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EXTRACTION AND CHARACTERIZATION OF ARABINOXYLANS FROM SPELT BRAN AND HEMICELLULOSES FROM SPELT HULL BY CHEMICAL AND ENZYMATICAL METHODS

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Summary

ESCARNOT Emmanuelle (2012) - Extraction and characterization of arabinoxylans from spelt bran and hemicelluloses from spelt hull by chemical and enzymatical methods. (PhD Thesis). Université de Liège - Gembloux Agro-Bio Tech, Belgique. 212 pages, 31 tables, 29 figures.

Spelt, a minor cereal, with hulled grain, high straw, suited to low input farming is mainly cultivated in Belgium, Germany and Switzerland. Development with high added value is sought after for spelt by-products. Dietary fibers have benefit effects for health and many applications. Through the methods from Prosky and Van Soest, were quantified 10.3% of total fiber in whole grain flour and 25.6% in spikelet flour; 18.6% hemicelluloses in bran and 35.5% in hull. Bran and hull were confirmed as rich sources of hemicelluloses. From bran, 7.1% of water-extractable arabinoxylans were isolated (molecular weight: 7-8 and 28 kDa) and 48% of water-unextractable were extracted trough alkaline peroxide hydrogen with molecular weight of 7-8 and 310-415 kDa. Hemicelluloses from hull were extracted through alkaline peroxide hydrogen, sodium and potassium hydroxyde with yields of 15.6-49.6% and with organic acids with yields of 0.9-64.2%. Molecular weights also covered a large range from 1 to 367 kDa and from 0.28 to 11.3 kDa, according the type of method. Enzymatic solubilization of arabinoxylans by xylanases and beta-glucanases combined with a cellulase, enabled to reach a yield of 69.7% for bran and of 6.4% for hull. Degree of polymerisation ranged between 1 and 1164 for spelt hydrolysates and between 2 and 17 for hull hydrolysates. The thesis provides the procedure of several methods and characterizes the obtained products.

ESCARNOT Emmanuelle (2012) - Extraction et caracterisation des arabinoxylanes du son d'épeautre et des hemicelluloses de la balle d'épeautre par des méthodes chimiques et enzymatiques. (Thèse de doctorat). Université de Liège - Gembloux Agro-Bio Tech, Belgique. 212 pages, 31 tableaux, 29 figures.

L'épeautre, céréale mineure, à grain vêtu, à paille haute, adapté à des conditions de culture « faibles intrants », est principalement cultivé en Belgique, en Allemagne et en Suisse. Une valorisation à haute valeur ajoutée est recherchée pour les coproduits d'épeautre. Les fibres alimentaires présentent des effets positifs pour la santé et plusieurs applications d'intérêt. Par les méthodes de Prosky et Van Soest, ont été quantifiés 10.3% de fibres totales dans la farine complète et 25.6% dans la farine d'épillet ; 18.6% d'hemicelluloses dans le son et 35.5% dans la balle. Le son et la balle ont donc été confirmés en tant que sources d'hemicelluloses. Du son, 7.1% des arabinoxylanes solubles dans l'eau ont été isolés (poids moléculaire : 7-8 et 28 kDa) et 48% des insolubles ont été extraits par peroxyde d'hydrogène en conditions alcalines avec des poids moléculaires de 7-8 et 310-415 kDa. Les hemicelluloses de la balle ont été extraits par peroxyde d'hydrogène en conditions alcalines, hydoxyde de sodium et de potassium avec des rendements allant de 15.6 à 49.6% et avec des acides organiques avec des rendements compris entre 0.9 et 64.2%. Les poids moléculaires couvraient également une large gamme, de 1 à 367 kDa et 0.28 à 11.3 kDa, selon le type de méthode. La solubilisation enzymatique des arabinoxylanes par des xylanases et beta-glucanases associées à une cellulase, permet d'atteindre un rendement de 69.7% pour le son et de 6.4% pour la balle. Les degrés de polymérisation oscillent entre 1 et 1164 pour les hydrolysats du son et 2 et 17 pour ceux de la balle. La thèse fournit le mode opératoire de plusieurs méthodes et décrit les produits obtenus.

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List of publications

This thesis is based on the following papers and is divided into chapters which must be read as a logical progress of the whole work. Each chapter (except chapter 1) includes the following sections: abstract, introduction, material and method, results, discussion, and references.

Article 1

Quantitative and qualitative study of spelt and wheat fibres in varying milling fractions.
Escarnot E., Agneessens R., Wathelet B., Paquot M.
Food Chemistry, 122, (2010) 857-863.

Article 2

Extraction and characterization of water-extractable and water-unextractable arabinoxylans from spelt bran: Study of the hydrolysis conditions for monosaccharides analysis.
Escarnot E., Aguedo M., Agneessens R., Wathelet B., Paquot M.
Journal of Cereal Science, 53, (2011) 45-52.

Article 3

Characterization of hemicellulosic fractions from spelt hull extracted by different methods.
Escarnot, E., Aguedo, M., Paquot, M.
Carbohydrate Polymers, 85, (2011) 419-428.

Article 4

Enzymatic hydrolysis of arabinoxylans from spelt bran and hull
Escarnot, E., Aguedo, M., Paquot, M.
Journal of Cereal Science, 55, (2012) 243-253.

Article 5

Comparative review of the content and profiles of macronutrients in spelt and wheat
Escarnot E., Jacquemin J.-M., Agneessens R., Paquot M.
Biotechnology, Agriculture, Society and Environment, (2012) in press.

List of abbreviations

ADL	Acid Detergent Lignin
ADF	Acid Detergent Fiber
AE1	First Alkali Extract
AE1D	First Alkali Extract After Dialysis
AE2	Second Alkali Extract
AE2D	Second Alkali Extract After Dialysis
AX	Arabinoxylan
AXOS	Arabinoxylooligosaccharides
A/X ratio	Arabinose to Xylose ratio
C	Cellulose
CRR	Cellulose Rich Residue
DF	Dietary Fiber
d.m.	dry matter
DP	Degree of Polymerization
DW	Dry Weight
exp.	experiment
F	Filtrate
GAX	Glucuronoarabinoxylan
H	Hemicelluloses
HMW	High Molecular Weight
IF	Insoluble Fiber

L	Lignin
LMW	Low Molecular Weight
LR	Landrace
MW	Molecular Weight
NDF	Neutral Detergent Fiber
NSP	Non Starch Polysaccharide
P	Precipitate
PCR	Polymerase Chain Reaction
PWEM	Purified Water-Extractable Material
R	Residue
RP-HPLC	Reversed Phase-High Performance Liquid Chromatography
RVA	Rapid Visco Analyser
S	Solubilized
SDS	Sodium Dodecyl Sulfate
SF	Soluble Fiber
TF	Total Fiber
V	Volume of ethanol for precipitation
WE	Water-Extractable
WEM	Water-Extractable Material
WU	Water-Unextractable
WUM	Water-Unextractable Material
XOS	Xylooligosaccharides

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1 CHAPTER 1

General introduction

In 2007, global cereal production was 2,520 million tons. The three main cereals were maize (826 million tons), rice (686 million tons) and wheat (683 million tons), together accounting for 87.1% of the world's cereal production. Europe produced 504 million tons of cereals, with wheat dominating (248 million tons), followed by barley (105 million tons) and maize (93 million tons), together accounting for 88.5% of Europe's cereal production (FAOSTAT, 2011a). Globally, 46.7% of the cereal produced were used for food, 36.1% for feed and 17.2% for seed and other uses. In contrast, cereal production in Europe is geared more towards feed (56.8%) than food (24.8%) and seed and other uses (18.5%) (FAOSTAT, 2011b).

