
*Research article***Influence of arabinoxylan and crosslinked arabinoxylan consumption on blood serum lipids and glucose levels of Wistar rats****Figuerola-Pizano Mar ía Dolores^{1*}, Campa-Mada Alma Consuelo¹, Canett-Romero Rafael², Paz-Samaniego Rita², Martínez-López Ana Luisa³ and Carvajal-Millan Elizabeth^{1,*}**¹ Biopolymers-CTAOA, Research Center for Food and Development (CIAD, AC). Carretera Gustavo Enrique Astiazar án Rosas No. 46, Hermosillo, Sonora 83304, Mexico² University of Sonora, Rosales y Blvd. Luis D. Colosio, Hermosillo, Sonora 83000, Mexico³ NANO-VAC Research Group, Department of Chemistry and Pharmaceutical Technology, University of Navarra, 31008 Pamplona, Spain

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Abstract: Several studies have described the health benefits of arabinoxylan as prebiotics; however, other authors have related them with an anti-nutrient effect as arabinoxylan increases the viscosity of the alimentary bolus. In this work, the impact of arabinoxylan and crosslinked arabinoxylan on blood serum lipids and glucose levels of Wistar rats was investigated. Arabinoxylan was extracted from maize bran, presented a Fourier Transform Infra-Red spectrum typical for this polysaccharide, and a molecular weight of 250 kDa. Arabinoxylan solution at 4% (w/v) formed covalent gels induced by laccase. Male Wistar rats were fed a standard diet supplemented with 5% (w/w) lyophilized arabinoxylan or crosslinked arabinoxylan. Blood glucose levels were determined, collecting a drop of blood from the tail vein of rats at 0, 2, and 10 h after food consumption. Total cholesterol, triglycerides, high-density lipoprotein (HDL) cholesterol, and low-density lipoprotein (LDL) cholesterol were also determined. Postprandial blood glucose of the treatment groups was maintained at the same level as the control group. The serum lipid profile levels also remained close to the control group, excepting total cholesterol and LDL-cholesterol, which were higher in crosslinked arabinoxylan treatment but in the range reported for this murine model. The obtained results revealed that consumption of arabinoxylan and crosslinked arabinoxylan at moderated levels does not interfere with the absorption of these nutrients.

Keywords: arabinoxylan; gels; blood glucose; serum lipids; viscosity

Abbreviations: AX: arabinoxylan; AX-Gel: crosslinked arabinoxylan; FA: ferulic acid; HDL: high-density lipoprotein; LDL: low-density lipoprotein; A/X: arabinose to xylose ratio; FT-IR: Fourier transform infrared; KBr: potassium bromide; CIAD: Research Center for Food and Development; G' : storage modulus; G'' : loss modulus.

1. Introduction

Arabinoxylan (AX) are non-starch polysaccharides in the cell walls of the most commonly consumed cereal grains, constituting a significant portion of dietary fiber [1]. AX are essential components in diets due to their potential beneficial effects on human health as prebiotics stimulating specific intestinal microbes and fermentation patterns, improving colon function [2]. Many of the biological activities of AX are correlated with their structural features [3]. Structurally, AX are composed of a linear backbone of (1→4) linked β -D-xylopyranosyl residues, which are substituted at C(O)–2 or C(O)–3 positions with L-arabinofuranosyl residues [4]. Around 50–60% of xylose residues are not replaced with arabinose units and present different substitution patterns, but variations in a structure depend on the extraction source of AX [5]. These arabinose units can be esterified with ferulic acid (FA) on (O)–5 position, forming di- or tri-FA structures. The esterification grade also varies in function of cereal source and the extraction methodology employed [3]. The FA units in AX chains can be crosslinking using chemical or enzymatic free radicals generating agents. Commonly, the laccase enzyme is used for FA oxidation and form additional di- or tri-FA structures [6]. The crosslinking of AX results in the formation of highly viscous solutions and gels (AX-Gel), presenting unique physicochemical properties and playing exciting roles in the food industry and human health [7].

