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Human colonic *in vitro* fermentation of water-soluble arabinoxylans from hard and soft wheat alters *Bifidobacterium* abundance and short-chain fatty acids concentration

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ABSTRACT

The human intestinal microbiome plays an important role in health due to the large number of beneficial effects related to the bacterial profile and the metabolites generated in the intestine. Arabinoxylans are compounds present in different cereals such as wheat and they can modulate the profile and functioning of some beneficial bacteria from human intestinal microbiota. In the present work, a colonic *in vitro* fermentation with human faecal inoculum was done using arabinoxylans extracted from Argentinian hard and soft wheat as substrates. Molecular size alteration of arabinoxylans were studied during fermentation and *Lactobacillus* and *Bifidobacterium* abundance as well as short chain fatty acids concentrations were determined. The arabinoxylans fermentation was proved to induce the growth of *Bifidobacterium* and the release of short-chain fatty acids. The speed and efficiency of fermentation were different for each of the arabinoxylan extracted from both wheat genotypes, perhaps because of differences in their chemical and physical structures. The consumption of water-extractable arabinoxylans (WE-AX) (either supplemented or enriched) to maintain the balance or modulate in a favorable way the profile of *Bifidobacterium* can be an important contribution to the human health.

1. Introduction

The human microbiome is the total population of microorganisms and metabolites which colonise the whole human body (Petrosino, Highlander, Luna, & Gibbs, 2009). The gut microbiota is one of the most densely populated communities (Ruiz Álvarez, Puig Peña, & Rodríguez Acosta, 2010). In the past several years, more attention has been given to gut microbiota, and several studies have shown modulating functions on different aspects of the digestive function (Salvucci, 2014). In more recent years, the intestinal microbiome has been considered a symbiotic partner for health maintenance (Qin et al., 2010). The intestinal microbiome defines and modulates host homeostasis by determining nutritional, immunological, and neuroendocrine balance (Bakhtiar et al., 2013; Salvucci, 2014). The nutrients which are not digested by human enzymes, such as nondigestible carbohydrates, reach the intestine and can be fermented by the gut microbiota, obtaining final products such as short-chain fatty acids (SCFA) (Karppinen, Liukkonen, Aura, Forssell, & Poutanen, 2000; Kasubuchi, Hasegawa, Hiramatsu, Ichimura, & Kimura, 2015).

Prebiotics are compounds which improve human health and reduce risk of diseases mediated by microbiota alterations (Gibson et al., 2017). Those compounds which are selectively fermented by gut bacteria induce specific changes in the composition and/or activity of the gastrointestinal microbiome, which confers benefits to the welfare of the host and health in general (Al-sheraji, Ismail, Yazid, & Mustafa, 2013; Gibson et al., 2017; Gibson & Fuller, 2000). The prebiotic effect in the intestine can be assessed by the enhanced proliferation of *Bifidobacterium* and *Lactobacillus* (Gibson, 1999), the decreased amount of intestinal pathogens, the increased production of metabolites related to the activity of beneficial bacteria such as SCFA, and the decrease in the

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Table 1
pH and pressure values during fermentation.[a]

<!–Col Count:4– > Time (h)	Samples	pH[b]	Pressure (psi)
0	Control	9.0 ± 0.5 a	0.0 ± 0.1 a
	Inulin	9.0 ± 0.5 a	0.0 ± 0.1 a
	AXs	9.0 ± 0.5 a	0.0 ± 0.1 a
	AXh	9.0 ± 0.5 a	0.0 ± 0.1 a
6	Control	8.2 ± 0.3 a	1.0 ± 0.1 a
	Inulin	6.2 ± 0.2 b	1.0 ± 0.3 a
	AXs	7.5 ± 0.2 a	1.0 ± 0.4 a
	AXh	7.5 ± 0.1 a	1.2 ± 0.3 a
12	Control	7.1 ± 0.1 a	1.3 ± 0.2 a
	Inulin	5.8 ± 0.2 b	2.5 ± 0.3 b
	AXs	6.2 ± 0.1 b	3.5 ± 0.3 c
	AXh	5.8 ± 0.3 b	4.2 ± 0.3 d
24	Control	6.7 ± 0.2 c	2.1 ± 0.2 a
	Inulin	5.3 ± 0.4 a	4.0 ± 0.3 b
	AXs	6.0 ± 0.4 b	5.3 ± 0.1 c
	AXh	5.7 ± 0.3 ab	7.2 ± 0.4 d
28	Control	6.2 ± 0.5 b	2.0 ± 0.3 a
	Inulin	5.2 ± 0.2 a	5.0 ± 0.1 b
	AXs	5.1 ± 0.1 a	5.0 ± 0.1 b
	AXh	5.3 ± 0.3 a	7.0 ± 0.1 c

[b] The results are expressed as mean average and standard error of a triplicate. Different letters in the columns mean significant differences ($p < 0.05$).
[a] Variation of pressure and pH during the 28 h of the fermentation test.

production of toxic metabolites (Al-sheraji et al., 2013).