As the figures above indicate, the production diversity is low. This fact is seen as a food 'oligoculture' which poses some risk because the likelihood of catastrophic crop failure through insect pests or fungal diseases is greater with reduced genetic diversity. In addition, the use of so few different types of grains for human nutrition appears to be detrimental to human health in the long term. Concerns about micronutrient deficiencies in the human diet are increasing, as is the incidence of food allergies and intolerance. Different plant foods can provide different functional substances that have positive health effects, indicating that eating a wider variety of plant foods is advantageous. This is supported by dietary guidelines such as those produced by the Food Guide Pyramid made by the United States Departments of Agriculture and Health and Human Services which recommends 6 to 11 servings per day of cereal-based foods (Belton and Taylor, 2002). Over the past two decades there has been increased recognition of the importance of locally cultivated food plants as sources of micronutrients and plant secondary metabolites (Scoones *et al.,* 1992). There is therefore increasing interest in the lesser known cereals not currently used to their full potential. The advantage of these cereals is that they can be cultivated in environments unsuitable for the 'big three' (maize, rice and wheat), without using intensive agricultural practises. The Green Revolution, which involved using intensive cultivation practices to obtain high yields of the 'big three', has led to environmental degradation through soil denudation and environmental pollution due to pesticide and fertilizer runoff (Belton and Taylor, 2002). The role of these biologically diverse species in maintaining human and environmental health is now being highlighted, particularly in relation to global food security, sustainable development and the United Nations Millenium Development Goals (Frison *et al.,* 2006; Johns and Eyzaguirre, 2006). The generation and analysis of food composition data from locally cultivated food species is fundamental to the aims and activities of the 'Cross-Cutting Initiative on Biodiversity for Food and Nutrition' that is part of the United Nations Convention on Biological Diversity (Convention on Biological Diversity, 2006 in Nesbitt *et al.,* 2010). As mentioned earlier, cereal 'oligoculture' is more pronounced in Europe than elsewhere in the world, even though some minor cereals are still cultivated in Europe. Among the minor cereals produced in Europe are rye (16.4 million tons), triticale (12.9 million tons) and buckwheat (1.4 million tons). Europe is the world's leading producer of these cereals, which in 2007 accounted for 92.7%, 93.0% and 75.9% of world production, respectively

(FAOSTAT, 2011a). Spelt (Fig. 1.1) belongs to the group of less widely grown cereals, with 10,260 ha on average over the 2006-2010 period in Belgium (DGSIE, 2011). In Germany, the area under spelt increased annually, from 11,300 ha in 2003 to 37,500 ha in 2009 (Federal Statistical Office Germany, 2011). In Switzerland, 9,095 tons of spelt and 1625 tons of spelt for feed were recorded in 2006-2009 (Office Fédéral de la Statistique suisse, 2011). In Belgium, no official figures are available on the uses of spelt, but Wal.Agri SA, one of the country's largest enterprises in buying, processing and selling spelt, uses 25% for milling and 75% for feed (Roiseux, 2011, pers. comm.).

Figure 1.1. Two lithographs of white not awned and black awned spelt ears, spikelets and grains (Vilmorin-Andrieux & Cie, 1880).

Globally, including Europe, cereals are very important in the daily food supply, (calculated as the number of kcal/capita/day). The global food supply is 2,796 kcal/capita/day, with cereals accounting for 1,289 kcal/capita/day, of which wheat accounts for 529 kcal/capita/day. In Europe, the food supply is higher, at 3,406 kcal/capita/day; cereals are less important in the diet, but are nevertheless significant, accounting for 1,001 kcal/capita/day, of which wheat accounts for 819 kcal/capita/day (FAOSTAT, 2011b). Cereals are important in the human diet because they are rich in carbohydrates. They provide 40-75% of total energy intake for humans, constituting the most important energy source in human diets. Starch and sugars provide 20-50% and 9-27% of total energy intake, respectively. The FAO/WHO Expert Consultation on Carbohydrates in Human Nutrition recommended an optimum carbohydrate level of at least 55% of total energy intake, obtained from a variety of food sources (Gray, 2003). Among the dietary carbohydrates, there are two main categories: available

carbohydrates and resistant carbohydrates. In the resistant carbohydrates category, there are three groups: non-starch polysaccharides (NSPs), resistant short chain carbohydrates (RSCCs) and sugar alcohols. (Englyst *et al.,* 2007). Wheat, as one of the main cereals both globally and in Europe, contributing significantly to the food supply, has been heavily researched and its composition is well known. Wheat kernels typically contain about 1.7% minerals, 2.5% lipids, 12.3% proteins and 79.8% carbohydrates of which 85% is derived from starch (Belitz and Grosch, 1999). Spelt belongs to the baking cereals group and its composition is very similar to that of wheat, as shown in Chapter 3.

The NSPs refer to those polysaccharides that lack the α-1-4- linked glucose that is characteristic of starch. There are various types of NSPs that differ in sugar composition and glycosidic linkages, which are important in determining their physico-chemical properties. The NSPs in plant cell walls have a structural function in determining the integrity of plant cells and tissues. Within NSPs are the dietary fibers. In terms of a plant-rich diet, dietary fiber consists of "intrinsic plant cell-wall polysaccharides"; from an indigestibility perspective it consists of "indigestible carbohydrate (DP>3) and lignin" (Englyst *et al.,* 2007). Dietary fibers are known for their beneficial effects on bowel functioning, the fermentation and physiological functions of colon, constipation, diverticulosis, inflammatory bowel disease, colorectal cancer, coronary heart disease, type 2 diabetes, satiety and body weight. Globalrecommendations for dietary fiber intake have been established by the WHO/FAO, and at the national level in several countries (Gray, 2006). Among the NSPs, hemicelluloses are an important and heterogeneous group. Their most important biological role is their contribution to strengthening the cell wall through interaction with cellulose and, in some walls, with lignin (Scheller and Ulvskov, 2010). Cell wall polysaccharides have been grouped into three classes: cellulose, hemicelluloses and pectins. Of these classes, only cellulose is well defined, consisting entirely of β-(1,4)-linked glucan chains. Pectins are highly heterogeneous polysaccharides, traditionally characterized by the relative ease with which they can be extracted with hot acid or chelators and by the large amount of galacturonic acid residues they contain. Hemicelluloses traditionally comprise the remaining polysaccharides, which can be extracted by alkaline treatment. These polysaccharides differ greatly from each other structurally and in physicochemical properties. Definition based on extractability is not useful because some pectins can be extracted by alkaline treatment, and β-glucans and some arabioxylans (AXs), considered as hemicelluloses, are quite readily extracted without alkaline treatment. Hemicelluloses are considered as a group of polysaccharides characterized by being neither cellulose nor pectin and by having β-(1,4)-linked backbones of glucose, mannose or xylose. These glycans have the same equatorial configuration at C1 and C4, and therefore the backbones have significant structural similarity. Xyloglucans, xylans, mannans, glucomannans and β-(1,3;1,4)-glucans are all hemicelluloses (Scheller and Ulvskov, 2010). There is considerable interest in hemicelluloses because they have several applications. Oligosaccharides have significant market potential as specialty pharmaceuticals, chemicals and food additives (Tuohy *et al.,* 2004; Moure *et al.,*

2006; Nabarlatz *et al.,* 2007). Other potential products include bioethanol, organic alcohols, polyols (Werpy *et al.*, 2004) specifically, arabitol and xylitol (Duarte *et al*., 2004; Rivas *et al.,* 2006; Da Silva *et al.,* 2007; Carvalheiro *et al.,* 2007) organic acids, yeast extract (Pessoa *et al* 1996; Duarte *et al.,* 2008), 1,2-propanediol, 2,3-butanediol, and aromatic chemical intermediates (Mielenz 2001; Saha 2003).

The components listed above are present in wheat grain, but the distribution is not uniform. Wheat grain will is taken as a reference point because no data of this type are available for spelt grain (Fig. 1.2). Wheat grain comprises three major parts: 1) the endosperm, with the starchy endosperm and the aleurone layer (80-85% of the grain); 2) seed coats formed by six tissues (nucellar epidermis, testa, tubular cells, crossed cells, mesocarp and epicarp) (13-17% of the grain); and 3) germ (3%), containing the embryo and scutellum (Feillet, 2000).