Generally, AX and AX-Gel consumption in humans is associated with beneficial postprandial effects such as lowering glucose and lipids levels to avoid disease development. The consumption of AX is related to anti-obesogenic impact, reduction of heart disease, and the attenuation of type 2 diabetes by improving carbohydrates, lipids, and amino acid metabolism [1,8–10]. The AX gels fabricated with covalent crosslinking can absorb large amounts of water, are stable at temperatures or pH changes, and have a neutral taste and odor, which are essential characteristics for food applications [11,12]. Besides, the AX crosslinking can promote a selective fermentation of these polysaccharides in the colon, limiting the growth of bacteria considered non-beneficial (*Bacteroides*) and favoring the growth of *Bifidobacteria*, a probiotic [13]. Furthermore, the AX gels are considered hypothetical protectors of the gut microbiota against consuming a high-fat diet when they were part of the Wistar rats' diet formulation [9].

A few human evaluations have reported minor effects or no changes in postprandial glucose after AX-rich diet ingestion [14,15]. The principal hypothesis for attenuation of metabolic responses caused by AX intakes is the increase of alimentary bolus viscosity in the gastrointestinal tract. It has been suggested that the high viscosity that AX and AX-Gel present in aqueous media would reduce the absorption rate of nutrients resulting in a subsequent lowering of blood glucose and lipids levels [16]. Some other studies point out that the AX structure may reduce the intestinal α -glucosidase activity avoiding the production of monomeric sugars units as glucose or fructose from dietary digestible carbohydrates [17].

In animals, contrasting results about the postprandial effects of AX and AX-Gel consumption are also presented. In some studies, postprandial glucose levels present a considerable reduction by the

AX or AX-Gel intake, while in others, glucose levels remain similar to control [18]. In addition, sometimes, the consumption of AX-rich diets has been considered anti-nutritive due to changes in nutrients digestion and absorption rate associated with the viscous nature of AX [19,20]. Despite the contradictory results by consuming AX or AX-Gel, dietary fiber is important as part of the diet. It does not always impart negative or anti-nutritive effects. For that reason, this work aimed to evaluate the impact of AX and AX-Gel on blood serum lipids and glucose levels of Wistar rats. A standard diet supplemented with 5% (w/w) lyophilized AX or AX-Gel was administered in a single meal, and, after that, blood samples were collected. The serum lipid profile, including total cholesterol, triglycerides, high-density lipoprotein (HDL) cholesterol, and low-density lipoprotein (LDL) cholesterol and glucose levels, were determined from blood samples.

2. Materials and methods

2.1. Materials

Maize bran AX was isolated following the methodology of Carvajal-Millan et al. [21]. The obtained AX contains 0.025 µg/mg AX of FA and arabinose to xylose (A/X) ratio of 0.8. Table 1 presents the composition of the AX obtained. Laccase enzyme (benzenediol: oxygen oxidoreductase, E.C.1.10.3.2) from *Trametes versicolor* and all other used chemicals were provided by Sigma Aldrich Co. (St. Louis, Missouri).

Table 1. Composition of maize bran arabinoxylans.

Component	Value
Arabinose	25.9 ± 0.138
Xylose	32.3 ± 0.210
Galactose	12.2 ± 0.203
Glucose	9.3 ± 0.071
Protein	3.4 ± 0.190
Ash	1.1 ± 0.001
Ferulic acid	0.025 ± 0.001
A/X ratio	0.8

*Note: Carbohydrates, and ash and protein are expressed in g/100 g of AX dry matter. Ferulic acid is expressed in µg/mg of AX dry matter. Values are the mean ± standard deviation.

2.2. Fourier transform infra-red spectroscopy

Fourier transform infrared (FT-IR) analysis of dry maize bran AX and AX-Gel was carried out on a Nicolet FT-IR spectrophotometer (Nicolet Instrument Corp., Madison, WI, USA), using KBr pellets (2 mg sample/200 mg KBr). A blank pellet was used as background. FT-IR spectra were acquired in absorbance mode in the mid-infrared region (400–4000 cm⁻¹) at 4 cm⁻¹ of resolution and 32 scans [22]. The obtained spectra were analyzed with the OMNIC 9.3.32 software (Thermo Fisher Inc). The characteristic bands of AX were detected according to previously reported FT-IR spectra [23].