Arabinoxylans (AX) are nondigestible carbohydrates considered as the main non-cellulosic polysaccharides in the cell walls of cereals such as wheat (Paesani, Salvucci, Moiraghi, Fernandez Canigia, & Pérez, 2019). The AX present in wheat are classified according to their solubility in water: soluble (water-extractable AX – WE-AX) or insoluble; between 25 and 33 g/100 g of wheat AX are WE-AX (Saulnier, Sado, Branlard, Charmet, & Guillon, 2007). AX are formed by xylose chains linked by a β1-4 bond, substituted by arabinose units linked by 1α-2 and 1α-3 bonds along the xylose chain (Grootaert et al., 2009; Mendis & Simsek, 2015). Ferulic acid can be attached at the C(O)-5 position of the arabinose. It is still not clear how the proportions of the number and type of substitution influence the heterogeneous nature of the AX (Saulnier et al., 2007).

The structural properties of AX determine their biological effects, fermentation processes, and modulation of gut microbiota. Some studies have reported the effect of molecular structure on the functionality of WE-AX, such as the lower the molecular weight of WE-AX is, the higher the prebiotic effect is (Van Craeyveld et al., 2008). The distinct fermentation effects of WE-AX could be due to AX structure variation depending on the plant tissue components and wheat variety (Sun, Sun, & Wang, 2017). For instance, hard and soft wheat produces flours with different particle sizes when milled, which can result in different WE-AX structures. However, there are limited reports regarding colonic fermentation of hard and soft WE-AX and their possible prebiotic effects. Thus, the aim of this work is to investigate the effect of WE-AX extract from hard and soft wheat on modulation of human gut microbiota in a colonic *in vitro* fermentation by analysing *Lactobacillus* and *Bifidobacterium* populations, SCFA production, and WE-AX molecular size changes during the fermentation, using inulin as a positive prebiotic control.

2. Material and methods

2.1. Extraction of WE-AX from hard and soft wheat

Two pools of water-extractable AX (WE-AX) of wheat were obtained: one pool of soft Argentinian wheat (AXs) (experimental lines identified by the acronym #PM carrying the alleles *Pina-D1a/Pinb-D1a*; triplicate), and another of hard wheat (AXh) (commercial Argentinian cultivars Klein Yarara, ACA 315, and Guerrero; triplicate). The WE-AX extraction was done according to Buksa et al. (2010), with modification (Paesani et al., 2020). The AX samples were extracted with water (12 h at 50 °C) and then centrifuged (1250×*g*, 10 min). The supernatant was treated with thermo-resistant α-amilase (Sigma, 350 kU) and pronase E (from Streptomyces griseus, Sigma Type XIV 3.5 U/mg), the solution was filtered and the WE-AX precipitated with acetone:alcohol (1:1). Finally, the extract was dried in an oven for 2 h at 50 °C. The characterization of the samples is depicted in Table S1 of the supplementary material and was discussed in detail by Paesani et al. (2020).

