before flash freezing in liquid nitrogen. Then, 8 µm thick cross-sections were cut with Leica Rotary Microtome Cryostat and transferred onto BaF2 windows (size 13 × 1 mm disk, Crystran Ltd, Poole, UK) and dehydrated overnight at room temperature. Infrared maps of whole seed/nodule sections with 5.5 µm pixel resolution were obtained using the FTIR spectrometer (Agilent Technologies, Santa Clara, CA, USA) with a Cary 620 Microscope (128 × 128 pixel Focal Plane Array detector) equipped with a 25× objective. The spectra were collected in the MI range of 4000–800 cm^{-1} at a resolution of 4 cm^{-1}. The FT-MIR data analysis was performed using Quasar[21] software (version 0.5.6) (https://doi.org/10.5281/zen-odo.4287478). The MI imaging data was water vapor corrected and, baseline corrected using a rubber band correction algorithm and visualized with the baseline integration of selected MI peaks.

CALCULATIONS

The IVDDM was calculated as below:

$$\text{IVDDM} = 1 - \frac{\text{undigested residue (mg)}}{\text{sample (mg)}}$$

where the undigested residue' is the insoluble residue left after digestion and 'sample' is the original sample weight before digestion.

In vitro digestibility of starch (IVD-starch) and *in vitro* digestibility of crude protein (IVD-CP) digestibility was calculated as below:

$$\text{IVD-starch or IVD-CP} = 1 - \frac{A \times B}{C \times D}$$

where A is weight of the insoluble residue sample, B is the percentage of nutrient in the insoluble residue sample, C is the weight of the cereal grain before digestion, D is the percentage of nutrient in the cereal grain before digestion.

Cellulose and dietary fiber were calculated as described by Bach Knudsen:[17]

$$\text{cellulose} = NSP_{\text{glucose}} - NCP_{\text{glucose}}$$

$$\text{dietary fiber} = NSP_{\text{total}} + \text{klason lignin}$$

where NSP_{glucose} is glucose content in the *NSP* (it includes glucose from cellulose), NCP_{glucose} is glucose content in the non-cellulosic polysaccharides (*NCP*; it excludes glucose from the cellulose), and NSP_{total} is total *NSP*.

Arabinoxylan oligosaccharides (AXOS) in the soluble flow-through were calculated according to Lærke *et al.*[22] as:

$$\text{AXOS} = (\text{arabinose} + \text{xylose})_{\text{total CHO}} - (\text{arabinose} + \text{xylose})_{\text{HMW-AX}}$$

where arabinose + $\text{xylose}_{\text{total CHO}}$ are the total AX in the soluble flow-through after acid hydrolysis and without ethanol precipitation, whereas arabinose + $\text{xylose}_{\text{HMW-AX}}$ are the high molecular weight soluble AX in the soluble flow-through after 80% ethanol precipitation followed by the acid hydrolysis.

Porosity was calculated using the following equation:

$$\text{porosity (\%)} = \frac{\text{volume of the voids within cells}}{\text{total volume of cells}} \times 100$$

Statistical analysis

The NSP composition, *in vitro* digestibility, and porosity were statistically analyzed with JMP 15 Pro (SAS Institute Inc., Cary, NC, USA) using a two-way analysis of variance (ANOVA) in a completely randomized design. Treatment means were separated by the Tukey honestly significant difference (HSD) test when the interactions between cereal grain and supplemental enzymes were significant.

RESULTS

Composition of cereal grains

Arabinose and xylose, and hence AX, comprised the predominant NSP in maize and wheat (Table 1). Wheat contained more T-NSP than maize. However, maize had a greater insoluble NSP (relative to total NSP) than wheat. The A:X ratio was also greater in maize than in wheat.

Digestion of the cereal grains

Cereal grain type and enzyme supplementation did not interact on IVDDM, IVD-starch, or IVD-CP (Table 2). Maize exhibited lower ($P < 0.05$) values for IVDDM, IVD-starch, and IVD-CP than wheat, whereas enzyme supplementation had no effect on these response criteria. Enzyme supplementation reduced ($P < 0.05$) arabinose and xylose in the insoluble residue of wheat but not of maize (Table 3). The insoluble fraction of wheat had higher ($P < 0.05$) AX content and lower ($P < 0.05$) T-NSP content than that of maize. Supplemental enzymes increased ($P < 0.05$) AXOS and decreased high molecular weight arabinoxylan (HMW-AX)

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Table 1. Composition of feedstuffs (g kg^{-1} dry matter).

