PAPER

ARABINOXYLANS AND β-GLUCANS ASSESSMENT IN CEREALS

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ABSTRACT

Arabinoxylans (AX) and β-glucans are the major source of soluble dietary fibre in cereals and have a significant role as functional/bioactive ingredients implicated in lowering plasma cholesterol, postprandial blood glucose and improving lipid metabolism. In this work, the variation in the content and solubility of AX and β-glucans in different cereal species and varieties were studied. Different methods (phloroglucinol, orcinol-HCl, HPAEC-PAD) for AX analysis were tested. The results confirmed the variability in contents of both polymers (AX and β-glucans) in cereal species and varieties as well as providing additional information useful for their characterization.

Keywords: Arabinoxylans, barley, β-glucans, emmer, spelt, wheat

1. INTRODUCTION

The most common source of dietary fibre are the outer layers and the endosperm cell walls of cereal grains (wheat, barley, oat, rye etc.). The non-starch polysaccharides (NSP) found in mature cereal grain include the arabinoxylans (AX), which make up the pentosan portion of the insoluble fibre fraction and the β-glucans which are major components of starchy endosperm and aleurone cell walls.

AX have been identified in a variety of tissues of major cereals: wheat, rye, barley, oats, rice, sorghum (FINCHER and STONE, 1986). Although these polysaccharides are minor components of the whole caryopsis, they still account for a substantial fraction of the cell walls and thus constitute a major portion of the dietary fibre of wheat flours (SKENDI *et al*., 2011). Wheat varieties differ in AX amount and properties (MASLEN *et al*., 2007) such as average molecular weight and distribution, branching pattern, extractability with water (FINNIE *et al*., 2006) and interaction with other cell wall components such as lignin or cellulose (REVANAPPA *et al*., 2007).

AX consist mainly of a xylan chain with *β*-1,4-linked D-xylopyranosyl residues (Xyl) to which mostly single *α*-L-arabinofuranose units (Ara) are linked at the O-2 and/or O-3 positions of the xylose units as side residues (IZYDORCZYK and BILIADERIS, 1995). In wheat AX, approximately 66% of the xylose residues, that form the backbone chain, are unsubstituted Xyl (SAULNIER *et al*., 2007b). Moreover, some Ara units carry ferulic acid residues esterified to O-5 of Ara linked to O-3 of the xylose residues (SOSULSKI *et al*., 1982). Extractable AX and unextractable AX structures are similar, showing only slightly differences in molecular weight and in the Ara/Xyl ratio (IZYDORCZYK and BILIADERIS, 1995).

AX represent the major polysaccharides in the aleurone fraction (65%) of the wheat caryopsis, and the arabinose to xylose ratio decreases from the pericarp to the endosperm; furthermore AX from the aleurone layer as well as AX from starchy endosperm have a lesser degree of branching than acidic AX from pericarp/testa, which may improve their solubility and their digestibility (BROUNS *et al*., 2012; SAULNIER *et al*., 2007a).

β-glucans are linear polymers of high molecular weight consisting of D-glucose molecules linked by β-(1-4) and β-(1-3) linkages; the presence of β-linkages (1-3) gives to the molecule an irregular shape that makes the β-glucans flexible and partially soluble in water (PAPAGEORGIOU *et al*., 2005; SHELAT *et al*., 2011).

From a nutritional point of view, as major non-starch polysaccharides of various cereals and main constituents of cell walls of wheat, rye, barley and oat, β-glucans and soluble AX have nutritional benefits in humans (WARD *et al*., 2008). The positive effects of AX are related to the ability to reduce postprandial blood glucose levels (GARCIA *et al*., 2007; LU *et al*., 2000), lowering levels of potentially toxic ammonia in the colon and the ability to reduce levels of triglycerides in the blood (GARCIA *et al*., 2006). Over the last two decades β-glucans have been considered as bioactive ingredients due to their capacity in lowering plasma cholesterol, improving lipid metabolism, and reducing the glycaemic index (MÄKELÄINEN *et al*., 2007; WOOD, 2007). These positive effects increased the popularity and consumption of cereal-based foods as well as of many other foods fortified with cell wall-enriched grain fractions, β-glucan concentrates and isolates (LAZARIDOU and BILIADERIS, 2007).