2.2. Colonic batch in vitro fermentation

The *in vitro* fermentation of the WE-AX samples was evaluated using the faecal content of three volunteers older than 18 years with no history of gastrointestinal diseases and who did not use antibiotics in the three months prior to the sample collection. This stage was undertaken with the approval of the Research Ethics Committee of the Faculty of Pharmaceutical Sciences of the University of São Paulo (Ethics Committee approval CAAE #43129115.7.0000.0067, approval #1.089.446). The faeces were collected in sterile containers and placed on the ice until the sample was delivered. After the reception, they were conditioned in CO_2 for maintenance of anaerobiosis. Samples from the three individuals were mixed and reconditioned in CO_2. Faecal fermentation was performed as described by (Jonathan, Wiechen, Souza, Schols, & Gruppen, 2012; M. T.; Williams & Hord, 2005). The fermentation medium was composed of basal solution (76mL/100 mL), vitamin/phosphate solution (1mL/100 mL), reducing solution (1mL/100 mL), and bicarbonate solution (4mL/100 mL) (Williams, Bosch, Boer, Verstegen, & Tamminga, 2005). Immediately after making the pool, the faeces were diluted six times (w/v) in sterile NaCl 0.9 g/100 mL and were homogenised and filtered in Miracloth (EMD Millipore). One hundred mg of WE-AX were used for the treatment, and inulin was used as a positive control in the same amount. Each weight was corrected for the purity of each extract (Table S1). A control experiment was done using the medium and the inoculum without added fibre and a negative control was done using only medium to guarantee aseptic handling. The bottles with 0.750 mL of the inoculum, in triplicate, were placed in a 37 °C bath with shaking, and 50 μL samples were taken at 0, 6, 12, 24, and 28 h. Kinetics of fermentation were assessed by measuring the medium pH variation and the pressure of the gas production over time point. The pressure of gas production was measured by the apparatus of Theodorou, Williams, Dhanoa, Mcallan, and France (1994) in each fermentation time.

2.3. Quantification of short-chain fatty acids

The collected aliquots from each fermentation vessels were centrifuged, and filtered in a 0.22 μm filter pore for the determination of SCFA at the times 0, 6, 12, 24, and 28 h. The quantification was done by gas chromatography (CG Agilent Technologies 7890 B GC System, USA) with flame ionizer detector (FID) using a fused silica column CP 7747 (WCTO, Varian, Palo Alto, CA, USA), under the same protocol as Paesani et al. (2020). The injection was carried out at 250 °C and 2,8 KPa. The initial temperature was 110 °C followed by an increase of 2 °C/min up to 140 °C and then 40 °C/min up to the final temperature of 200 °C. The total SCFA was calculated by adding the total of the acids measured.

2.4. Molecular size distribution

Aliquots of the fermentation were collected for the analysis of the molecular size distribution of the polysaccharides at each time point. High pressure molecular size exclusion chromatography coupled with a refractive index detector (HPSEC-RID) was performed using the Infinity 1250 System (Agilent, Santa Clara, CA, USA). The determinations were made as specified in Paesani et al. (2020).