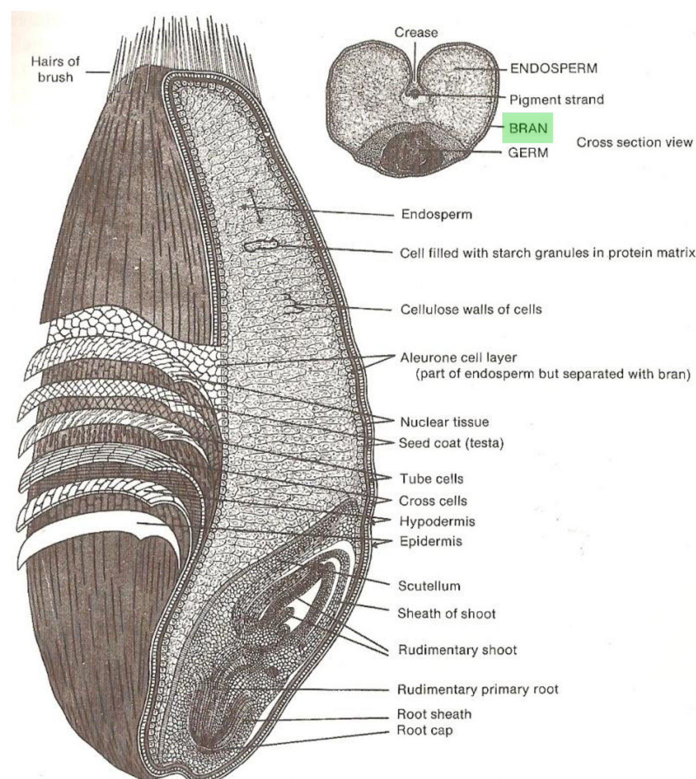

Figure 1.2. Longitudinal and cross-sections of a wheat kernel (according to Delcour and Hoseney, 2010).

Milling is performed to separate the starchy endosperm from the external parts (coats and aleurone layer) and from the germ. After milling, four main products are obtained: flour, which consists of the endosperm and a few external parts of the grain; shorts, which are fine particles made from the coats of the grain and contain an important amount of starch from the endosperm; the fine brans, and the coarse brans (Fig. 1.3) (Feillet, 2000).

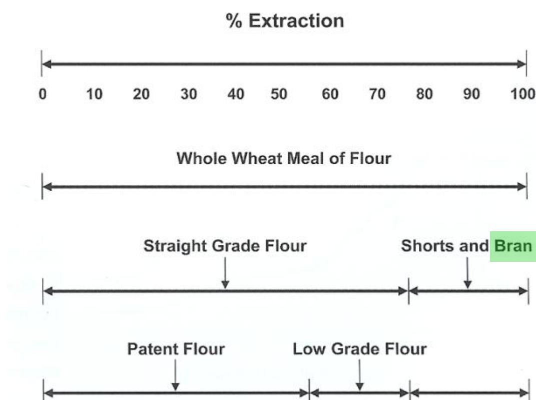

Figure 1.3. Grades of flour obtained from wheat. Whole wheat flour corresponds to 100% extraction (line 2) but can be divided into straight-grade flour plus shors and bran (line 3). The straight-grade flour can be further divided into patent and low-grade flours (line 4) (according to Delcour and Hoseney, 2010).

Shorts, germ and fine and coarse brans are by-products of the milling industry, used mainly for feed where they provide an economic source of energy for livestock farming. For spelt, dehulling before milling is necessary and produces a by-product, the hull. Wheat endosperm typically consists of more than 80% carbohydrates (mostly starch), 12% proteins, 2% lipids, and 1% mineral and minor constituents. The outer layers typically contain 70% carbohydrates (almost equally divided between cellulose and NSPs), 17% proteins, 5% lipids, and 7% minerals and minor constituents. Wheat germ contains relatively high proportions of carbohydrates (about 50%) and proteins (about 32%), as well as high levels of lipids (about 11%; Barnes[1], 1982, cited by Pomeranz, 1988). These by-products are rich in NSPs and consequently in hemicelluloses. The detailed composition of some of these by-products is known; for example, the composition of wheat bran is well known, but spelt bran and hull have not been characterized. Wheat bran represents 10-21% of wheat grain (Pomeranz, 1988) and about 11% for commercial bran (Hoseney, 1994b). Even where plant variety, environment and climatic conditions modify wheat bran composition (Salomonsson *et al.*, 1984), it remains fairly stable. Industrial wheat bran contains 47% NSPs (Ralet *et al.*, 1990), 10-30% starch (originating from residual endosperm attached to bran) and 15-20% proteins. The rest consists of minerals (4-6%), lipids (about 5%), lignin (3-10%) and other minor components (Ralet *et al.*, 1990; Bergmans *et al.*, 1996; Bataillon *et al.*, 1998). The main NSPs in bran are AXs (70%) (Fig. 1.4), cellulose (24%) and (1-3), (1-4)-β-D-glucans, called β-glucans (6%) (Brillouet et Mercier, 1981; Carré et Brillouet, 1986). The AX content in bran varies from 19 to 35% (Lee and Stenvert, 1973; Ralet *et al.*, 1990; Bataillon *et al.*, 1998), while the cellulose content varies from 9 to 11% (Anderson et Clydesdale, 1980 ; Bataillon *et al.*, 1998). Wheat bran contains 24.1% water-unextractable arabinoxylan (WU-AX) and 0.9% water-

[1] Barnes P.J., 1982b. Lipid composition of wheat germ and wheat germ oil. Fette, Seifen, Anstrichm, 84: 256-269.

extractable arabinoxylan (WE-AX) (Maes and Delcour, 2001). Miniscule quantities of glucomannans (Mares and Stone, 1973; Bacic and Stone, 1981a; Gruppen *et al.*, 1989) and arabinogalactans (Fincher *et al.*, 1974), originating from the endosperm and aleurone, are also present in wheat bran. Low levels of xyloglucans associated with AXs in the pericarp tissue have been reported (Dupont and Selvendran, 1987). There are residues of neutral predominant sugars in wheat bran (glucose, xylose and arabinose) and galactose and mannose are minor components (Maes and Delcour, 2001).

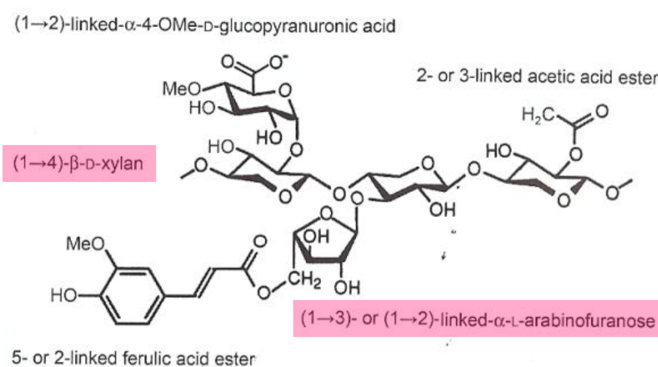

Figure 1.4. Substituents in (1-4)-β-D-xylan chain residues, α-L-arabinofuranose might substitute at position 3, position 2, or both on one xylopyranosyl residue. Some arabinofuranosyl residues might carry an esterifed hydrocinnamic acid at position 5, which could be either p-coumaric acid (R=H) or ferulic acid (R=OCH_3). 4-O-methyl α-D-glucopyranuronic acid (4MeGA) is also a substituent at position 2 of the xylopyranosyl residues, and acetyl esterification can occur at position 2 or position 3 (according to Stone and Morell, 2009).