2.3. Arabinoxylan gel preparation

The AX-gel was prepared following the methodology of Berlanga-Reyes et al. [24] and Martínez-López et al. [25] with some modifications. Maize bran AX were dispersed in 0.1 M sodium acetate buffer at pH 5.5 to obtain a 4% (w/v) solution. The solution was maintained at constant stirring and room temperature for 24 h. Laccase was dispersed in 0.1 M sodium acetate buffer at pH 5.5 (0.4 U/ μ L) and incorporated as a crosslinking agent into the AX solution to prepare the AX-Gel (1.675 nkat per mg AX). The mixture was stirring for a few minutes. Then, the gel was allowed to set at 25 °C overnight. Both AX solution and AX-Gel were frozen at -20 °C and freeze-dried at -40 °C/0.125 mbar (Labconco lyophilizer, Kansas, USA) for two days.

2.4. Mechanical spectrum

The mechanical spectrum of AX-Gel was studied using a strain-controlled rheometer (Discovery HR-2 rheometer, TA Instruments, New Castle, DE, USA) in oscillatory mode. The storage (G') and loss (G'') moduli and $\tan \delta$ (G''/G') were monitored to evaluate the gel hardness by a frequency sweep from 0.01 to 10 Hz at 5% strain and 25 °C. Measurements were made at the end of the network formation in the linearity range of viscoelastic behavior. The rheological test was carried out in duplicate, and the results were reported as the means [26].

2.5. Scanning electron microscopy (SEM)

The microstructure and surface morphology of the freeze-dried AX and AX-Gel were evaluated by field emission scanning electron microscopy (JEOL JSM-7401F, Peabody, MA, USA) without coating at low voltage (1.8 kV). The SEM images were obtained a 2000 \times magnification in secondary and backscattered electrons image mode [22]. ImageJ software was used to evaluate the external morphology of AX powder and AX-gel.

2.6. Animals

Twelve male Wistar rats were housed individually in metabolic cages in an environment-controlled room (temperature: 23 \pm 2 °C; relative humidity: 60 \pm 5%) with a 12 h day/night cycle. During a week, the organisms were acclimatized, and they were fed a standard pellet diet with free access to water. The weight of the animals on the study day was between 300–320 g. Animal handling and all experiments were approved by the animal ethical committee of the Research Center for Food and Development (CIAD, AC), following the procedures and specifications of the Official Mexican Standard Norm (NOM-062-ZOO-1999).

2.7. Bioassay

The animals were fasted for 15 h with free access to water and randomly divided into three groups (4 per treatment). The rats were weighed, and their tail tip was washed with ethanol. A drop of blood was taken from the end of the tail vein, and the blood glucose concentration was determined with the Accu-Check Performa glucometer (Roche, Mannheim, Germany). This first measure was considered as the baseline blood glucose level. After that, the rats were divided into three groups. Two

groups were fed with 20 g of a standard pellet diet containing 5% (w/w) of lyophilized AX or AX-Gel. The control group was fed with 20 g of only standard pellet diet. The rodent consumed the whole meal after 4 h of presentation. Blood glucose concentrations were determined by collecting a new drop of blood from the tail vein at 2 and 10 h after food presentation. The next day, all groups were fed with 20 g of standard pellet diet.

The concentration of total cholesterol, triglyceride, HDL-cholesterol, and LDL-cholesterol were determined on blood serum after 10 h of food consumption. A sample of blood (approximately 500 μL) from the tail vein of each rat was taken. The samples were allowed to stand for 2 h to let that coagulation process take place. The serum lipid profile was determined using a clinical chemistry autoanalyzer based on dry chemistry micro-slide technology (VITRO® 350 chemistry system, Johnson & Johnson, USA).

2.8. Statistical analysis

The blood glucose, total cholesterol, triglyceride, HDL-cholesterol, and LDL-cholesterol values were performed in triplicate the data are reported as the means \pm standard deviation.