Item	Maize		Wheat	
Starch	729		689	
Crude protein	69.7		98.7	
Non-cellulosic polysaccharides	57.5	(11.8)[a]	88.6	(37.1)
Arabinose	17.5	(2.85)	25.7	(8.88)
Xylose	23.9	(3.49)	39.4	(13.2)
Mannose	1.55	(0.655)	2.76	(1.36)
Galactose	4.57	(2.97)	3.57	(2.90)
Glucose	5.37	(2.93)	11.1	(5.98)
Uronic acids	5.05	(0.802)	6.33	(4.77)
Arabinoxylan	41.4	(6.34)	65.1	(22.1)
Arabinose:xylose	0.755	0.816	0.652	0.672
Cellulose	18.0		17.4	
Total non-starch polysaccharides	75.4		106	
Klason lignin	4.70		11.1	
Dietary fiber	80.1		117	

[a] Values in parentheses are soluble components.

Table 2. Effects of enzyme supplementation on coefficient of *in vitro* digestibility of feedstuffs

Item[†]	IVDDM	IVD-starch	IVD-CP
Maize			
Enzyme (–)	0.799	0.937	0.755
Enzyme (+)	0.814	0.952	0.767
Wheat			
Enzyme (–)	0.894	0.996	0.886
Enzyme (+)	0.902	0.998	0.895
Main effects of feedstuffs			
Maize	0.807[b]	0.944[b]	0.760[b]
Wheat	0.898[a]	0.997[a]	0.892[a]
Main effects of enzymes			
Enzyme (–)	0.847	0.967	0.820
Enzyme (+)	0.858	0.975	0.832
SEM	0.005	0.010	0.007
P-value			
Cereal grain	<0.001	<0.001	<0.001
Enzyme	0.125	0.360	0.138
Cereal grain×enzyme	0.603	0.499	0.839

[†] IVDDM = *in vitro* digestibility of dry matter; IVD-starch = *in vitro* digestibility of starch; IVD-CP = *in vitro* digestibility of crude protein.

content in the soluble fraction of wheat but had no effect in maize (Table 4). The AX content in the soluble fraction was higher ($P < 0.05$) in wheat than in maize.

Microscopy analysis

Micro-computed tomography

Cereal grain and supplemental enzymes did not interact on the porosity of undigested residue (Fig. 2). The porosity of undigested residue for wheat was greater ($P < 0.05$) than that for maize. Supplemental enzymes did not affect porosity. From the SR-μCT images, the starch granules in maize were more closely packed together than those of wheat (Fig. 3(A,D)). The fragments of the aleurone layer attached to the endosperm were detected in undigested residue obtained from maize after *in vitro* digestion without enzyme (Fig. 3(B)) or with supplemental enzymes (Fig. 3(C)). Some endosperm and aleurone layer cells were empty, whereas others had their contents for undigested residue from maize, with or without supplemental enzymes. Fragments of aleurone layer attached to the pericarp, along with endosperm fragments, were observed for undigested residue from wheat without supplemental enzyme (Fig. 3(E)). Some aleurone cells retained their content but almost all endosperm cells lacked content for undigested residue of wheat without supplemental enzymes. In the image of undigested wheat residue with supplemental enzymes (Fig. 3(F)), endosperm fragments were absent, but aleurone layer fragments remained attached to the pericarp. Most aleurone cells lacked contents. Three-dimensional volume rendering images of undigested maize residue (Fig. 4) showed pericarp and endosperm fragments attached to aleurone fragments.