So far as we known, no data are available on the spelt hull *per se*, but its composition is thought to be similar to that of wheat straw, which contains, on average, 8% lignin, 26% hemicelluloses and 38% cellulose (Sauvant *et al.,* 2002). The spelt hull might also be a source of hemicelluloses, be rich in cellulose and contain lignin. Although spelt remains a minor cereal, it is a source of interest. Several teams in Europe, mainly in public institutions are conducting spelt breeding research; there is one team at the Centre wallon de Recherches agronomiques in Gembloux, Belgium, one at the University of Hohenheim in Stuttgart, Germany, and one at a foundation managed by Peter Kunz in Hombrechtikon, Switzerland. In the Healthgrain project under the EU 6th Framework Programme, spelt has been included among the cereals being studied, with different components being analysed (for example, phytochemicals, dietary fibers, alkylresorcinols, tocopherols, tocotrienols, pytosterol, folate, steryl ferulate, phenolic acids, xylanase and xylanase inhibition activity). This project has produced new data on spelt. The present work is focusing on fibers, especially hemicelluloses. It aims to describe the spelt by-products from milling, develop methods of extracting hemicelluloses and characterize the fractions obtained in order to develop new ways of better exploiting the by-products of spelt milling. The objectives and strategy of this work are described in detail in Chapter 2. In Chapter 3, spelt grain is discussed. In Chapter 4, the potential of spelt bran and hull as a rich source of hemicelluloses is

evaluated. Chapters 5, 6 and 7 focus on the extraction of the hemicelluloses from the bran and hull, using several methods. In Chapters 8 and 9 the results are discussed and the new opportunities presented by these results are described.

2 CHAPTER 2

Objectives and strategy

Spelt is a minor cereal in production terms, but is a valuable crop in terms of biodiversity, cultivation method, food cereal diversity and, nutritive feed value, and it is therefore important to develop new ways of using this cereal in order to strengthen the spelt market and to increase, or at least maintain, the level of spelt breeding and production in Europe and elsewhere.

Developing spelt production means new uses for spelt, preferably high added value. Currently, many aspects of the crop's production are researched in response to human needs. Several by-products making up the biomass have been specifically researched: for example, wheat bran has been treated in order to extract AXs (Maes and Delcour, 2001); straw has been analyzed for hemicelluloses (Sun *et al.,* 1998; Sun *et al.,* 2000) and cellulose extraction (Rémond *et al.*, 2010); and hulls mainly from barley (Hoije *et al.*, 2005) and buckwheat (Hromadkova and Ebringerova, 2003), have been used to extract hemicelluloses.

In cereals such as wheat, maize, barley and rice, cellulose and hemicelluloses are well known, but lignins have only recently been researched. No data on spelt bran and hull are available with regard to cellulose, hemicelluloses and lignin in terms of their extraction potential or their characterization. There is a lack of scientific knowledge about fibers from spelt bran and hull, although they are available as a food and feed resource in Europe. Among the fibers present in the bran and hull, only the extraction of hemicelluloses has been the best mastered, leading to a wide range of applications (Ebringerova *et al.*, 2005).

The objectives of the current research are to extract, quantify and characterize the hemicelluloses from spelt bran and hull. This work includes studying the parameters of the methods of extraction or solubilization, and the systematic quantification and characterization of the fractions. The description focuses on the composition of the fraction with the content, the A/X ratio and the distribution of molecular weights or degree of polymerization of AXs. Another objective of the current research is to compare the various methods developed and the products obtained (Fig. 2.1).

First, several fractions of spelt spikelets will undergo two fractionations, following Van Soest and Wine (1967) and Lee *et al.* (1992), in order to evaluate the content initially of cellulose, hemicelluloses and lignin and then of soluble and insoluble fiber. Spelt bran and whole spikelet flour are the fractions that have attracted most interest. The bran is a by-product that will be used in itself, and the hull is one of the components of the whole spikelet flour.

Second, hemicelluloses will be extracted from the spelt bran, using a chemical method. Bran is close to the grain and is an important by-product of wheat milling. Wheat bran has been well researched and is thought to be similar to spelt bran. Among the methods available, the alkaline method is widely used to extract hemicelluloses (Persson *et al.,* 2009). One alkaline method has been tested successfully

on wheat bran (Maes and Delcour, 2001) and will be tried with spelt bran, making a comparison of wheat and spelt bran possible.

Third, hemicelluloses will be extracted from the spelt hull, another spelt by-product from milling, using the same method used for spelt bran, and then using methods used for wheat straw, which is thought to be similar to spelt hull (Sun *et al.,* 1998; Sun *et al.,* 2000). As spelt hull is a poorly known material from the vegetative part surrounding the grain that has never been analyzed in this way, it will be interesting to use several extraction methods to produce diverse products, giving better characterization of this material.

Finally, a more environmentally friendly method will be tried, such as an enzymatic method. Several commercially available enzymatic preparations will be tested on the bran and hull in order to evaluate their efficiency and the type of products they release. These enzymatic preparations contain β-xylanase activity as well as other activities, such as cellulase and β-glucanase, which will broaden the experiment.

All the extracted products will be quantified, their composition of monosaccharides studied and their protein and ash content established. The yield of extracted AXs will be calculated. The molecular weight of the extracted polysaccharides will be determined by High Performance Size Exclusion Chromatography (HPSEC) and the degree of polymerization will be calculated when necessary.

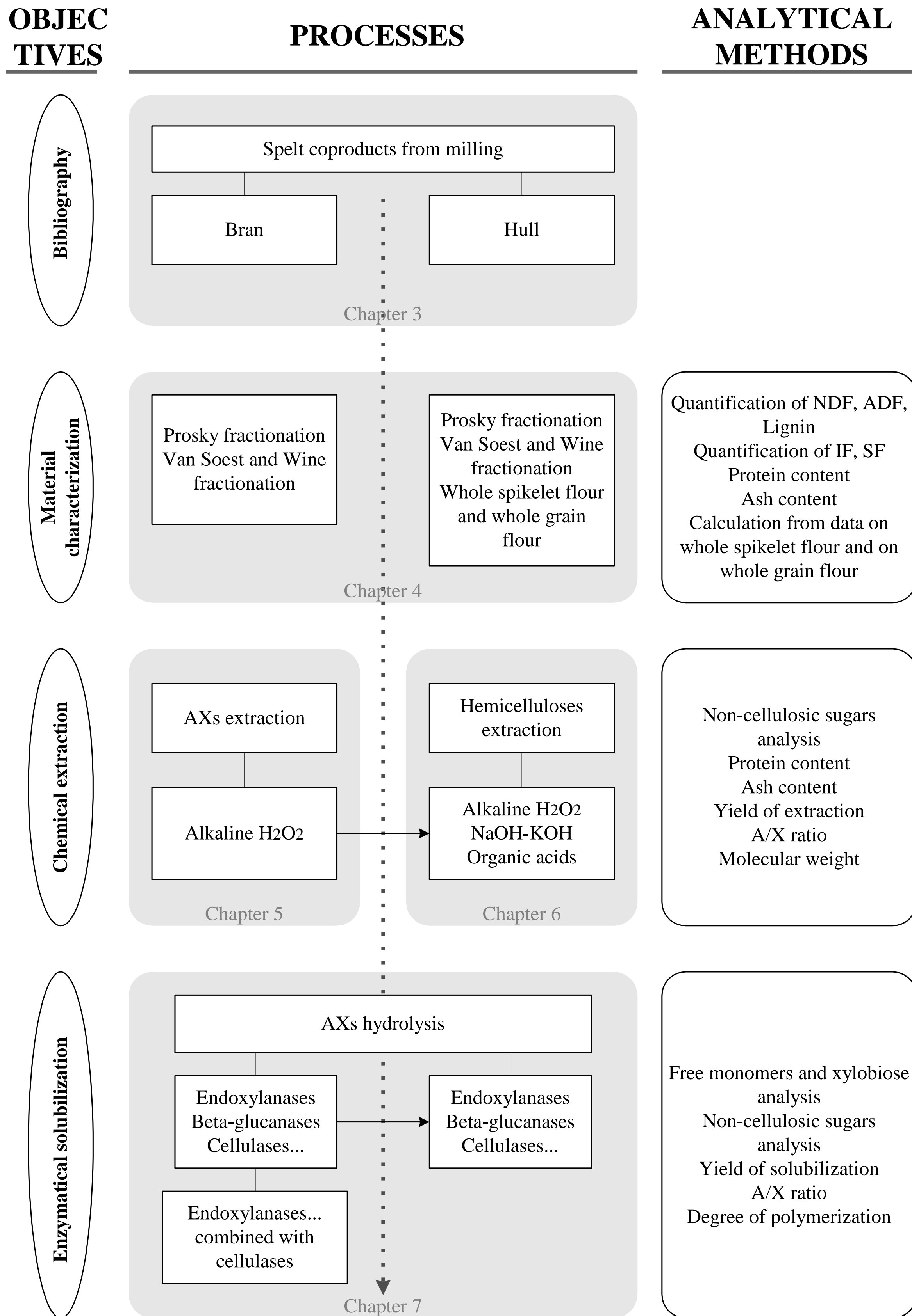

Figure 2.1. Diagrammatic representation of the current work, showing objectives, process and analytical methods

3 Chapter 3

Comparative review of the content and profiles of macronutrients in spelt and wheat

Excerpt from Article 5:

Escarnot E., Jacquemin J.-M., Agneessens R., Paquot M., 2012. Comparative review of the content and profiles of macronutrients in spelt and wheat. *Biotechnology, Agriculture, Society and Environment*, in press.