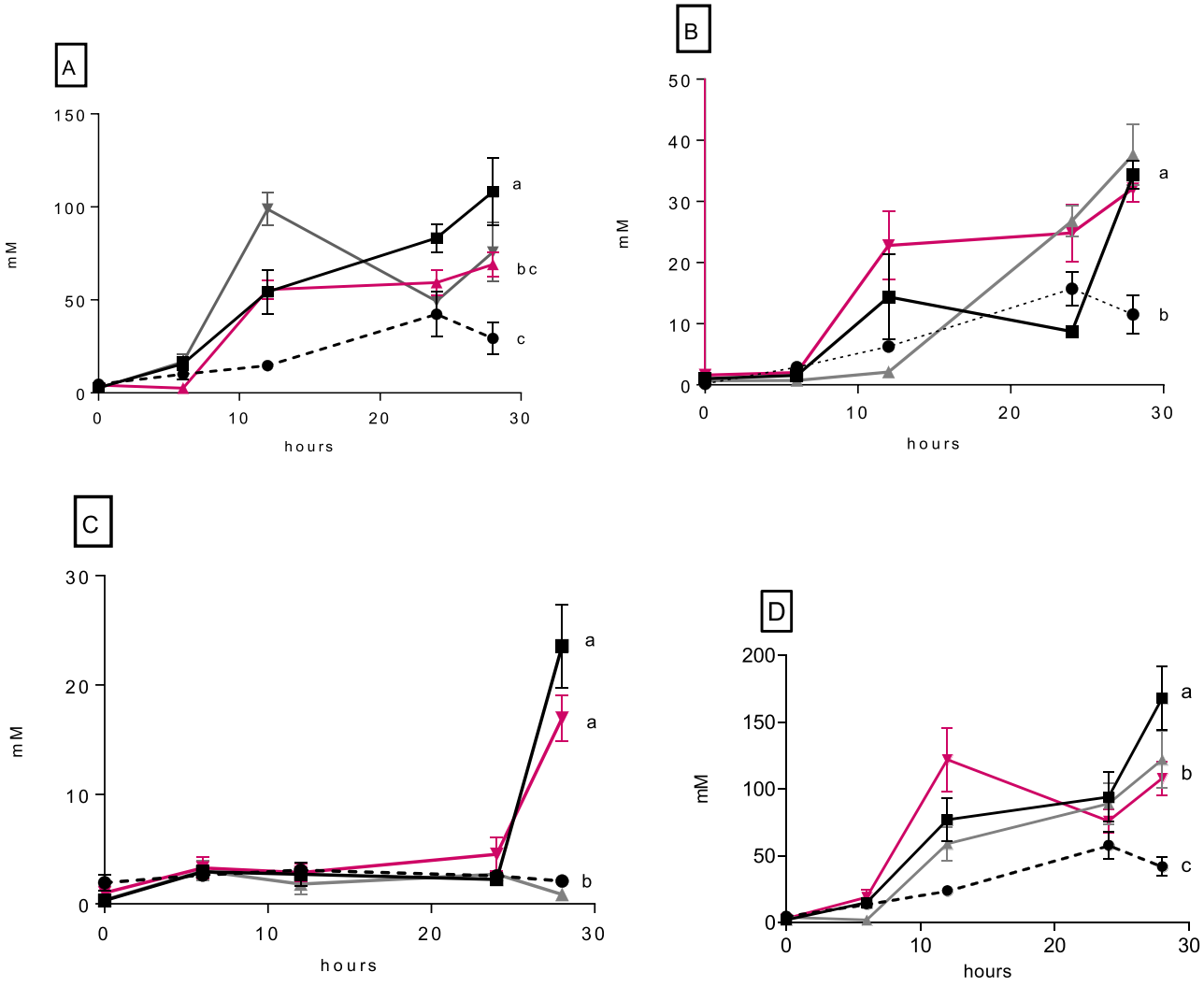

Fig. 1. Concentration of acetic acid (A), propionic acid (B) butyric acid (C) and (D) the total SCFA during the fermentation with human faecal inoculum (mM). The discontinuous black lines represent Control, the continuous black lines represent Inulin, grey lines are AXs and pink lines are AXh. Different letters indicate significant differences in the final concentrations with $p < 0.05$. The curve is made from the average of the measurements made in triplicate. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

2.5. *Bifidobacterium and Lactobacillus analysis*

After 28 h of fermentation (inefficacy of fermentation medium), the samples were centrifuged, and DNA was extracted from the precipitate for the quantification of *Bifidobacterium* and *Lactobacillus* by qPCR. The whole process was carried out following exactly the protocol detailed by Paesani et al. (2020). Bacterial groups were expressed as a number of copies for μL of the cDNA.

2.6. *Statistical analysis*

The data collected from biological and technical triplicates in experiments were treated statistically by analysis of variance (ANOVA), and the results were compared by the Fisher Method at a significance level of 0.05. The values were reported as the arithmetic mean, and each letter indicates significant differences. These analyses were performed using the INFOSTAT software (School of Agricultural Sciences, UNC, Córdoba, Argentina).

3. Results and discussion

3.1. *AX colonic in vitro fermentation*

We obtained the WE-AX from hard and soft Argentinian wheat, evaluated its composition, and performed colonic *in vitro* fermentation to evaluate the WE-AX prebiotic potential. The characterisation of WE-AX extracts was analysed in detail by Paesani et al. (2020) and is shown in Table 1 of the supplementary material. The pressure increased significantly during the fermentation for both AX extracts and showed greater values when compared to control sample (2.0 psi) at 28 h (Table 1). Since the human inoculum might have unfermented saccharides, it was expected a subtle increase in pressure and decreased pH during fermentation in control group. The negative control experiment did not change any parameter.

AXh showed the higher increase in pressure (7.0 psi) even when compared with the prebiotic control inulin (5 psi). The pH decreased in all samples, reaching the lowest values at 28 h, when fermented medium was inefficient to maintain fermentation (Table 1). There was no significant difference amongst AXh, AXs, and inulin. The control had a final pH value of 6.2, possible due to fermentation of reminiscent