3. Results

FT-IR spectra of AX extracted from maize bran and the AX-Gel are presented in Figure 1. Both samples showed almost identical IR spectra, indicating similar molecular identity, and they presented the typical AX spectrum. The spectrum displays the characteristic absorbance bands for polysaccharides in the region of 1200–800 cm^{-1} , especially the signal at 1028 cm^{-1} is related to the C-OH bending of xylans [27,28]. Two little dissimulated shoulders at 1069 cm^{-1} and 904 cm^{-1} are detected, indicating the antisymmetric C-O-C stretching mode of the glycosidic links and the arabinose substitutions C-3 of xylose units [23,28]. The band at 1639 cm^{-1} has been associated with the carbonyl stretching vibration of FA at a low degree of esterification [27]. At 2923 cm^{-1} , the signal for the group CH_2 was observed, and a wide band at 3320 cm^{-1} was presented for the OH group.

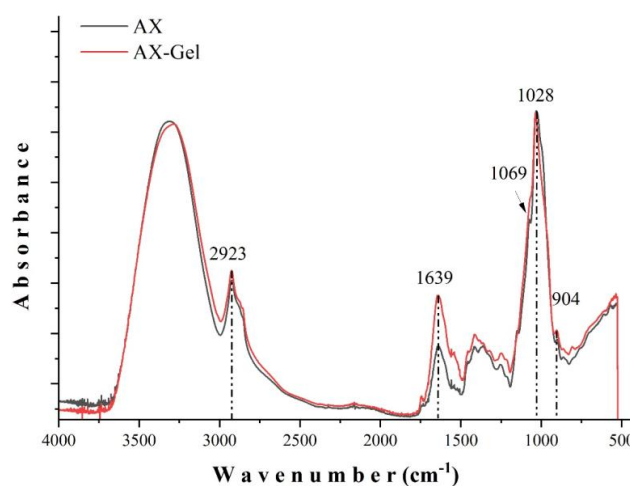


Figure 1. FT-IR spectrum of maize bran arabinoxylan.

The mechanical spectrum of the AX-Gel after 12 h of gelation showed that G' was higher than G'' and independent of frequency (Figure 2). The values of G' were linear, ranging from 170 to 200 Pa, while G'' values were lower and changed depending on the frequency. The values of G' and G'' were considered to calculate $\tan \delta$ (G''/G'). The $\tan \delta$ values increased from 0.007 to 0.1 when frequency increased.

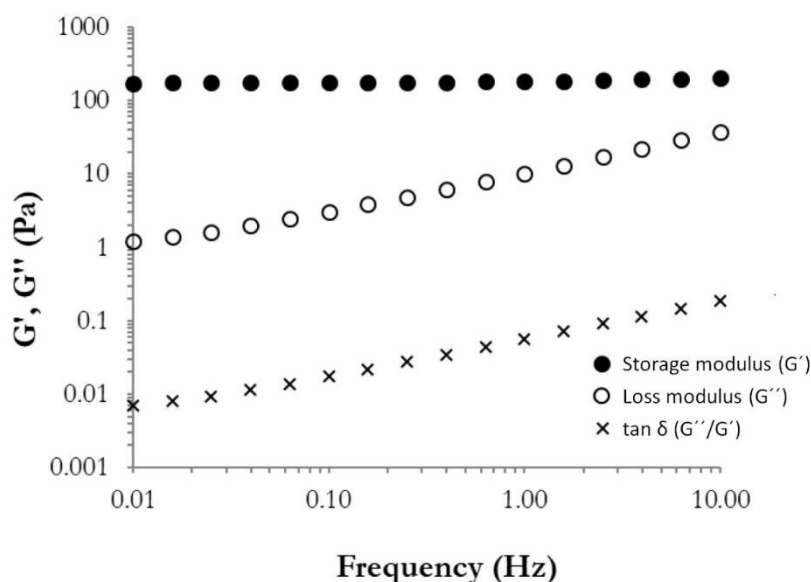


Figure 2. Mechanical spectra of AX-Gel at 25 °C.