Fourier transform mid-infrared. In maize and wheat grain (before *in vitro* digestion), the MI images (Fig. 5 MA-i and 5 WA-i) show three tissue types consisting of starch granules embedded in protein matrix and cellulose/hemicellulose ribbons (Fig. 5 MA-i and 5 WA-i). After digestion, protein was absent for both grains. In the undigested residue for maize, with or without supplemental enzymes, starch-protein complexes associated with low quantity of hemicellulose were detected (Fig. 5MB-i and MC-i), but their proportions decreased and they appear more fragmented. Cellulose and hemicellulose ribbons associated with lignin or phenolic compounds remained and were the dominant components of the residues (Fig. 5 MB-ii and MC-ii). In contrast to maize, no starch-proteins were detected in the undigested residue for wheat without supplemental enzymes (Fig. 5 WB-i). Some protein that was associated with hemicellulose was detected in the undigested residue of wheat without supplemental enzymes (Fig. 5 WC-i) and none was detected in the residue with supplemental enzymes (Fig. 5 WC-II). Notably, less hemicellulose was detected in comparison with cellulose in the undigested residue of wheat with supplemental enzymes than in the undigested residue of wheat without supplemental enzymes (Fig. 5 WB-ii *vs*. Fig. 5 WB-iii).

DISCUSSION

The NSP composition and lignin content of the maize and wheat were similar to those reported by Bach Knudsen[23] and Jha and Berrocoso[24] for the same cereal grains. Maize had a different AX structure, as indicated by a greater proportion of arabinose (A) relative to xylose (X) compared to wheat. The greater A:X ratio in maize than in wheat was due to a higher substitution of AX in maize and higher levels of ferulic acids in maize than in wheat.[14,25]

Maize had a lower IVDDM than wheat, partly due to its lower IVD-starch and IVD-CP. Synchrotron-based micro-computed tomography images revealed that some endosperm cells in the undigested residue of enzyme-unsupplemented maize retained their contents, whereas nearly all endosperm cells in the undigested residue of enzyme-unsupplemented wheat were empty. This finding aligns with MI imaging, which showed that the undigested residue of maize contained some starch, whereas that of wheat did not, consistent with starch being localized in the endosperm cells of cereal grains. Maize grain contains two types of

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Table 3. The NSP composition of the insoluble residues (g kg^{-1}) after *in vitro* digestion

Item[†]	Arabinose	Xylose	Mannose	Galactose	Glucose	Arabinoxylans	Arabinose:xylose	T-NSP
Maize								
Enzyme (–)	17.8^{ab}	24.2^{c}	1.13	4.67	133	42.0^{b}	0.736	180
Enzyme (+)	18.8^{ab}	25.9^{bc}	1.00	5.07	114	44.8^{b}	0.726	165
Wheat								
Enzyme (–)	19.8^{a}	33.3^{a}	1.17	1.63	27.7	53.1^{a}	0.593	83.4
Enzyme (+)	17.4^{b}	28.5^{b}	1.07	1.57	27.3	45.9^{b}	0.610	76.0
Main effects of feedstuffs								
Maize	18.3	25.0^{b}	1.67	4.87^{a}	123^{a}	49.5^{a}	0.731^{a}	172^{a}
Wheat	18.6	30.9^{a}	1.12	1.60^{b}	27.5^{b}	43.4^{b}	0.602^{b}	79.7^{b}
Main effects of enzymes								
Enzyme (–)	18.8	28.7	1.03	3.15	80.4	47.5	0.665	132
Enzyme (+)	1.82	27.2	1.15	3.32	70.8	45.4	0.668	121
SEM	0.462	0.842	0.074	0.128	5.34	1.30	0.005	6.35
P-value								
Cereals	0.586	<0.001	0.424	<0.001	<0.001	0.002	<0.001	<0.001
Enzyme	0.188	0.108	0.137	0.240	0.109	0.131	0.597	0.107
C × E[‡]	0.007	0.005	0.945	0.159	0.121	0.005	0.232	0.537

[†] T-NSP = total non-starch polysaccharides.
[‡] Interaction between cereal grain type and supplemental enzymes.