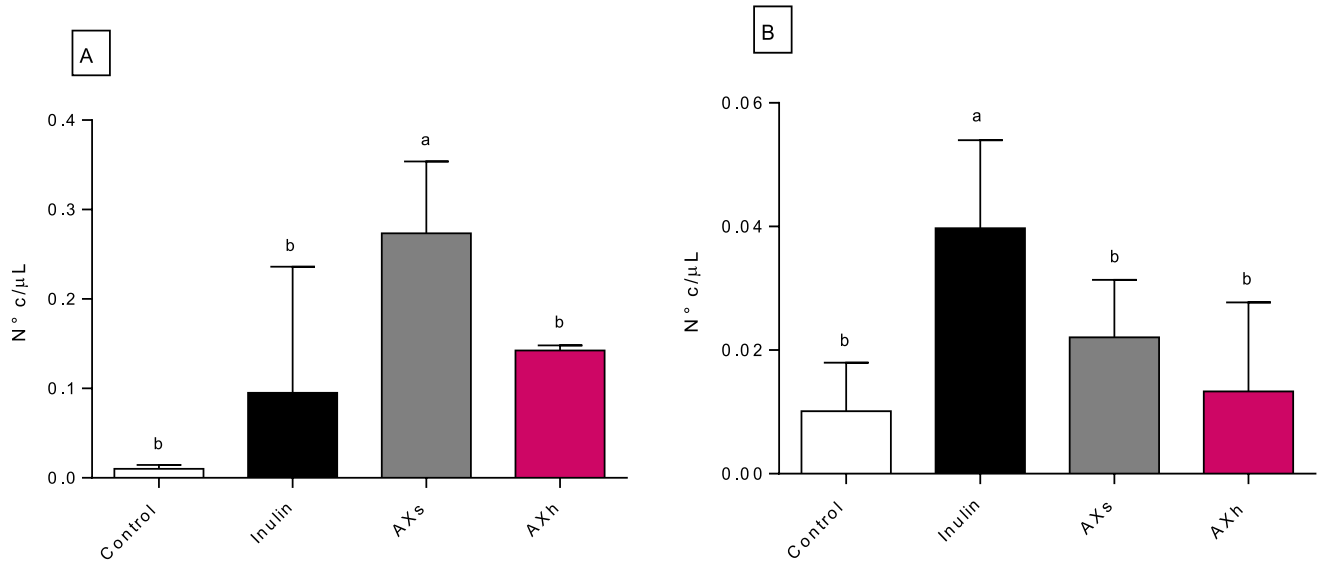

Fig. 2. Number of beneficial bacteria found at the end of fermentation. Number of copies of *Bifidobacterium* (A) and *Lactobacillus* (B) for µL of DNA. White columns represent Control, black columns are Inulin, grey columns are WE-AX of soft wheat (AXs) and pink columns are WE-AX of hard wheat (AXh). The columns show the average and standard error of each determination made in duplicate. Different letters indicate significant differences with $p < 0.05$. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

carbohydrates in human faeces, whilst the WE-AX samples and inulin reached values between 5.1 and 5.3. The colonic pH decrease is caused by the accumulation of SCFA, a biological health consequence which could decrease the solubility of free bile acids, for example (Grubben et al., 2001). The decrease in pH, therefore, would not only demonstrate the ability of the human inoculum, partially represented by the gut microbiota, to metabolise the WE-AX, thus generating SCFA, but it would also exert a protective effect in the intestine since the acidification inhibits most opportunistic pathogens (Roberfroid, Loo, & Gibson, 1998, pp. 11–19).

3.2. Short-chain fatty acids and gas produced during fermentation

The consequence of polysaccharides fermentation is the production of some types of gasses and SCFA (Topping & Clifton, 2001). The variation in SCFA concentrations was evaluated at each *in vitro* fermentation time point. The SCFA are a source of energy for the intestinal epithelial cells, and they could also modulate cells growth. The SCFA also modulate enzymatic activity and transcription factors (Corrêa-Oliveira, Fachi, Vieira, Sato, & Vinolo, 2016; Kasubuchi et al., 2015). The production of these metabolites is determined by numerous factors, including the amount and type of bacteria present in the colon and the substrate source (Roberfroid, 2007). Thus, the decreased values of the medium pH are a direct consequence of SCFA formation (Sun et al., 2017). The total production of SCFA was significantly higher for both WE-AX extracts and inulin than control (Fig. 1D).

Acetic acid concentration increased throughout the fermentation for all samples, except for the AXs which showed the highest concentration at 12 h and then decreased until the end of the fermentation (Fig. 1A). This could be due to a faster fermentation of AXs than AXh. Nevertheless, both WE-AX showed significantly higher values of C:2 than the control. Acetic acid usually has the faster and higher production (Rumpagaporn et al., 2015) and it has been associated with changes in the expression profile of regulatory neuropeptides, favouring appetite suppression and activation of acetyl-CoA carboxylase (Sun et al., 2017).