The scanning electron micrographs of AX and AX-Gel are presented in Figure 3. The AX powder (Figure 3a) displayed a segmented and granular structure with an irregular and rough morphology which simulate small porous in the surface. In the case of the AX-Gel (Figure 3b), they had a continuous surface characterized by an irregular three-dimensional arrangement. They presented continuous aggregates of nodular structures that create the porosity of the networks.

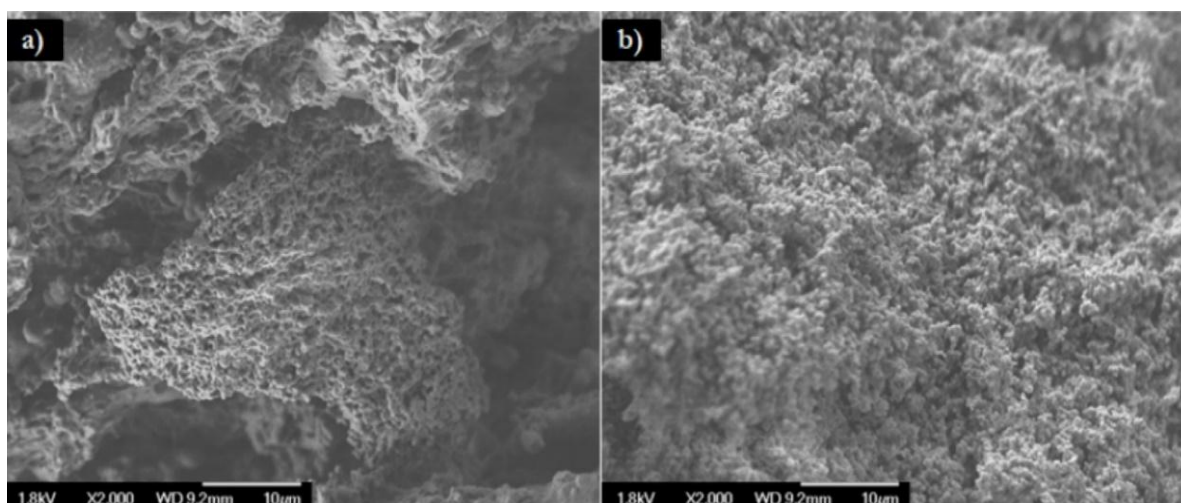


Figure 3. SEM micrographs of AX powder (a) and freeze-dried AX-Gel (b).

The rat's blood glucose levels registered before and after food consumption are shown in Figure 4. The mean of fasting blood glucose in each group, considered basal level or time 0, was 125 ± 1 , 132 ± 10 , and 129 ± 9 mg/dL for control, AX-Gel, and AX group, respectively. After 2 and 10 h of food consumption, the blood glucose levels were measured. In general, the glucose values were from 130 to 150 mg/dL, and in a few cases, they exceeded the 160 mg/dL, especially in the AX-Gel group. Although postprandial blood glucose values trended to increase in both AX-Gel and AX groups, they do not show significant differences compared with the control group.