Table 4. Composition of soluble non-glucose carbohydrates after *in vitro* digestion (g kg^{-1})

Item[†]	Arabinose	Xylose	Mannose	Galactose	Arabinoxylans	Arabinose: xylose	HMW-AX	AXOS	T-NGC
Maize									
Enzyme (–)	1.87	0.77	3.30^{b}	9.83^{c}	2.63	2.58	0.04^{b}	2.59^{b}	15.8
Enzyme (+)	3.23	1.30	5.67^{ab}	8.90^{d}	4.53	2.46	0.21^{b}	4.32^{b}	19.1
Wheat									
Enzyme (–)	7.27	7.87	8.50^{a}	11.5^{b}	15.1	0.93	11.0^{a}	4.13^{b}	35.1
Enzyme (+)	7.87	11.2	6.50^{ab}	15.0^{a}	19.1	0.70	0.27^{b}	18.8^{a}	40.6
Main effects of feedstuffs									
Maize	2.55^{b}	1.03^{b}	4.48^{b}	9.37^{b}	3.58^{b}	2.52^{a}	5.63^{a}	3.46^{b}	17.4^{b}
Wheat	9.53^{a}	9.53^{a}	7.50^{a}	13.3^{a}	17.1^{a}	0.81^{b}	0.13^{b}	11.5^{a}	37.9^{a}
Main effects of enzymes									
Enzyme (–)	4.57	4.32	5.90	10.7^{b}	8.87	1.69	5.52^{a}	3.36^{b}	25.4
Enzyme (+)	5.55	6.25	6.08	11.9^{a}	11.8	1.64	0.24^{b}	11.6^{a}	29.6
SEM	1.148	1.487	0.886	0.196	2.618	0.173	0.213	2.659	3.554
P-value									
Cereals	0.002	<0.001	0.009	<0.001	<0.001	<0.001	<0.001	0.017	<0.001
Enzymes	0.421	0.224	0.849	<0.001	0.295	0.762	<0.001	0.015	0.248
C × E[‡]	0.749	0.369	0.040	<0.001	0.703	0.336	<0.001	0.041	0.767

[†] HMW-AX = high molecular weight arabinoxylans; AXOS = arabinoxylans oligosaccharides; T-NGC = total non-glucose carbohydrates.
[‡] Interaction between cereal grain type and supplemental enzymes.

endosperm: floury and hard.[23] The floury endosperm is located at the center of the grain, whereas the hard endosperm is located on the outer parts of the grain, and hence it is associated with the aleurone layer. The starch granules of the floury endosperm are loosely packed together, whereas the starch granules of the hard endosperm are closely packed together.[26] The fact that maize had lower IVD-starch and IVD-CP than wheat could have been due to incomplete *in vitro* digestion of starch and protein in cells of the hard endosperm of maize. In maize, hard endosperm starch is more intensely associated with protein and has a greater proportion of amylose than the floury endosperm, which results in greater compressibility of the starch granules in the former than in the latter.[27] The compactness and close arrangement of starch granules in the hard endosperm may make it less susceptible to pepsin and pancreatin digestion, resulting in lower IVD-starch and IVD-CP for hard endosperm than for floury endosperm. Indeed, the SR-μCT images revealed that the endosperm cells in the undigested maize residue retained their content, which was associated with the aleurone layer, indicating that the undigested starch originated from the hard endosperm. The MI images also showed that some proteins in the undigested residue of maize were associated with hemicellulose and others were associated

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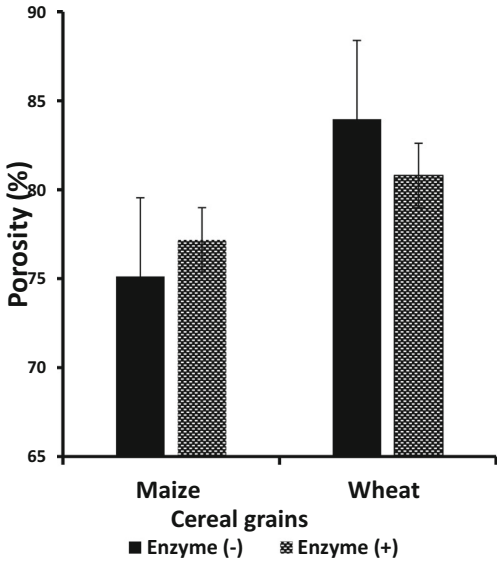

Means of main effects of feedstuffs, %	
Maize	76.2[b]
Wheat	82.4[a]
Means of main effects of enzymes, %	
Enzymes (-)	79.5
Enzymes (+)	79.0
SEM	1.81
P-value	
Cereal grain	0.003
Enzyme	0.761
C × E [†]	0.168

Figure 2. Porosity of the insoluble residue after *in vitro* digestion as visualized under synchrotron based micro-CT spectroscopy. [†]*C* × *E* = Interaction of cereal grain and supplemental enzymes.