The fermentation of AX also produced high amounts of propionic acid compared to other studies (Van Laar, Tamminga, Williams, & Verstegen, 2002; Rose, Inglett, & Liu, 2010). It increased during the experiment with higher concentrations observed for the WE-AX and inulin. At 12 h fermentation, the AXs had a lower concentration than control with a sharp increase after. At 12 h, propionic acid in AXh was the highest and then increased progressively (Fig. 1B). Rumpagaporn et al. (2015) have also observed that the propionic acid increased during the fermentation of AX. Propionic acid is related to the synthesis of cholesterol and influences the glucose metabolism and postprandial blood glucose levels (Cook & Sellin, 1998).

Until 24 h of fermentation, none of the fermentation samples showed a significant increase in the concentration of butyric acid. However, at 28 h, the values of C:4 were significantly higher for AXh with a similar value to inulin, but with neglected values for control and AXs. An increase in the production of butyric acid, may result in a protective effect in the distal colon (Wong et al., 2011), since it plays an important role in the regulation of cell proliferation and differentiation (Roberfroid, 2007; Topping & Clifton, 2001). AXs fermentation might not be considered beneficial in terms of butyric acid production, whereas AXh, which showed molecules with higher molecular weight, displayed a greater increase in butyric acid content.

The SCFA contents were significantly different between WE-AX samples and this might be due to variances in the AXs and AXh structures (Sun et al., 2017). The AXh presented a fraction of bigger molecules (50–450 KDa) which were not found in the soft wheat. In accordance with that, Hughes et al. (2007) performed an *in vitro* batch fermentation and observed higher concentrations of acetic, propionic, and butyric acid after the addition of high molecular size AX fractions.

3.3. Bifidobacterium and Lactobacillus abundances

The fermentation promoted a significantly increase in *Lactobacillus* abundance only in the inulin sample as expected and *Bifidobacterium* showed only significant higher concentrations in the AXs sample (Fig. 2). *Bifidobacterium* promote human health through different pathways (Meulen et al., 2016; Wang, Sun, Cao, & Wang, 2010). *Bifidobacteria* presence in human gut can lower the uptake of lipopolysaccharide from the gut lumen, reinforcing tight junctions of epithelial cells. It can also prevent inflammatory diseases, contributing to the equilibrium of the immune system, and intervening in gut-brain axis (Turroni et al.,

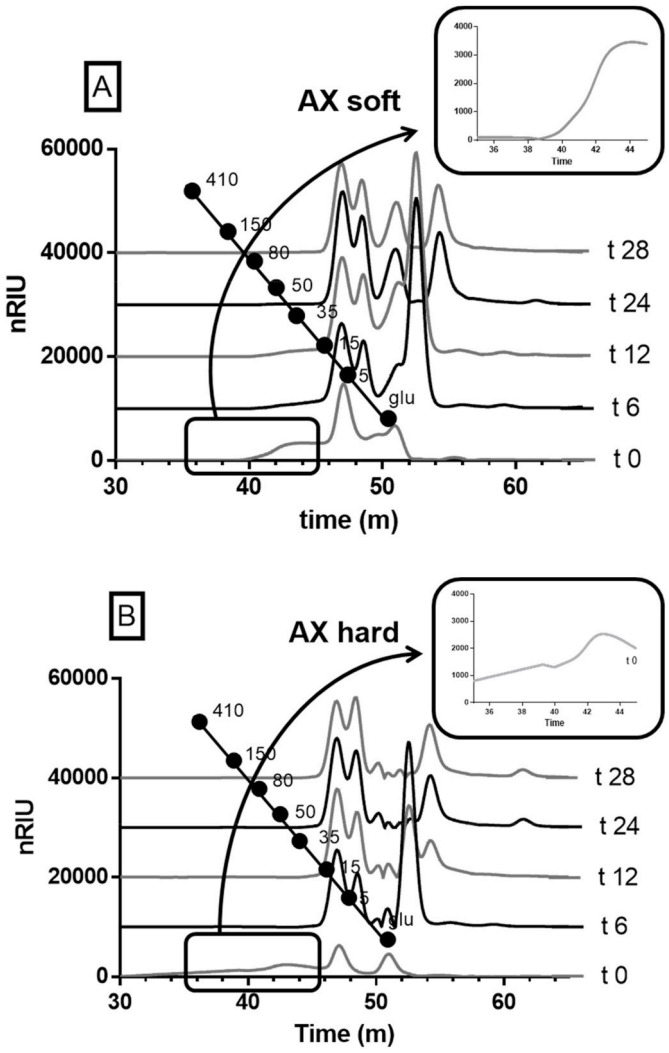

Fig. 3. Variation of the molecular size profile of the WE-AX of the soft (A) and hard (B) wheat during the fermentation with human fecal samples. The curve is made from the average of the measurements made in triplicate. The standard curves are depicted in the graphics and each point represents the peak and the retention time of each standard molecule with a determined molecular weight (kDa) as described in Material and Methods. nRIU: Refractive Index Unit.