3.1 The context of the study

Spelt is the cereal under study in the present thesis but is not very well known. Consequently, a state of the art is necessary to understand the stake of this species.

If the geographical origin of spelt seems clear, the phylogeny of spelt is still controversial and a quick point on the matter will be done in the following chapter. Spelt displays distinct agronomic characteristics which make it an alternative cereal which can respond to some specific needs. Besides, the particular morphology of spelt will be commented.

Spelt composition has been studied but not so extensively than for wheat and the different studies often establish a comparison with this species. The reference to wheat is convenient but it should be made with caution. Indeed, the genotypes must be grown in the same conditions (environment, cultivation practices and year), must follow the same processing and must be stored identically. When considering the information in the following chapter, attention must be paid to these elements in order to evaluate the relevance of the data. However as nowadays no table of reference is available for spelt, the few studies existing represent the only sources of information on spelt composition and are important. Consequently, a review gathering the most scientific work on spelt is of great interest.

The composition of the grain can be studied under two aspects, based on the two main categories of nutrients, the macronutrients and the micronutrients. As a first approach, it seems that the knowledge of the macronutrients was the most important and was in the framework of this research. Fibers belong to the group of macronutrients and consequently a preliminary state of the art on spelt fibers was necessary. To complete the group of the macronutrients, the carbohydrates in general will be presented with the starch and the sugars. Proteins will also be included with a point regarding the content and a point regarding the quality. Gliadins, glutenins and amino acids will be described. Finally, the last group of macronutrients is lipids for which the contents in fatty acids and in sterols will be displayed.

For each component, a comparison with data from wheat will be considered to highlight the differences between both subspecies. Generally, the whole grain flour is studied but in some studies the spikelet flour, the white flour and the bran were investigated and are consequently displayed in the review.

The micronutrients have not been tackled in the following chapter as they are not the core of the present work. However, they are important in human and animal nutrition and some differences have been highlighted between spelt and wheat. Besides, technological aspect has neither been treated in the following chapter as it was not the subject of this work. However, one should know that the technological behaviour from spelt is very different from that of wheat (Cubadda and Marconi, 2002), especially for baking which is one of the main uses in human food. Besides, several other applications

of spelt have been successfully investigated for instance cereal breakfast, pasta (Abdel-Aal *et al.*, 1998b; Bonafaccia *et al.*, 2000) and beer.

3.2 Comparative review of the content and profiles of macronutrients in spelt and wheat

Abstract

Spelt (*Triticum spelta*) is a hexaploid wheat, hulled and with a brittle rachis, and it has interesting agronomic properties. It is used in feed and food, and is becoming more widely used in the growing natural foods market. Spelt differs from wheat in that it has a higher protein content (15.6% for spelt, 14.9% for wheat), higher lipid content (2.5% and 2.1%, respectively), lower insoluble fiber content (9.3% and 11.2%, respectively) and lower total fiber content (10.9% and 14.9%, respectively). There are no important differences in starch, sugar and soluble fiber content, and there is a qualitative diversity at the protein, arabinoxylan and fatty acid levels.

3.2.1 Introduction

Spelt (*Triticum spelta*) is a hexaploid cereal belonging to the *Triticum* genus in the Gramineae family. Spelt grains are hulled and the hull represents 21-32% of the harvested product (Percival, 1921). Spelt spikes are pyramidal and the rachis is brittle (Luo *et al.*, 2000). The relationship between spelt and wheat has been investigated extensively, with most studies postulating that they belong to the same species, but to separate gene pools. The literature continues to consider them as distinct species, more from the point of view of use rather than genetics (Abdel-Aal and Hucl, 2005). The most recent studies on the phylogenic origin of spelt support the hypothesis that spelt results from several hybridizations between club wheat and a hulled tetraploid emmer (Yan *et al.*, 2003; An *et al.*, 2005). It has been established that spelt originated from the Middle East and migrated northwards along the Black Sea and the Danube from east to west, reaching Austria, southern Germany and northern Switzerland (Andrews, 1964). In Europe, during a period of climatic cooling (750-15 BC), spelt replaced einkorn (*Triticum monococcum*) and emmer (*Triticum dicoccum*) and then, in turn, was replaced by free-threshing wheat almost throughout Europe during the first millennium (Nesbitt and Samuel, 1996).

In terms of agronomic characteristics, spelt displays high resistance to environmental factors such as diseases and stress, and can produce good yields under disadvantageous growing conditions such as wet, cold soils and high altitudes (Campbell, 1997). In addition, as the hull covers the seed, chemical treatment before sowing is not always necessary and, because of its long straw, spelt cannot withstand a high level of nitrogen fertilization (Bonafaccia *et al.*, 2000). It is suitable for organic farming and contributes to agro-biodiversity, thus meeting the objectives of the European Union with regard to growing practices. Spelt is now cultivated in Europe, Asia (Iran), North Africa, the USA and Canada (Abdel-Aal *et al.*, 1998a; Dvoracek *et al.*, 2002). It is used mainly in animal feed in order to provide a balanced intake for animals fed primarily on grass silage (Lecomte *et al.*, 1996). As an ancient crop, however, spelt occupies a niche market in North America and Europe in the natural, organic, health and specialty-food markets (Abdel-Aal and Hucl, 2005). It has the potential for a variety of uses,

including bread, pasta and breakfast cereals (Abdel-Aal *et al.*, 1998b; Bonafaccia *et al.*, 2000). These uses are similar to those associated with wheat, although the characteristics differ. The composition of the two cereals has been investigated for several decades and over the past 5 years a number of studies have produced more detailed information. The renewed interest in spelt requires an update on its composition. This paper discusses the macronutrients of spelt and wheat and highlights the differences between these cereals. The focus is on non-fiber carbohydrates, fibers, proteins and lipids.

3.2.2 Non-fiber carbohydrates

Carbohydrates provide 40-75% of total energy intake, constituting the most important energy source in human diets (Gray, 2003). They are usually classified according to their degree of polymerization: sugars, oligosaccharides and polysaccharides (FAO, 1998). For both spelt and wheat, carbohydrates are the main components (59-71%) of the grain kernel (Belitz and Grosch, 1999). Various studies have indicated that there is no great difference in total carbohydrate, starch and sugar content between spelt and wheat whole flour (Abdel-Aal *et al.*, 1995; Ranhotra *et al.*, 1995; Grela, 1996; Ranhotra *et al.*, 1996a) (Table 3.1).

3.2.2.1 Starch

Starch is the main storage carbohydrate in spelt and wheat kernels, accounting for 61-68% of the grain, whereas sugars account for 2-3% (Abdel-Aal and Hucl, 2005). According to Abdel-Aal *et al.* (1999a and 1999b), spelt has a lower amylose content than wheat, but Wilson *et al.* (2008) reported a higher amylose content (2-21%) in spelt starch than in the hard red winter wheat control. Amylose and amylopectin are two components of the starch granule, accounting for 26-28% and 72-74%, respectively; amylose is a linear glucose homopolymer of glucose, and amylopectin is a branched homopolymer of glucose (Feillet, 2000).