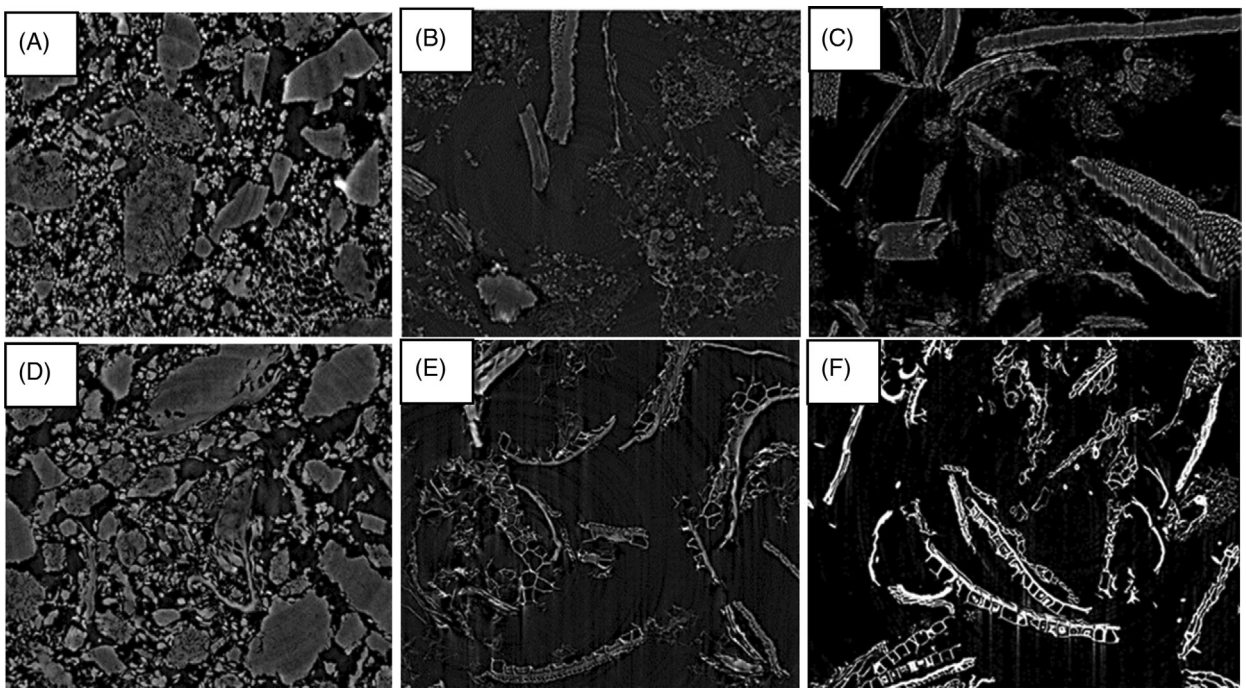

Figure 3. Synchrotron-based micro-CT images of (A) ground maize before *in vitro* digestion, (B) maize insoluble residues after digestion without supplemental enzymes, (C) maize insoluble residues after digestion with supplemental enzymes, (D) ground wheat before *in vitro* digestion, (E) wheat insoluble residues after digestion without supplemental enzymes, and (F) wheat insoluble residues after digestion with supplemental enzymes.

with starch, unlike in the undigested residue of wheat where some proteins were associated with hemicellulose but not with starch. The proteins that were associated with starch were mainly from the endosperm, whereas the proteins that were not associated with the starch were mainly from the aleurone layer.