Furthermore, the Commission Regulation (EU) No 432/2012 of 16 May 2012 (Official Journal of the European Union 25-05-2012) included arabinoxilans and β-glucans in the list of authorized health claims.

In this study, the content of AX and β-glucans in several cereal wholemeal flours was determined using different techniques, whose efficacy was compared.

2. MATERIALS AND METHODS

2.1. Samples

- Five barley varieties, Acquarelle, Braemar, Kelibia, Naturel, USA 2 (waxy variety), were provided by Agroalimentare Sud S.p.A. (Melfi, Potenza, Italy).
- Five spelt varieties, Ebners Rotkorn, Hercule, Oberkulmer, Redoute, Triventina, were provided by Agenzia Regionale per lo Sviluppo Agricolo, Rurale e della Pesca (ARSARP, Campobasso, Italy).
- Five emmer varieties, Molise, Angelo, Garfagnana, Molise Colli, Guardiaregia, were provided by ARSARP (Campobasso, Italy).
- Two durum wheat varieties, Cappelli, Saragolla, were provided by ARSARP (Campobasso, Italy).
- Three soft wheat varieties, Roscetta, Solina 1, Bianchetta, were provided by ARSARP (Campobasso, Italy).

Samples were milled in a refrigerated laboratory mill IKA A 10 Labortechnic (Tanke & Kunkel, Gmbh & Co., Staufe, Germany) and stored in aliquots of 100 g at 4°C in a closed containers until analysis.
Data reported for all parameters are the average values of three different aliquots of each sample. All results are expressed as % dry weight (d.w.). Moisture content was determined according to ICC method 109/1 (ICC, 1995).

2.2. Reagents

NaOH 50% (p/v) was purchased from Baker (Mallinckrodt Baker B.V., Deventer, Holland), high-purity laboratory water was produced by means of a MilliQ-Plus apparatus (Millipore S.p.A., Milano, Italy); glucose, xylose, arabinose, fructose, standards were from Sigma Chemical Co. (St. Louis, MO, USA); all other chemicals and reagents of HPLC grade were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

2.3. AX analysis by phloroglucinol method

Total AX analysis was performed as reported by DOUGLAS (1981). Flour (5.0 mg) was added to 2 mL of water followed by 10 mL of a solution of glacial acetic acid, hydrochloric acid and phloroglucinol in a stoppered tube. The tube was placed in a boiling water bath for 25 min and the absorbance of the resulting solution measured at 552 nm and 510 nm.

2.4. AX analysis by orcinol-HCl method

Water soluble AX and total AX analysis by orcinol-HCl method was performed as reported by HASHIMOTO *et al*. (1987).
Water soluble AX: 100 mg of flour sample were shaken in water at 30°C for 2 h and centrifuged. Aliquots of the supernatant were hydrolyzed with 4N HCl at 100°C. The AX content was estimated, after treatment in boiling water bath with $FeCl_3$ and orcinol and by reading the absorbance at 670 nm.
Total AX: flour (10 mg) was weighed into a glass tube, where 2 mL of 2N HCl was added, and the mixture was hydrolyzed at 100°C for 150 min. After cooling, neutralization was carried out by adding 2N sodium carbonate and fermentable sugars were removed by means of fresh compressed yeast. The mixture was then centrifuged and an aliquot of the supernatant was treated with $FeCl_3$ and orcinol in boiling water bath; the absorbance was read at 670 nm.