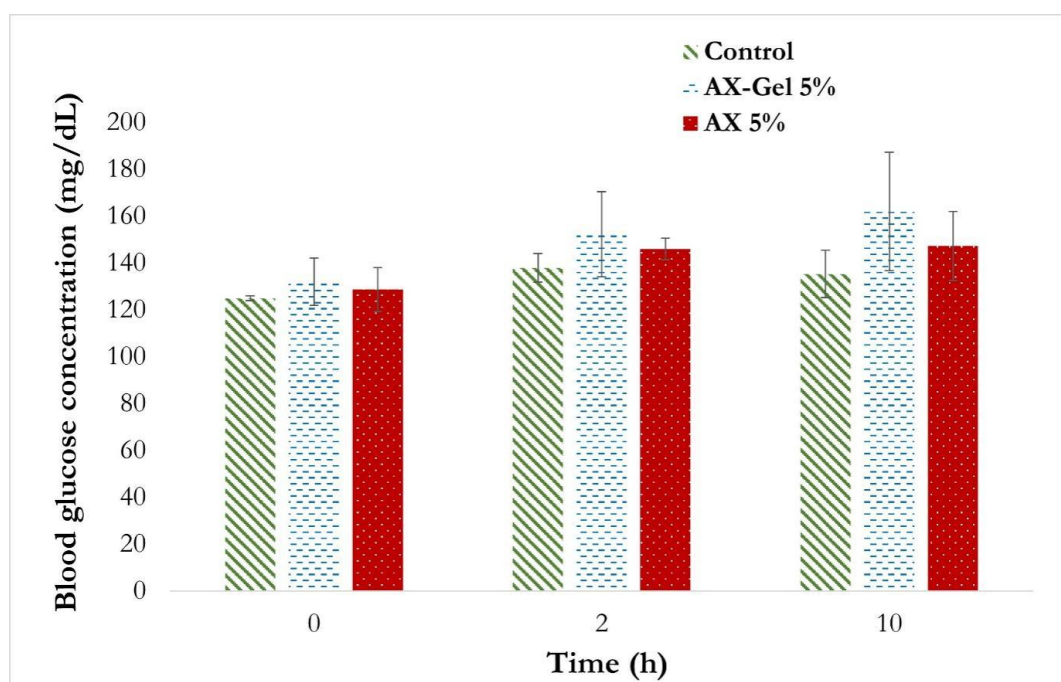


Figure 4. Blood glucose levels in rats before (0 h) and after consumption of food containing AX or AX-Gel at 5% (w/w) (2 and 10 h).

The serum lipid profile levels were also assessed after 10 h of food consumption. The total cholesterol mean values (Figure 5a) were similar for the AX and the control group, while the mean value increased significantly ($p \leq 0.05$) for the AX-Gel group. In triglyceride levels (Figure 5b), the mean of the AX, AX-Gel, and control group were not significantly different. The HDL-cholesterol levels (Figure 5c) were similar between the three groups presenting values around 50 mg/dL. The LDL-cholesterol level (Figure 5d) for AX and AX-Gel groups was significantly higher ($p \leq 0.05$) with 14 and 7 mg/dL, respectively, compared with the control group (1.6 mg/dL).