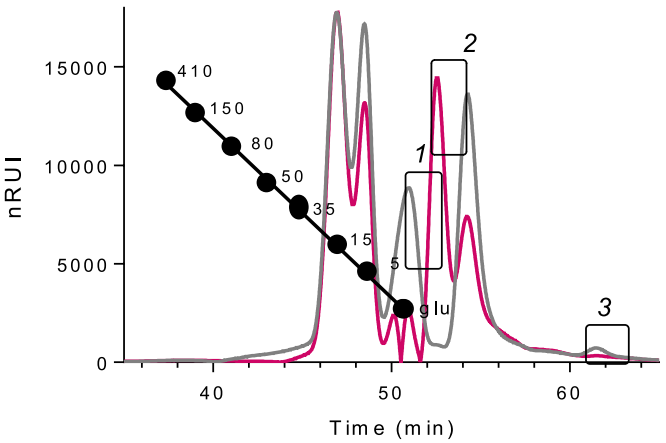

Fig. 4. Differences on the profile of molecular size between WE-AX of the soft arabinoxylans as grey line (AXs) and hard arabinoxylans as pink line (AXh) at 12 h of the fermentation with human fecal sample. The 12 h fermentation was the time point with the highest differences in HPSEC profiling for both samples. The squares with numbers indicate the most important differences. The standard curve is depicted in the graphic and each point represents the peak and the retention time of each standard molecule with a determined molecular weight (kDa) as described in Material and Methods. 1) 51 min peak; 2) 53 min peak; 3) 62 min peak. nRIU: Refractive Index Unit. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

2014). In previous study, Aguirre, De Souza, and Venema (2016) illustrated that the fermentation of arabinogalactan and inulin with whole faecal samples from lean and obese subjects increased the abundance of *Lactobacillus,* and *Bifidobacterium.* Other researchers have suggested that the increase in the consumption of whole wheat is associated with a higher abundance of *Bifidobacterium* and *Lactobacillus*, compared with the intake of refined wheat (Costabile et al., 2008; Rosa-sibakov, 2015).

The increase in *Lactobacillus* and *Bifidobacterium* was different in both extracts obtained from soft and hard genotypes. Different wheat pools had possible unequal effects in the microbiome due to bacteria amount and SCFA quantities results, which could be related to the AX structure and composition; this agrees with Gong, Chi, Wang, Zhang, and Sun (2019), who studied the *in vitro* fermentation of whole and refined wheat flour using human faeces samples.

Bifidobacteria and *Lactobacillus* can produce xylosidases, α-L-arabinofuranosidases (ARF), and xylanases (XYL) for the initial steps of AX fermentation (Crittenden et al., 2002). Because AXs fermentation significantly increased *Bifidobacterium* abundance, it can be speculated that AXs and AXh have different structures, as has already been described in an earlier work from our group (Paesani et al., 2020). In the case of AXs, it can be metabolised more efficiently than AXh. This ability to metabolise AX is strain dependent and depends on different types of enzymes produced which break the specific AX glycoside bonds. The presence of arabinose substituents could prevent the access of xylanases to hydrolyse the xylose skeleton (Rose et al., 2010). The differences on the WE-AX degradation might be related to xylosidases and ARF produced by *Bifidobacteria* and xylanases produced by other microorganisms in gut microbiota (Flint, Duncan, & Scott, 2007; Sun et al., 2017). ARF and XYL enzymes perform very specific hydrolysis of the backbone of β1-4 xylose with branched arabinose 1α-2 and 1α-3 (Correia et al., 2011; McCleary et al., 2015).

3.4. Variation of the molecular size of the WE-AX during fermentation

The utilisation of the WE-AX by inoculum was confirmed by the decrease in the molecular weight of these compounds during the fermentation. The first AXs peak found at the beginning of the fermentation (t 0) corresponds to the fraction of molecular size between 100 and 20 KDa, which decreased significantly after 6 h and disappeared after 12 h (Fig. 3). The 49 min peak appears for the first time at 6 h of fermentation and corresponds to molecules of approximately 3 KDa. The utilisation of AXs can be observed by the higher peak of around 5 kDa (47 min) after 12 h of fermentation. The peaks after 52 min (glucose) represent metabolites from fermentation, such as organic acids, with clear differences at various time points.