2.5. AX analysis by HPAEC-PAD

Determination of arabinoxylans was performed as reported by MESSIA *et al*. (2016). Briefly, for water soluble AX: 100 mg of a flour sample were shaken in 10 mL of water at 30°C for 2 hours and centrifuged. Aliquots (1 mL) of the supernatant were hydrolyzed with 1 mL of 4N HCl for 2 hours. For total AX, flour sample (10 mg) was weighed into a glass tube, 2 mL of 2N HCl were added, and the mixture was hydrolyzed at 100°C for 150 min. After cooling, neutralization was carried out by the addition of 2N sodium carbonate. Diluted samples were injected in a chromatographic system equipped with a Rheodyne injector (Cotati, CA, USA) with a 25 μL loop. The chromatographic separation was carried out with a Carbopac PA1 (250x2 mm) (Dionex Corporation, Sunnyvale, CA, USA) analytical column. The chromatographic run (22 min) and the quantitative determination were conducted with a 0.25 mL/min flow rate, using a mobile phase of water and 200 mM sodium hydroxide (90%-10%). The control of the instrument, the data collection and the total quantification were carried out by the chromatographic software Chromeleon (Dionex). An HPAEC-PAD Dionex system (Dionex Corporation, Sunnyvale, CA, USA) composed of a gradient pump (mod GP50) with an on-line degaser and electrochemical detector (model ED40) was used. The flow-through electrochemical cell (Dionex) consisted of a 1 mm diameter Gold Working Electrode, a pH reference electrode, and a titanium body of the cell as the counter electrode. The optimized time-potential waveform used was: 0.1 V at 0-0.40 s, -2.00 V at 0.41-0.42 s, 0.60 V at 0.43 s, -0.10 V at 0.44-0.50 s.
Total and soluble AX were quantified on the basis of the Ara and Xyl content in the hydrolyzed sample: ([Ara] + [Xyl] x D x 0.88), where: D = dilution factor; 0.88 =adjustment for free sugar to anhydrous sugar.

2.6. β-glucans analysis

Total β-glucans were determined using the K-BGLU assay kit (Megazyme International Ltd., Ireland). Insoluble fractions were determined after extraction of soluble β-glucans with water for 2 h at 38°C (ÅMAN and GRAHAM, 1987). Soluble β-glucans were calculated as the difference between the total and insoluble components.

2.7. Statistical analysis

All analyses were carried out in triplicate. Results were expressed as means±Standard Deviation (SD).
A one-way analysis of variance (ANOVA) was performed, considering species, variety or analytical method as factor. When significant differences ($p < 0.05$) were detected, Fisher's least significant difference (LSD) was computed. All the statistical tests were performed using the software IBM SPSS statistics 23.

3. RESULTS

3.1. AX analysis and quantification

A comparison between three different analytical methods (HPAEC-PAD and colorimetric methods) was carried out in order to devise a practical system for screening of different varieties of grains such as barley, wheat, spelt and emmer (Table 1).

Table 1. Total and soluble AX content in different cereal wholemeals determined by three different methods (g/100g d.w.±SD).