Porosity is a measure of the volume of voids within the cells relative to the total volume of the cells in the undigested residue. It can therefore be considered to reflect the digestibility of the nutrients within the cells. The greater porosity of the undigested wheat residue in comparison with maize was due to the higher IVDDM in wheat. This is further supported by the SR-μCT images, which showed that most endosperm cells in the undigested maize residue retained their content, whereas nearly all endosperm cells in the undigested wheat residue lacked content. Only a few of the aleurone cells in the undigested residue of wheat retained their content, unlike in maize where most of the aleurone cells had their content. The nutrients within the cells of the endosperm were more susceptible to enzymatic digestion than the nutrients in the cells of the aleurone layer and pericarp.[28] Due to greater bioaccessibility for enzymatic digestion of nutrients in the cells

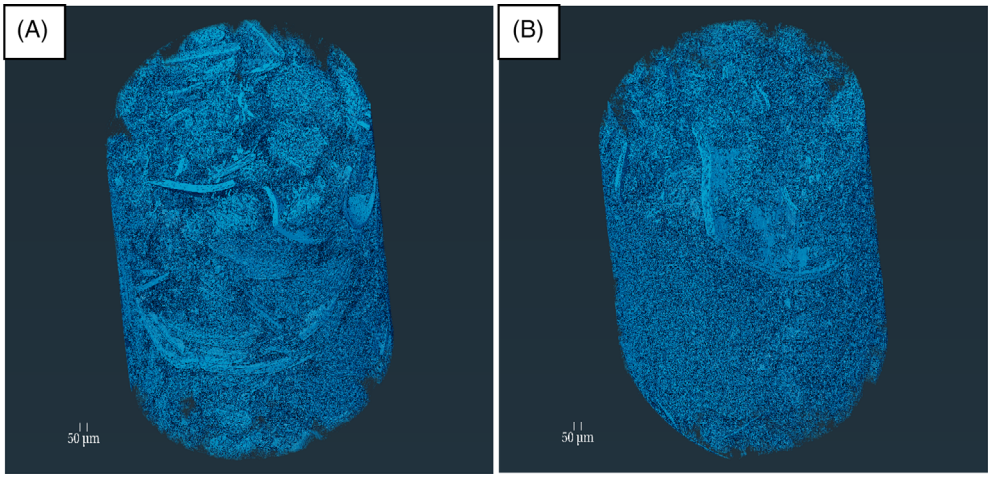

Figure 4. Three-dimensional volume rendering images (generated using synchrotron based micro CT) of maize insoluble residues after digestion without (A) or with (B) supplemental enzymes.

of endosperm than in the aleurone layer and pericarp, more nutrients are likely to be digested in the endosperm resulting in greater porosity of the cells in the endosperm than in the aleurone layer and the pericarp.

In cereal grains, the complexity of the cell wall increases from the core towards the periphery; the cell walls in the endosperm are thin, single layered and highly soluble in comparison with those in the aleurone layer and pericarp.[29] Thus, the degradation of the NSP by the supplemental enzymes in the endosperm is greater than that in the aleurone layer and outer layer.[30] Extensive degradation of some of the cell walls (especially the cell walls in the endosperm) surrounding the porous cells due to the supplemental enzymes can result in the complete disintegration of the cell wall into lower molecular weight fragments. These lower molecular weight fragments are not recovered in the insoluble residue, resulting in an undigested insoluble residue that is rich in aleurone layer and pericarp fragments, hence the low number of voids. Thus, in the current study, the reduction in porosity of insoluble residues of cereal grains due to supplemental enzymes can be attributed to the fact that the supplemental enzymes degraded more NSP in the endosperm cell walls than in the aleurone layer and pericarp cell wall. The degradation of more NSP in the endosperm cell walls than in the elaurone layer and pericarp cell walls by the supplemental enzymes resulted in a lower content of porous endosperm cells than cells of the aleurone layer and pericarp of the cereal grains after the pepsin and pancreatin digestion.