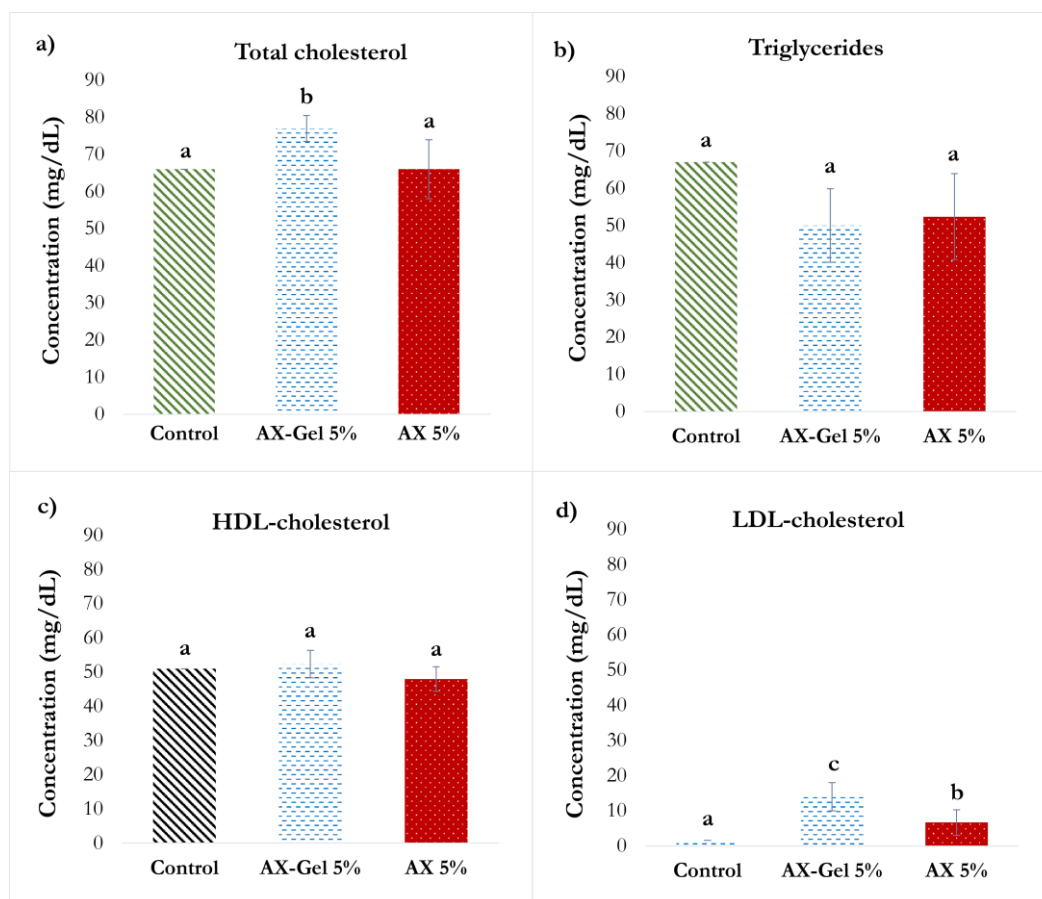


Figure 5. Lipid profile in rats after more than 10 h of food consumption. The different letter indicates significant differences ($p \leq 0.05$).

4. Discussion

The maize bran AX presented an integral structure corroborated with the FT-IR spectrum, in which all typical signals of AX polysaccharides were detected. Besides, the A/X value (0.8) is similar to that reported for other AX obtained from maize bran and indicates that they have a highly branched structure [29,30]. However, the FA content (0.025 $\mu\text{g}/\text{mg}$) was lower than the value reported in the literature for other maize bran AX (7.8 $\mu\text{g}/\text{mg}$) [31]. Differences in FA content have been associated with the length of the alkaline process. The increase of AX alkaline extraction time decreases the FA content, impacting the gelling capacity of the AX directly because less crosslinking points are formed, leading probably to a low viscosity solution [32,33].

The viscoelastic properties of AX-Gel were similar to those shown by other authors for crosslinked solutions of AX at different concentrations using laccase [23,25,34,35]. Typical behavior of solid-like material was proved in the AX-Gel through the mechanical spectra, with $G' > G''$; a linear and independent G' value, and the G'' value-dependent of frequency [30,34]. In previous studies, high G' values in AX gels have been attributed to the content of covalent crosslinking combined with physical entanglements between their chains [7,25]. The frequency-independent behavior of G' displayed in AX-Gel reflects the stability between crosslinking points in the network, which has been seen in many AX gels [25,31]. According to Mendez-Encinas et al. [36], the behavior

of AX gels is related to the structural and conformational characteristic of AX, especially the FA content, which is the molecule for covalent crosslinking. Furthermore, the small values obtained for $\tan \delta$ describe the nature of the network, and in this case, it proves an elastic character of AX-Gel [22,37]. $\tan \delta$ values lower than 0.1 are associated with an elastic system, while $\tan \delta$ values higher than 0.1 suggest a more liquid-like character in the network [38].

The differences between the surface morphology of AX and AX-Gel have also been shown by previous works that point out how several factors as the branching degree of the xylan backbone, the content of FA, and the crosslinking of the AX chains generate specific morphologies. Martinez-Lopez et al. [25] observed similar surfaces with nodular clusters in AX microparticles crosslinked from a 4% AX solution. They have associated this heterogeneous structure with the content and distribution of ferulic acid due to other studies have reported that the crosslinking of gels via phenolic groups present nodular clusters, which determine the pore size in the network.