Sample	Phloroglucinol	Orcinol-HCl		HPAEC-PAD				
				Total		Soluble		Soluble AX/ Total AX
	Total AX	Total AX	Soluble AX	Total AX	A/X	Soluble AX	A/X	
Barley								
Acquarelle	4.92±0.05^{b}	6.42±0.06^{d}	0.09±0.03^{a}	6.18±0.03^{d}	0.85	0.08±0.02^{a}	0.65	0.013
Braemar	4.30±0.04^{d}	5.94±0.05^{e}	0.06±0.06^{a}	5.21±0.07^{e}	0.75	0.07±0.03^{a}	0.60	0.013
Kelibia	5.08±0.06^{c}	7.30±0.08^{b}	0.08±0.04^{a}	6.87±0.05^{b}	0.73	0.10±0.03^{a}	0.66	0.015
Naturel	4.85±0.03^{b}	7.01±0.03^{c}	0.08±0.06^{a}	6.76±0.04^{c}	0.72	0.09±0.04^{a}	0.62	0.013
USA 2	6.17±0.08^{a}	9.75±0.04^{a}	0.11±0.05^{a}	8.96±0.03^{a}	0.47	0.12±0.05^{a}	0.71	0.013
mean	**5.06$^{b\alpha}$**	**7.28$^{a\beta}$**	**0.08$^{A\beta}$**	**6.80$^{a\beta}$**	**0.70**	**0.09$^{A\alpha}$**	**0.65**	**0.013**
Wheat								
Cappelli*	5.10±0.04^{c}	8.78±0.05^{a}	0.09±0.02^{a}	8.21±0.04^{a}	0.78	0.10±0.03^{a}	1.25	0.012
Saragolla*	5.18±0.03^{bc}	8.17±0.04^{d}	0.09±0.03^{a}	7.90±0.02^{b}	0.80	0.10±0.06^{a}	0.80	0.013
Roscetta**	5.49±0.05^{a}	8.76±0.05^{a}	0.11±0.03^{a}	8.16±0.10^{a}	0.66	0.12±0.03^{a}	0.71	0.015
Solina 1**	5.15±0.04^{bc}	8.54±0.07^{b}	0.10±0.04^{a}	7.97±0.04^{b}	0.71	0.12±0.04^{a}	0.71	0.015
Bianchetta**	5.23±0.06^{b}	8.32±0.04^{c}	0.11±0.03^{a}	7.98±0.03^{b}	0.74	0.13±0.05^{a}	0.71	0.016
mean	**5.23$^{c\alpha}$**	**8.51$^{a\alpha}$**	**0.10$^{A\alpha}$**	**8.04$^{b\alpha}$**	**0.74**	**0.11$^{A\alpha}$**	**0.84**	**0.014**
Spelt								
Ebners Rotkorn	4.18±0.05^{b}	5.57±0.04^{b}	0.14±0.04^{a}	5.41±0.05^{a}	0.66	0.11±0.02^{a}	0.77	0.020
Hercule	4.29±0.06^{a}	5.97±0.05^{a}	0.14±0.03^{a}	5.34±0.07^{a}	0.68	0.12±0.02^{a}	0.85	0.022
Oberkulmer	3.99±0.04^{d}	5.18±0.06^{c}	0.12±0.03^{a}	4.75±0.05^{c}	0.64	0.12±0.04^{a}	0.95	0.025
Redoutè	3.89±0.07^{c}	5.13±0.05^{c}	0.14±0.03^{a}	4.60±0.06^{d}	0.61	0.10±0.02^{a}	0.80	0.022
Triventina	4.20±0.04^{b}	5.63±0.10^{b}	0.11±0.02^{a}	5.03±0.03^{b}	0.65	0.11±0.03^{a}	0.78	0.022
mean	**4.11$^{c\beta}$**	**5.50$^{a\gamma}$**	**0.13$^{A\alpha}$**	**5.03$^{b\gamma}$**	**0.65**	**0.11$^{A\alpha}$**	**0.83**	**0.022**
Emmer								
Molise	3.65±0.04^{c}	6.48±0.04^{b}	0.23±0.04^{a}	5.89±0.04^{b}	0.67	0.18±0.05^{a}	1.04	0.031
Angelo	3.76±0.03^{b}	5.26±0.06^{c}	0.11±0.05^{b}	5.03±0.05^{d}	0.70	0.10±0.06^{b}	1.16	0.020
Garfagnana	4.12±0.09^{a}	5.63±0.03^{d}	0.11±0.03^{b}	5.29±0.03^{c}	0.62	0.11±0.05^{b}	1.00	0.021
Molise Colli	4.06±0.05^{a}	5.31±0.05^{c}	0.09±0.05^{b}	4.90±0.08^{e}	0.58	0.07±0.04^{b}	1.10	0.014
Guardiaregia	3.60±0.06^{c}	6.76±0.07^{a}	0.12±0.04^{b}	6.32±0.04^{a}	0.69	0.08±0.05^{b}	1.40	0.013
mean	**3.84$^{c\gamma}$**	**5.89$^{a\gamma}$**	**0.13$^{A\alpha}$**	**5.49$^{b\gamma}$**	**0.65**	**0.11$^{A\alpha}$**	**1.14**	**0.020**

*durum wheat; **soft wheat
Different superscript letters (total AX=lower case, soluble AX=upper case) between species means within a row indicate statistically significant differences at P< 0.05.
Different superscript letters between means (species=Greek font, variety=italic font) within a column indicate statistically significant differences at P< 0.05.