AXh showed a start of a peak detection at 32 min and presented two peaks of high molecular weight at t 0, one eluted at 39 and the other at 41 min (refer to the zoomed pictures inside Fig. 3). Both peaks disappeared completely after 6 h, indicating that the high molecular weight compounds were hydrolysed by the inoculum bacteria. The high molecular weight peaks were not visualised in AXs samples, and a peak detection started at 40 min. In the same way as AXh fermentation, at the time of 6 h, the proportion of the peaks after 40 min disappeared, and peaks approximately corresponding to 5 KDa (47 min) and 1 KDa (51 min) increased. These peaks were still observed at 12 h and decreased significantly at 24 and 28 h.

In both cases (AXs and AXh), the overall molecular size of polysaccharides decreased during the fermentation. No changes were observed after 24 h, confirming that fermentation of the AX had already

been completed. However, after 6 and 12 h, the profiles found for the AX extracts at the same time of fermentation presented different profiles (Fig. 4). On the 12 h of fermentation, at 51 min, a large peak (1) was observed, and at 62 min, a small one (3) was observed for AXs but not for AXh. In addition, at 53 min, a peak (2) was only observed for AXh. Since theses peaks are below the glucose elution, they are not characterize by carbohydrates but for organic acids formed during fermentation, demonstrating AXs and AXh hydrolysis could have produced different compounds because of different structures. More studies should be done to characterize these compounds.

Hughes et al. (2007) have reported that all fractions of AX showed bifidogenic impact, but this bifidogenic effect was clearly greater as the molecular mass decreased. They have also reported that AX with lower molecular weights were particularly selected for *Lactobacillus* and *Eubacterium*, and the high molecular weight fractions significantly increased the *Bifidobacterium* quantities. In our work, the molecular size of WE-AX could have influenced the fermentation speed as well as the selective growth or activity of beneficial intestinal bacteria. AXs fermentation favoured *Bifidobacterium* growth while AXh increased butyric acid production. The differences found in our study could be due not only to the molecular weight of the extracts but also to the types of linkages of the structure, which can influence bacteria enzyme activity and fermentation. The differences found between the speed and the fermentation capacity of the two AX samples (AXs and AXh), as well as the effects they exert in the intestine, would be due to the different size range of the molecules and the degree of substitution. Since different types of bonds and substitutions are known to exist in arabinoxylans, they can be affected by the action of several different enzymes, including XYL and ARF (Grabber, Hatfield, & Ralph, 1998; Grabber, Ralph, & Hatfield, 1998; Sorensen, Pedersen, Jorgensen, & Meyer, 2007).

4. Conclusion

WE-AX from soft wheat showed a prebiotic effect thus possibly while WE-AX from hard wheat produced more butyric acid. This could be due to modulation of the human intestinal microbiota in a beneficial way, but more studies should be done to confirm this. Both WE-AX colonic *in vitro* fermentation showed a high production of total SCFA, which exert health benefits at local and systemic levels. The use of WE-AX by intestinal bacteria can be verified by the decrease of pH and the increase of pressure inside flasks, and also the decreasing molecular sizes during colonic fermentation. The different utilisation of the arabinoxylans deriving from various genotypes of wheat reinforces the important influence of the nondigestible carbohydrate source on their chemical structures and the consequent fermentation. The consumption of foods which contain WE-AX (either supplemented or enriched) would have beneficial effects on human health, even on some disorders or diseases characterised by intestinal microbiome dysbiosis. The consumption of WE-AX to maintain the balance of or favourably modulate the profile of the intestinal microbiome can be an important contribution to the quality of human life.

CRediT authorship contribution statement

Candela Paesani: Methodology, Validation, Formal analysis, Investigation, Writing - original draft, Visualization. **Lorena S. Sciarini:** Formal analysis, Writing - review & editing. **Malena Moiraghi:** Validation, Formal analysis. **Emiliano Salvucci:** Writing - review & editing. **Samira Prado:** Validation, Writing - review & editing. **Gabriela Teresa Pérez:** Conceptualization, Writing - review & editing, Supervision, Project administration, Funding acquisition. **João Paulo Fabi:** Conceptualization, Writing - review & editing, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare they have no conflict of interest declared.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2020.110253.

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