Gelatinization is the transformation of starch granules in four stages: loss of crystallinity (fusion of the crystalline phase, due mainly to amylopectin); water absorption (swelling); bursting of the granules; and amylose solubilization. This phenomenon is irreversible and results in increased viscosity and starch jellification when temperatures fall (Feillet, 2000). Jorgensen *et al.* (1997) reported that the gelatinization temperature, measured with a Brabender amylograph, was higher in spelt (87-93.2°C) than in common wheat (84.6°C) varieties. Abdel-Aal *et al.* (1999b) found a wide range of transition temperatures for spelt starches compared with wheat, but the difference in the enthalpy of the gelatinization of spelt starch was very similar to that of common wheat starch.

With regard to starch size distribution, Abdel-Aal *et al.* (1999a) observed little difference. Wilson *et al.* (2008) studied the relationship between the type of granules and technological characteristics in spelt. Negative correlations were observed between the large A-type granules and breadcrumb score, amylose level, pasting viscosity for cultivars grown in 1999 and pasting temperature for those grown

in 1998. Positive correlations were found between the small B- and C-type granules and crumb score, loaf volume, amylose, Rapid Visco Analyser (RVA) final pasting viscosity for cultivars grown in 1999 and RVA pasting temperature for those grown in 1998.

Intra-species variability in starch content has been observed, which could be explained by the genotype and growing season conditions, as reported by Massaux *et al.* (2008) for wheat.

Wheat				Spelt				Growing conditions		Reference
n	Starch	Sugars	Total	n	Starch	Sugars	Total	Number of locations	Number of years	
1 (spring)	62.4	3.3	65.7	5	63.8 (60.9-65.8)	2.1 (1.7-2.5)	65.9 (63.4-67.6)	4 (1993) and 5 (1992)	2	Abdel-Aal *et al.* (1995)
1	-	-	69	1	-	-	66.2	1*	1	Ranhotra *et al.* (1995)
1	68.1	3.1	71.2	4	66.7 (65.9-67.6)	3 (2.4-3.4)	69.7 (68.4-70.9)	1	1	Grela (1996)
2	-	-	66.7 (63.6-68.3)	3	-	-	67 (59.2-69.8)	5 (spelt) and 2-3 (wheat)	1	Ranhotra *et al.* (1996a)
-	-	-	-	5	-	-	65.2 (59.5-69.4)	4*	-	Marconi *et al.* (1999)
	63.0 (62.4-68.1)	3.3 (3.1-3.3)	66.5 (63.6-71.2)		64.0 (60.9-67.6)	2.2 (1.7-3.4)	66.1 (59.2-70.9)			Weighted mean and range

* samples cultivated in different countries
n: number of genotypes

Table 3.1. Starch and sugar content (%, d.m.) of wheat and spelt whole grain

3.2.2.2 Sugars

Sugar content in spelt samples has been found to be more variable than in wheat samples, but the number of samples that have been investigated is limited (Abdel-Aal *et al.*, 1995; Ranhotra *et al.*, 1995; Grela, 1996; Ranhotra *et al.*, 1996a). With regard to free sugars, there is no difference in the total concentration between spelt and modern wheat (Zorb *et al.*, 2007).

3.2.3 Fibers

Dietary fiber has beneficial physiological effects, such as laxation. Specifically, insoluble dietary fiber reduces transit time and increases fecal bulk and defecation frequency (AACC, 2001). Dietary fiber fermentation results in the production of short-chain fatty acids conducive to bowel health (Moore *et al.,* 1998). In addition, high dietary fiber intake reduces the risk of diverticular disease, hemorrhoids and colorectal cancer (AACC, 2001). Reduced blood cholesterol and/or blood glucose has also been attributed to dietary fiber, which is linked to a reduced risk of cardiac disease through reduced blood cholesterol and the prevention of the development of type 2 diabetes (AACC, 2001; Gray, 2006). Fibers help control body weight, mainly through inducing satiety (Gray, 2006).

3.2.3.1 Fibers in whole grain

According to several studies (Bognar and Kellermann, 1994[2] in Ruibal-Mendieta, 2004; Abdel-Aal *et al.*, 1995; Ranhotra *et al.*, 1995; Ranhotra *et al.*, 1996a; Bonafaccia *et al.*, 2000; Escarnot *et al.*, 2010), the range of total dietary fiber content is greater in spelt than in wheat. This was not the finding in one study, however, conducted by Gebruers *et al.* (2008), but this could have been due to the higher number of wheat genotypes (131) considered (Table 3.2). Contrary to the range, in most studies the mean content of dietary fiber is higher in common wheat than in spelt, and the same is true for the mean content of insoluble fiber (Abdel-Aal *et al.*, 1995; Ranhotra *et al.*, 1995, 1996a; Escarnot *et al.*, 2010). Escarnot *et al.* (2010) attributed this difference to hemicelluloses and cellulose. For soluble fiber, the range is similar in spelt and wheat. Uniform values have been reported by most authors, including Abdel-Aal *et al.* (1995), Ranhotra *et al.* (1996a) and Bonafaccia *et al.* (2000); Escarnot *et al.* (2010) found no statistical difference between spelt and wheat for soluble fiber content. Work done by Gebruers *et al.* (2008) shows that the total arabinoxylan content is 1.75% (1.60-2.25) for spelt and 1.90% (1.35-2.75) for wheat, and the water-extractable arabinoxylan content is 0.35% (0.30-0.45) for spelt and 0.50% (0.30-1.40) for wheat. The large range for wheat may be due to the high number of samples analysed (131). The average arabinose/xylose ratios for total and water-extractable arabinoxylans are identical: spelt 0.60 (0.55-0.60) and 0.50 (0.45-0.55); and wheat 0.60 (0.50-0.70) and 0.50 (0.40-0.55).

[2] Bognar A. and Kellermann C., 1994. Ballaststoffgehalt von Dinkel. Ernährungs-Umschau 41: 454-455

According to Marconi *et al.* (1999), the β-glucan content of spelt is similar to that of common wheat, with a mean value of 1.2%. Gebruers *et al.* (2008), however, found a higher content in wheat (0.75%) than in spelt (0.65%), with ranges of 0.55-0.70% and 0.50-0.95%, respectively, displaying a higher diversity in spelt despite the low number of genotypes (5). These values accord with those noted by Loje *et al.* (2003), who reported 0.7% in spelt and 0.8% in wheat (based on different years of harvest, three spelt cultivars and one wheat cultivar). The concentration of fructans, such as 1-kestose and kestotetraose, is higher in spelt than in wheat (Zorb *et al.*, 2007).

With regard to crude fibers, the range is wider for wheat than spelt (Grela, 1996; Moudry and Dvoracek, 1999; Jorgensen *et al.*, 1997), but no general rule can be established on content (Table 3.3). The two studies on hemicelluloses (Grela, 1996; Escarnot *et al.*, 2010) produced contradictory results.

Lignins are non-polysaccharide cell wall substances derived mainly from the three monolignols: *p*-coumaryl, coniferyl, and synapyl alcohols. The monolignols are targeted to different and distinct regions of various cell wall types, where they are polymerized to form wall-reinforcing biopolymers with distinctive biophysical properties (Davin and Lewis, 2005). Lignin content is similar in spelt and wheat (Grela, 1996; Escarnot *et al.*, 2010). The Klason lignin was evaluated by Gebruers *et al.* (2008), with similar levels being observed for spelt and wheat (2.25% and 2.20%, with ranges of 1.85-2.90% and 1.40-3.25%, respectively). Cellulose content is lower in spelt than in wheat (Grela, 1996; Escarnot *et al.*, 2010).

These studies were based on enzymatic-gravimetric methods derived from Prosky for insoluble, soluble and total fiber and from Van Soest for cellulose, hemicelluloses and lignin. A minor part of the variability can therefore be attributed to the evolution of the method over the years. Intra-species variability is not surprising as it has been demonstrated that genotype and environment (including management practices), and the interaction between them, have influenced the pentosan content of wheat (Li *et al.,* 2002; Jiang *et al.,* 2007).