The systematic quantification of AX in cereal wholemeal flours revealed substantial differences between the colorimetric procedures. Although phloroglucinol and orcinol-HCl procedures are both based on colorimetric assessments, for the measurement of total AX, the phloroglucinol method provided values that were significantly lower than those of the orcinol-HCl method. This underestimation of total AX can be attributed to the type of extraction performed for the phloroglucinol assay, that leads to a relevant release of hexoses from starch hydrolysis, which greatly exceeds pentoses, influencing their

evaluation. In the orcinol-HCl method, the high glucose concentrations are removed by the action of the yeast *Saccharomyces cerevisiae*, thus providing a better assessment of the total AX; additionally the orcinol-HCl method allows to evaluate not only the total AX but also the soluble AX, that cannot be quantified with the phloroglucinol method.
A significant advantage of the HPAEC-PAD method is to provide more detailed information, as it offers the possibility to assess the content of individual sugars Ara and Xyl, which are constituents of the AX chain, and it allows to calculate the ratio Ara/Xyl (A/X), an important factor related to the behavior of the flour during technological processes. HPAEC-PAD assessments also give a more accurate estimation of the sugars present in the chain of AX without suffering problems caused by glucose interference, thus providing a more accurate and precise quantitative analysis. Finally, the HPAEC-PAD can be applied to matrices with high contents of AX (bran) as well as to refined flour (low AX content).
To sum up, the assessment made using the phloroglucinol is not reliable, whereas the values of total and soluble AX found in varieties of barley, wheat, spelt and emmer by using of either the orcinol-HCl or HPAEC-PAD methods are similar and comparable to the values of AX reported in the literature (BERGER and DUCROO, 2005, HENRY, 1987) for these cereals.
GEBRUERS *et al*. (2008) have published data about the content of AX in refined flours and in bran of wheat, spelt and einkorn showing a comparable value of total AX in the different types of flour examined. Instead, the results obtained in this trial show a much lower content of total AX in spelt an emmer (≈ 5.26%) compared to barley and wheat (6.80% and 8.04% respectively). This is in spite of the fact that spelt and emmer are phylogenetically close to wheat.
The data obtained also show a generally close relationship between AX content and cereal varieties. Among barleys, USA2, a waxy (i.e. having a low amylose content) variety has the highest content of β-glucans (9.5%) (Table 2) and of total AX (6.2%). The content of AX in barley also depends on genetic and environmental factors (IZYDORCZYK and DEXTER, 2008) but appears to be less variable than that of β-glucans.
The HPAEC-PAD method allows to compute the ratio A/X which indicates the degree of branching in the polymer chain, thus permitting to deduce information about the AX structure of different species and varieties. A high A/X ratio corresponds to a higher proportion of mono-substituted xylosyl residues and a lower proportion of unsubstituted xylosyl residues. The degree of substitution of xylan backbone is relevant for predicting the cereal behavior when subjected to different technological processes. Emmer varieties showed an A/X ratio of soluble AX (1.14), much higher than the ratios of barley (0.65), wheat (0.84) and also spelt (0.83).
From a technological point of view, the quantification and the assessment of variations in the overall AX content is relevant because AX is generally considered to have a significant effect on wheat functionality and also to affect suitability of flours for certain applications (GEBRUERS *et al*., 2008). Cereal varieties rich in AX, have a strong potential for the production of healthy or even health promoting food products that contain not only a high overall dietary fiber content but also increased levels of soluble dietary fiber as well as prebiotic oligosaccharides which are produced by the in situ action of xylanases (GEBRUERS *et al*., 2008).