From the wet chemistry analysis, the extent to which the supplemental enzymes solubilized the AX in the insoluble residue for wheat was greater than the extent to which the supplemental enzymes solubilized the AX for maize. The NSP solubilization in the cereal grain was monitored by a reduction of NSP in the insoluble residue due to enzyme supplementation. Although it was not significant, there was a decrease in levels of AX in the insoluble residue of wheat after *in vitro* digestion relative to the amount of AX in the wheat grain before the *in vitro* digestion, but not in maize. The SR-µCT images of the undigested residue of enzyme-supplemented maize illustrated that the fragments of the cell wall from the endosperm, aleurone, and outer layer remained after *in vitro* digestion, whereas in the undigested residue of enzyme-supplemented wheat, the cell walls that remained were from the aleurone layer and outer layer. The MI images illustrated that some of the hemicelluloses in the enzyme-supplemented undigested residue for maize were associated with the starch, which confirmed that they were from the endosperm cell wall. This implies that the supplemental enzymes were capable of solubilizing the endosperm cell wall in wheat into digestible sugars, but not in maize, thus improving the solubilization of NSP for wheat. The lower susceptibility of AX in maize than in wheat to solubilization by xylanase agrees with results from several studies that have reported the recalcitrance to xylanase degradation of the maize cell-wall polysaccharides.[25,31] The lower susceptibility of endosperm AX in maize than in wheat to solubilization by xylanase can be attributed to the greater content of phenolic compounds of endosperm cell walls and the greater intensity of cross-linkages between AX and other components of the cell wall, as evidenced by the greater A:X ratio in maize than in wheat. Soluble AX is highly fermentable in the hindgut with studies showing that most of the soluble AX is completely fermented in the proximal part of the colon,[32] whereas insoluble NSP is slowly fermented and hence a significant part can pass the colon undegraded.[33-35] In this sense, solubilization of the insoluble NSP into soluble NSP may increase the VFA production in the hindgut providing energy to the pig and improves gut health.[24]

In this study, the AXOS in the soluble fraction was quantified to measure the ability of the supplemental enzymes to reduce the HMW-AX into AXOS. The greater content of AXOS observed in the enzyme-supplemented wheat in comparison with the enzyme-unsupplemented wheat in the soluble fraction after enzymatic *in vitro* digestion supports the view that the supplemental enzymes not only solubilize the insoluble AX into HMW-AX but also further fragment HMW-AX into AXOS in wheat.[36] The HMW-AX is associated with high digesta viscosity, which negatively affects nutrient digestion and absorption[37] and, therefore, further hydrolysis into (AXOS) of lower molecular weight is beneficial.[38]

The insignificant increase in IVDDM for wheat due to enzyme supplementation, despite the increased solubilization of AX for wheat by enzyme supplementation, can be attributed to the fact that the enzymes solubilized the AX in walls of endosperm cells whose content was emptied by digestion with pepsin and pancreatin alone. Hence there were no cell wall-encapsulated nutrients