3.2.3.2 Fibers in grain milling fractions

Escarnot *et al.* (2010) observed that spelt bran is richer in soluble fibers and lignin than wheat, but less rich in hemicelluloses and cellulose (Table 3.3). In the bran, total-arabinoxylan content is much higher in wheat than in spelt (18% [13.2-22.1] and 12.7% [11.1-13.9], respectively), and the same is true for water-extractable arabinoxylan content (0.40% [0.30-0.85] and 0.30% [0.30-0.35]). The average arabinose/xylose ratio for total arabinoxylans is similar (spelt 0.50% [0.45-0.55] and wheat 0.60% [0.55-0.70]), but the average arabinose/xylose ratio for water-extractable arabinoxylans is higher for spelt (1.40% [1.20-1.6]) than for wheat (1.00% [0.70-1.65]) (Gebruers *et al.*, 2008). Escarnot *et al.* (2011a) extracted 55% of the arabinoxylans in spelt bran; 13% were water-extractable and 87% were

water-unextractable. The populations of water-extractable arabinoxylans were 7-8 kDa and 28 kDa, and of water-unextractable arabinoxylans they were 7-8 kDa and 310-415 kDa.

In hulled grain, Lecomte *et al.* (1996) reported absolute higher values of cellulose, hemicelluloses and lignin, unlike those reported by Escarnot *et al.* (2010). The analysis of the whole spikelet flour displayed great diversity among the several genotypes studied (Escarnot *et al.*, 2010).

Xylanase and xylanase-inhibitors affect grain quality, production parameters and, consequently, product quality. The only study conducted on enzymatic activity (Gebruers *et al.*, 2010) indicates that spelt white flour and bran do not display high xylanase activity. Spelt and wheat have similar *Triticum aestivum* xylanase inhibitor activity and display high xylanase-inhibiting protein activity. In addition, inhibitor activity is much higher in spelt bran than in spelt flour (Gebruers *et al.*, 2010).

Material	Wheat				Spelt				Growing conditions		Reference
	n	Insoluble	Soluble	Total	n	Insoluble	Soluble	Total	Number of locations	Number of years	
Whole grain	1	-	-	12.5	4	-	-	9.3 (8.8-10.3)	1	1	Bognar and Kellermann, 1994**
Whole grain	1			12.7	4	-	-	8.7 (7.7-9.3)	1	1	
Whole grain	1 (spring)	10.8	1.7	12.5	5	8.22 (8.0-8.4)	1.76 (1.7-1.9)	10 (9.8-10.3)	4 (1993) and 5 (1992)	2	Abdel-Aal *et al.* (1995)
Whole grain	1	13.2	2.2	15.4	1	10.8	1.8	12.4	2*	1	Ranhotra *et al.* (1995)
Whole grain	2	11.7 (10.7-12.6)	1.8 (1.7-1.9)	13.5 (12.5-14.3)	3	9.1 (8.5-9.9)	1.4 (1.2-1.7)	10.5 (10.1-11.6)	5 (spelt) et 2-3 (wheat)	1	Ranhotra *et al.* (1996a)
Whole grain					5	10.7 (8.7-12.9)	2 (1.2-2.4)	12.7 (10.5-14.9)	4*	-	Marconi *et al.* (1999)
Whole grain	1	11.4	1.4	12.8	3	11.5 (11.2-12.1)	1.7 (1.7-1.8)	13.2 (12.9-13.8)	1 (spelt) et 1* (wheat)	3 (spelt) et 1 (wheat)	Bonafaccia *et al.* (2000)
Whole grain	131			15.2 (11.5-18.3)	5			12.0 (10.7-13.9)	1	1	Gebruers *et al.*, (2008)
Whole grain	3	10.1 (9.8-10.5)	1.6 (1.4-1.9	11.7 (11.3-11.9)	4	8.8 (7.8-10.1)	1.4 (0.8-2.0)	10.3 (8.5-11.9)	1	1	Escarnot *et al.* (2010)
Whole grain	141	11.2 (10.7-13.2)	1.7 (1.4-2.2)	14.9 (12.3-15.4)	34	9.3 (8.0-12.9)	1.7 (1.2-2.4)	10.9 (7.7-14.9)			Weighted mean and range
White flour	1	0	2.52	2.52	1	0.58	2.07	2.65	1 et 1	1	Marques *et al.* (2007)
Bran	3	32.1 (32.0-32.2)	1.2 (0.3-2.6)	33.3 (32.3-34.7)	4	29.4 (21.2-34.4)	2.9 (2.5-3.5)	32.6 (23.7-37.1)	1	1	Escarnot *et al.* (2010)

* samples cultivated in different countries
** in Ruibal-Mendieta, 2004
n: number of genotypes

Table 3.2. Insoluble and soluble fiber content (%, d.m.) of wheat and spelt whole grain, white flour and bran

Material	Wheat					Spelt					Growing conditions		Reference
	n	Lignin	Hemicellulose	Cellulose	Crude	n	Lignin	Hemicellulose	Cellulose	Crude	Number of locations	Number of years	
Whole grain	1				1.83	10				2.18 (1.71-2.76)	1	3	Moudry et Dvoracek (1999)
Whole grain	1				3.3	7				2.77 (2.5-3.26)	2	1	Jorgensen *et al.* (1997)
Whole grain	1	1.18	9.17	2.16	2.73	4	1.02 (0.88-1.08)	10.57 (7.8-18.84)	2.07 (1.93-2.29)	2.64 (2.39-2.97)	?	3	Grela (1996)
Whole grain	3	0.7 (0.6-0.7)	7.3 (7.0-8.1)	2.4 (2.1-2.8)		4	0.7 (0.6-0.8)	5.4 (4.6-6.4)	1.7 (1.4-1.9)		1	1	Escarnot *et al.* (2010)
Whole grain	6	0.9 (0.6-1.2)	8.2 (7.0-9.2)	2.3 (2.1-2.8)	2.4 (1.8-3.3)	25	0.9 (0.6-1.1)	9.3 (4.6-18.8)	2.0 (1.4-2.3)	2.4 (1.7-3.3)			Weighted mean and range
Spikelet						24	2.7	13.2	12.8		1	1	Lecomte *et al.* (1996)
Spikelet						4	1.3 (1.2-1.3)	12.3 (10.0-13.2)	10.4 (8.1-11.3)		1	1	Escarnot *et al.* (2010)
Bran	3	2.1 (2.0-2.2)	22.6 (22.2-22.9)	7.5 (7.2-8.2)		4	2.3 (1.8-2.6)	18.6 (12.2-23.3)	5.7 (3.3-7.9)				

n: number of genotypes

Table 3.3. Lignin, hemicelluloses and cellulose content (%, d.m.) of wheat and spelt whole grain and bran, and spelt spikelet

3.2.4 Proteins

Proteins provide essential amino acids and are a source of energy. The wheat and spelt proteins are albumins, globulins, glutenins and gliadins. Most of the physiologically active proteins (enzymes) are albumins and globulins. They are concentrated in the cells of the aleurone layer, the pericarp and the germ, with a lower content in the endosperm. Glutenins and gliadins are storage proteins known as prolamins. They are limited to the endosperm, including the aleurone layer, and are therefore absent from the pericarp and the germ (Hoseney, 1994a).