3.2. β-glucans analysis and quantification

Total, insoluble and soluble β-glucans were quantified in different cereals (barley, wheat, spelt, emmer) flours (Table 2). Results showed a different β-glucans distribution in tested species and between waxy and non waxy barley varieties.

Table 2. β-glucans content, AX+ β-glucans and AX/β-glucans in different cereal wholemeals (g/100g d.w.±SD).

Sample	β-glucans				AX+β-glucans	AX/β-glucans
	Total	Insoluble	Soluble	Soluble/ Insoluble		
Barley						
Acquarelle	4.43±0.13^{c}	1.50±0.01^{e}	2.94	1.97	10.61	1.40
Braemar	3.89±0.11^{e}	1.92±0.07^{b}	1.97	1.03	9.07	1.34
Kelibia	4.17±0.07^{d}	1.61±0.02^{d}	2.57	1.60	11.04	1.65
Naturel	4.67±0.11^{b}	1.81±0.04^{c}	2.86	1.58	11.43	1.45
USA 2	9.49±0.09^{a}	3.42±0.08^{a}	6.08	1.78	18.45	0.94
mean	**5.33^{A}**	**2.05^{A}**	**3.28**	**1.59**	**12.12**	**1.36**
Wheat						
Cappelli *	0.52±0.03^{c}	0.41±0.03^{b}	0.11	0.27	8.73	15.79
Saragolla *	0.42±0.02^{d}	0.22±0.01^{c}	0.20	0.93	8.32	18.81
Roscetta **	0.60±0.05^{b}	0.42±0.06^{b}	0.19	0.45	8.76	13.60
Solina 1 **	0.53±0.03^{c}	0.37±0.03^{b}	0.16	0.42	8.50	15.00
Bianchetta **	0.79±0.01^{a}	0.60±0.03^{a}	0.19	0.31	8.77	10.10
mean	**0.57^{B}**	**0.40^{B}**	**0.17**	**0.47**	**8.62**	**14.66**
Spelt						
Ebners Rotkorn	0.68±0.02^{ab}	0.44± 0.05^{ab}	0.24	0.55	6.09	7.96
Hercule	0.63±0.05^{b}	0.42± 0.04^{ab}	0.21	0.50	5.97	8.48
Oberkulmer	0.70±0.02^{a}	0.49± 0.03^{a}	0.21	0.43	5.45	6.78
Redoutè	0.65±0.03^{ab}	0.47± 0.03^{a}	0.18	0.38	5.25	7.08
Triventina	0.57±0.02^{c}	0.40±0.03^{b}	0.17	0.43	5.60	8.82
mean	**0.65^{B}**	**0.44^{B}**	**0.20**	**0.46**	**5.67**	**7.82**
Emmer						
Molise	0.48±0.03^{b}	0.37±0.01^{a}	0.12	0.32	6.37	12.27
Angelo	0.41±0.02^{c}	0.29±0.05^{b}	0.12	0.42	5.44	12.27
Garfagnana	0.37±0.01^{c}	0.28±0.03^{b}	0.09	0.30	5.66	14.30
Molise Colli	0.53±0.02^{a}	0.35±0.03^{ac}	0.18	0.51	5.43	9.24
Guardiaregia	0.48±0.03^{b}	0.30 ±0.02^{bc}	0.18	0.61	6.8	13.17
mean	**0.45^{B}**	**0.32^{B}**	**0.14**	**0.43**	**5.94**	**12.25**

*durum wheat; **soft wheat.
Different superscript letters between means (species=upper case, variety=lower case) within a column indicate statistically significant differences at $P< 0.05$.

In barley, the variable β-glucans content can be influenced by genotype, culture practices and environmental growing conditions (NEWMAN and McGUIRE,1985; Newman and

Newman, 2008). The presence of waxy genes can influence polysaccharides biosynthesis and their composition in the kernel. In the "normal" barley genotype, the starch is composed of about 25% of amylose and 75% amylopectin while in the waxy genotypes the starch is almost exclusively composed of amylopectin (95-100%). The reduced level of starch is usually accompanied with an increased content of β-glucans in the cell walls of the starchy endosperm (ANDERSSON *et al*., 2008). In fact, according to many research reports (ABDEL-AAL *et al*., 2005; WOOD *et al*., 2003) a waxy barley variety (USA2) showed a β-glucans content which was twofold that of non waxy varieties.