In the present research, the consumption of a standard diet supplemented with 5% of AX or AX-Gel by Wistar rats did not significantly change the levels of postprandial glucose after 2 and 10 h in relation to control. Contrasting results have been reported in Wistar rats fed with a diet containing 4% of crosslinking AX which significantly reduced the postprandial glucose levels [39]. According to those authors, the crosslinking of AX dramatically increases its viscosity in solution, which may delay the bowel transit and absorption rate, causing the blunt of glucose values. The absence of changes in glucose levels observed in the present work, after the ingestion of 5% of the AX-Gel by the Wistar rats, could be associated with the low G' values (170–200 Pa) in these gels. AX gels exhibit viscoelastic properties and more compact microstructure than non-crosslinked AX [40]. However, the formation of covalent crosslinking points to set the gel mainly depends on the content of FA in the AX chain [4]. Probably, the low FA content in the structure of the AX used (0.025 $\mu\text{g}/\text{mg}$) generates few crosslinking points impacting directly on gel characteristics such as viscoelasticity. [40]. The small $\tan \delta$ values (0.007–0.100) support the elastic character of AX-Gel. Although, the $\tan \delta$ registered in the present study is higher than that reported for highly crosslinked AX gels (0.001) [31], which can be attributed to the lower FA content in the sample in relation to that study (7.18 $\mu\text{g}/\text{mg}$ AX). Thus, several structural characteristics of AX and AX gels, including the FA content, would modify their physiological functions. In a previous report [39], supplementation of food with 4% of non-cross-linked AX did not blunt the postprandial blood glucose response in Wistar rats, which agrees with the results found in the present study. Those authors attribute the similar effect of AX and control to the low viscosity of AX in relation to AX-Gel. Comparable results have also been reported in pigs where no diet-induced differences were found in postprandial glucose levels, even when the food contained 10% or 17% of native AX as dietary fiber [41,42]. For humans, AX consumption has been shown to reduce postprandial glucose even if only 3.2% of AX has been incorporated as dietary fiber in food [43,44].

Regarding the lipid profile behavior, the total cholesterol and LDL-cholesterol values were higher in AX-Gel in relation to control and AX treatments, but the values were low than those reported by previous studies in rats [45,46]. At the same time, triglycerides and HDL-cholesterol remain close to the control for both treatments. These results are different from those presented in other studies where the lipid profile has been evaluated after 4 or 5 weeks of AX consumption. One study with rats showed that the ingestion of the dietary fiber from diverse plants, which included AX, reduced the total cholesterol, triglycerides, and LDL-cholesterol and increased the production of HDL-cholesterol [47]. Those authors proposed the increase in bowel transit rate as a mechanism of action, causing an alteration in lipid absorption as cholesterol and fatty acids, which may bind to fiber preventing the

formation of micelles. Chen et al. [48] also presented decreased total cholesterol, triglycerides, and LDL-cholesterol after ingesting a high-fat diet supplemented with 6% AX. The contrasting results of this study suggest that the AX-Gel diet does not bind the cholesterol coming from a standard diet, probably due to the short time of treatments consumption. Similar results to those of our study were found in pigs fed with the AX diet, where the serum triglycerides resulted in higher concentrations compared with the control diet. Another study in pigs showed that an 8% AX-rich diet, consumed during 4 weeks, does not significantly affect LDL, HDL, or total cholesterol but decreases triglyceride levels [49]. In humans with metabolic syndrome or type 2 diabetes, the consumption of the AX diet does not improve the lipids profile [50,51].

In the case of LDL-cholesterol levels, they were low in all groups, in relation to those shown by previous studies in rats [45,46]. However, the AX-Gel group presented a higher LDL-cholesterol value and a higher total cholesterol level in relation to control and AX treatments. The biological role of LDL is to transport the cholesterol from the liver to tissues, where it is incorporated into the cellular membranes [46,52]. The increased serum levels of LDL-cholesterol in the AX-Gel group are probably related to the augmented total cholesterol in the same group, indicating its transport from the liver to extra-hepatic tissues.

5. Conclusions

The supplementation of maize bran AX and AX-Gel at 5% in food for Wistar rats does not interfere with the absorption of nutrients. The levels of postprandial glucose, triglycerides, and HDL cholesterol were close to control treatment. Only total cholesterol and LDL-cholesterol showed an increase in AX-Gel treatment, but the values were within normal ranges that have been reported for healthy organisms. The variation in postprandial responses after the consumption of AX and AX-Gel rich diets suggests that further studies are necessary to clarify the underlying mechanisms and the effect of polysaccharide structural variation and the characteristics of the gel formed.

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Conflicts of interest

The authors declare no conflict of interest.

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