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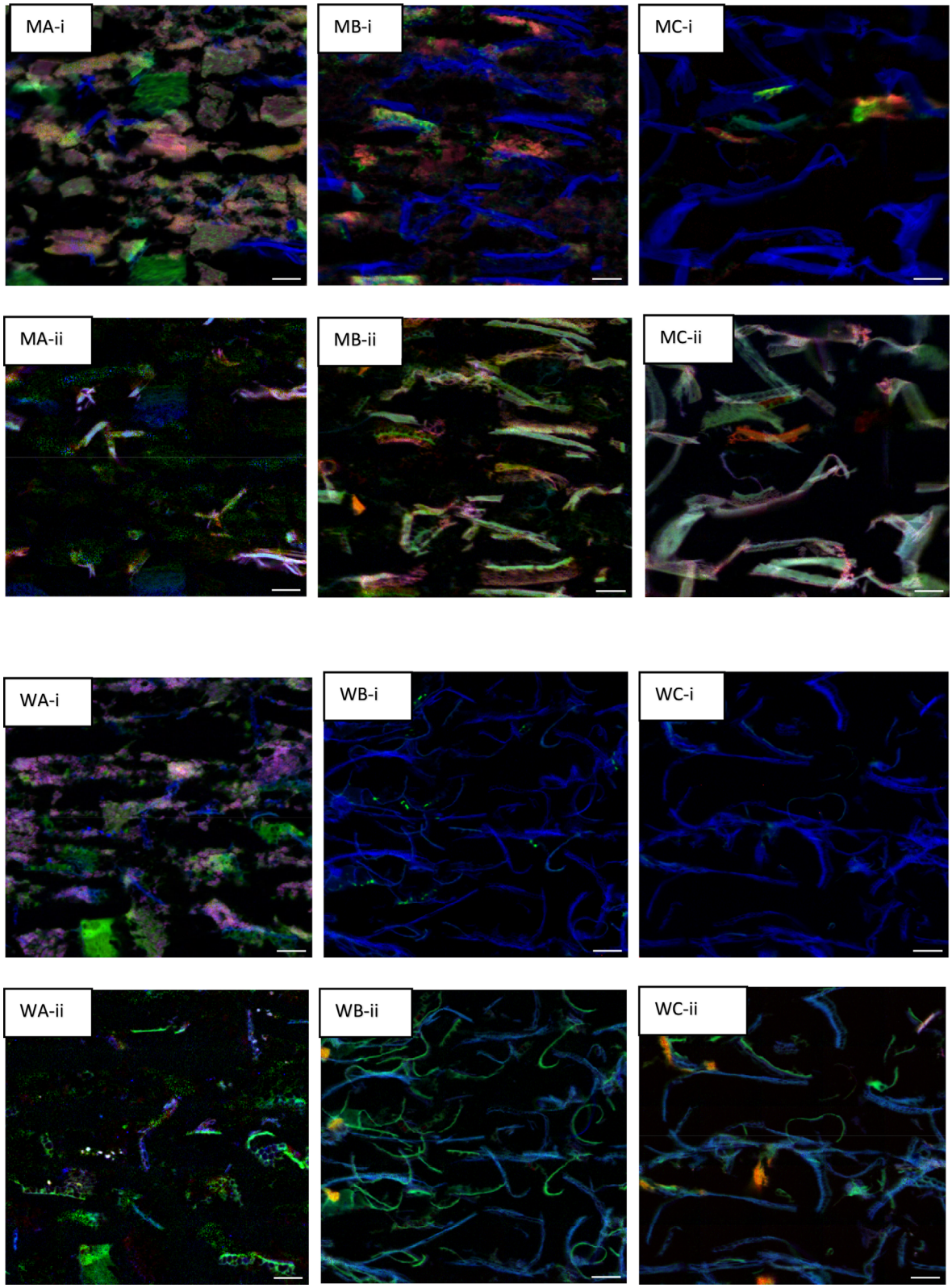

Figure 5. Infrared images (generated using synchrotron based Fourier transform mid-infrared spectroscopy) of maize before *in vitro* digestion (MA), maize insoluble residue after *in vitro* digestion without supplemental enzymes (MB), maize insoluble residue after *in vitro* digestion with supplemental enzymes (MC), wheat before *in vitro* digestion (WA), wheat insoluble residue after *in vitro* digestion without supplemental enzymes (WB), wheat insoluble residue after *in vitro* digestion with supplemental enzymes (WC). Color coding: (MA-i, MB-i, MC-i, WA-i, WB-i, and WC-i) red – starch, green – protein, blue – hemicellulose, (MA-ii, MB-ii, MC-ii, WA-ii, WB-ii, and WC-ii) red – lignin; green – hemicellulose; blue – cellulose.

to be released by the solubilization of the AX in endosperm cell walls. The amount of AX solubilized by the supplemental enzymes in wheat may also have been too small to affect IVDDM. The insignificant effect of enzyme supplementation on IVDDM of maize could be due to an inability of the enzymes to solubilize the NSP in the endosperm cell walls to release all the encapsulated nutrients, and the presence of hard endosperm in the maize.

CONCLUSIONS

Maize was less digestible than wheat due to the presence of hard endosperm in the former. The xylanase and cellulase did not improve the digestibility of wheat. They were effective in degrading the walls of endosperm cells but this did not increase the availability of nutrients for digestion, as most nutrients from the endosperm cells were released by digestion with pepsin and pancreatin alone. The xylanase and cellulase did not improve the digestibility of maize because of the presence of phenolic compounds in endosperm cell walls, high substitution of AX in cell walls, and the presence of hard starch in the endosperm of maize.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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