Comparing the data from different species (Table 2), it is evident that there is a significant difference in the average content of total β-glucans among barley and wheat, spelt and emmer (5.33% d.w. for barley, 0.57% d.w for wheat, 0.65% d.w. for spelt and 0.45% d.w. for emmer), confirming the data reported in the literature by other authors (SKENDI *et al*., 2003).

With regard to soluble β-glucans content, varieties of wheat, spelt and emmer are characterized by very low levels of soluble β-glucans (average: 0.17%, 0.20% and 0.14% d.w., respectively), probably related to a higher ratio of cellotriose/cellotetraose, which generally amounts to 4.6 and 3.3 respectively in wheat and barley. Statistically, although the distribution of cellotriose and cellotetraose units linked by β (1-3) is random (WOOD *et al*., 2003), the probability of a repetition of ordered cellotriose units is greater in wheat than in barley and oats (CUI *et al*., 2000). Since the structure is more ordered and more inter chain associations are favoured, the water solubility of β-glucans derived from wheat is lower than that of other cereals (LAZARIDOU and BILLIADERIS, 2007).

The soluble β-glucans content affects the soluble/insoluble ratio, which is greater in barley (1.59) than in wheat, spelt and emmer (0.47, 0.46 and 0.43 respectively). Data on total content of AX + β-glucans and their ratio (AX/β-glucans) (Table 2) showed significant differences between the analyzed wholemeals. The high content of AX + β-glucans in barley corresponded to a low AX/β-glucans ratio, which is further reduced in the waxy barley variety USA 2 (0.94). While β-glucans are mainly present in barley, the AX are distributed in all the analyzed species and varieties. The relevant presence of two polymers in barley makes this cereal an excellent ingredient for the preparation of products with an increased content of total and soluble dietary fiber, capable of enhancing both the physiological effects and health benefits (VERARDO *et al*., 2011a; VERARDO *et al*., 2011b; VITAGLIONE *et al*., 2010).

Moreover, β-glucans and AX are the chief structural constituents of cell wall in various tissues of the barley grain. In the starchy endosperm of mature barley grain, the matrix-phase AX and β-glucans may represent up to 85% of total cell wall polysaccharides. The endosperm cell walls are mainly made up of β-glucans and contains a smaller amount of AX, while aleurone cell walls are composed primarily of AX (67-71%), with smaller amounts of β-glucans (26%). Various studies have been carried out as to the possibility of producing barley flour enriched in β-glucans using air classification and dry milling methods to produce an enriched flour that can be used for producing different cereal products, such as bread, muffins, and pasta (MARCONI *et al*., 2000).

4. CONCLUSIONS

The present study revealed a wide variability in the content of AX and β-glucans in wholemeal of different cereal species and varieties.

Barley varieties showed a higher β-glucans and AX content compared to other cereal species/varieties. The assessment of the A/X ratio for AX and soluble/insoluble β-glucans ratio, which directly affect the behavior of cereal flours during transformation, are useful for deciding the end use of grains and evaluating the nutritional quality of the analysed grains.
The HPAEC-PAD method proved to be advantageous compared to colorimetric methods. It allows to compute the ratio A/X which indicates the degree of branching in the polymer chain, thus permitting to deduce information about the AX structure of different species and varieties.
The quantification and the assessment of variations in the overall AX content is relevant because AX is generally considered to have a significant effect on cereal flours functionality and also to affect suitability of flours for certain applications.
Cereal varieties rich in AX and β-glucan, have a strong potential for the production of healthy or even health promoting food products that contain not only a high overall dietary fiber but also increased levels of soluble dietary fiber, that should meet the health claims listed in the Commission Regulation (EU) No 432/2012.

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