3.2.4.1 Total content

Most literature data indicate higher protein content in spelt than in wheat (Abdel-Aal *et al.*, 1995; Ranhotra *et al.*, 1995; Grela, 1996; Codianni *et al.*, 1996; Piergiovanni *et al.*, 1996; Ranhotra *et al.*, 1996a; Jorgensen *et al.*, 1997; Marconi *et al.*, 1999; Moudry et Dvoracek, 1999; Bonafaccia *et al.*, 2000; Matuz *et al.*, 2000a Abdel-Aal and Hucl, 2002; Marconi *et al.*, 2002) (Table 3.4). This has been confirmed under low nitrogen fertilization: Oliveira (2001) found higher protein content in spelt than in wheat, and Dvoracek *et al.* (2002) observed that spelt grains had 0.5% more nitrogen than common wheat grain, but this difference was not statistically significant. For white flour, Pruska-Kedzior *et al.* (2008) found significantly higher protein content in spelt flour (14.7%) than in common wheat flour, and Wilson *et al.* (2008) reached the same conclusion about spelt and a hard winter wheat control. Wilson *et al.* (2008) also noted highly variable protein content in spelt white flour (from five cultivars after 3 years of cultivation). As the degree of nitrogen absorption from the soil and its conversion into proteins depend greatly on genotype and cultivation conditions, Gräber and Kuhn (1992) recommended comparing samples grown under the same conditions. The general attribution of high protein content in spelt could be a consequence of the low grain yield. When comparing protein yields (kg/ha), the values for spelt are lower than those for conventional durum wheat, with a difference of up to 25% (Piergiovanni *et al.*, 1996). In addition, a negative heterosis effect has been observed for spelt-common wheat crosses in terms of protein content (Schmid *et al.*, 1994).

Intra-species protein content varies widely in the different studies. All analyses were performed using the Kjeldhal method, and therefore the variability could be explained by the growing conditions (environment and nitrogen fertilization) and genetic background that influence protein content (Dupont and Altenbach, 2003).

Wheat		Spelt		Growing conditions		Reference
n	Crude proteins N*5,7	n	Crude proteins N*5,7	Number of locations	Number of years	
1 (spring)	15.3	5	15.4 (14.9-16)	4 to 5	2	Abdel-Aal *et al.*, (1995)
1	14.4	1	14	1*	1	Ranhotra *et al.*, (1995)
1	12.3	4	11.1 (9.8-13.1)	1	1	Grela, (1996)
1 (durum)	15	1	16.1	1	2	Codianni *et al.*, (1996)
3 (durum)	15.3 (14.1-16.1)	37	17.1 (15-19.4)	1	1	Piergiovanni *et al.*, (1996)
2	15.4 (13.4-17.5)	3	18.5 (15.8-25.5)	2-3 wheat and 5 spelt	1	Ranhotra *et al.*, (1996a)
1	10.9	7	13.3 (12.5-14.3)	1	1	Jorgensen *et al.*, (1997)
		5	15.7 (14.3-18.4)	4*	unknown	Marconi *et al.*, (1999)
1	12.9**	10	13.5** (12.2-15.2)	1	3	Moudry and Dvoracek, (1999)
1	13.8	3	16.4 (15.9-17.1)	1* wheat and 1 spelt	1 wheat and 3 spelt	Bonafaccia *et al.*, (2000)
4	15.8** (13-16.7)	1	20.6**	unknown	unknown	Matuz *et al.* (2000)
1 (spring)	16.4	2	16.9 (16.5-17.4)	1	2	Abdel-Aal and Hucl, (2002)
		5	14 (12.8-16)	1	1	Marconi *et al.*, (2002)
17	14.9 (10.9-17.5)	84	15.6 (9.8-25.5)			Weighted mean and range

* samples cultivated in different countries

**assumption of dry matter of 90%

n: number of genotypes

Table 3.4. Crude protein content (%, d.m.) of wheat and spelt whole grain

Wheat		Spelt		Growing conditions		Reference
n	% essential amino acids/total amino acids	n	% essential amino acids/total amino acids	Number of locations	Number of years	
1	39	4	40 (39-41)	1	1	Grela, (1996)
1	37	7	36 (35-37)	1	2	Jorgensen *et al.,* (1997)
2 spring and durum	34	2	34	1	2	Abdel-Aal and Hucl, (2002)
4	35.6 (34-39)	13	36.4 (34-41)			Weighted mean and range

n: number of genotypes

Table 3.5. Percentage (d.m.) of essential amino acids within total amino acids in wheat and spelt whole grain

3.2.4.2 Fractional composition and nutritional quality

Albumins and globulins account for about 20% of the protein content in spelt (Pruska-Kedzior *et al.*, 2008). Reversed Phase-High Performance Liquid Chromatography (RP-HPLC) has revealed a much higher content of total gliadins and a lower content of total glutenins in spelt than in wheat. The gliadin/glutenin ratio is significantly higher in spelt than in wheat (Wieser, 2000): 3.5 for spelt and 2 for common wheat (Koenig *et al.*, 2009). In spelt, α-gliadins and γ-gliadins are predominant, whereas low and high molecular weight (LMW and HMW) glutenin subunits and ω-gliadins are generally minor components (Wieser, 2000). Acetic acid soluble prolamins accounted for 94.1% of total gluten protein in spelt flours and 85-87% of the total gluten protein in wheat flours (Pruska-Kedzior *et al.*, 2008).

Gliadins and glutenins in spelt differ in structure from those in common wheat (Abdel-Aal *et al.*, 1996a; Harsch *et al.*, 1997; Radic *et al.*, 1997; Von Buren *et al.*, 2000) and spelt storage proteins form gluten in which the properties and quality differ from those of common wheat (Abdel-Aal *et al.*, 1995; Schober *et al.*, 2002).

The *in vitro* digestibility of spelt and wheat proteins is similar (86.7% on average), but it is 97.6% for casein (Abdel-Aal and Hucl, 2002). Ranhotra *et al.* (1995) observed a protein digestibility of 80.1% for spelt, 78.9% for wheat and 91.6% for casein, and concluded that this could suggest that spelt grain is better digested than common wheat, but the differences are minor.

Gliadins

Through hydration, gliadins form the gluten network that makes wheat and spelt unique bread-making cereals (Hoseney, 1994c). The MW of spelt gliadins is between 34 and 75 kDa (Abdel-Aal *et al.*, 1996). Abdel-Aal *et al.* (1996) and Harsch *et al.* (1997) found that the gliadin profile for common wheat and spelt differed. Spelt gliadins do not have slow-moving ω-gliadin or strong-staining fast-moving ω-gliadin, but these are present in common wheat. Spring and winter spelt were characterized by a large number of slow-moving α-gliadins. A γ-gliadin band was also observed in spring spelt, but

not in winter spelt or wheat, and this could be a useful point of distinction. Von Buren *et al.* (2000) discovered an unknown γ-gliadin gene in 18 spelt and spelt-wheat crosses and in the cultivar Chinese Spring, whereas the 16 wheat cultivars had a previously documented allele. In 2001, Von Büren *et al.* (2001) developed a polymerase chain reaction (PCR)-based method on the allelic difference in the γ-gliadin gene GAG56D to determine the proportion of wheat in spelt flour and products, with a minimal detection level of 5%. Piergiovanni and Volpe (2003) found that lines belonging to the same species could be differentiated mainly by comparing the pattern of β- and ω-gliadins. Federmann *et al.* (1992) reached the same conclusion and recommended slow-moving ω-gliadins for discriminating spelt flour. Using RP-HPLC and sodium dodecyl sulfate (SDS) electrophoresis, Koenig *et al.* (2009) observed that spelt was deficient in the so-called ω-bound gliadins (a minor portion of the ω-gliadins with a MW of 50-55 kDa) that are present in the glutenin fraction due to one cysteine residue in the amino acid sequence. This group of proteins could therefore be used to detect and quantify small amounts of common wheat in spelt and spelt products.

Glutenins

Most of the LMW-glutenin subunits of spelt seem acetic acid soluble. Spelt flour has been found to contain half as many NaOH-soluble glutenins (5.1% of total gluten protein) as common wheat flour (about 10% of total gluten proteins) (Pruska-Kedzior *et al.*, 2008). HMW and LMW glutenins have been associated with bread and pasta-making quality in common and durum wheat, respectively (Pogna *et al.*, 1990; Shewry *et al.*, 1995). From a cross between *T. aestivum* and *T. speltoides*, Moonen *et al.* (1985) found that two HMW glutenin subunits (5 and 9) of common wheat coded by a gene locus on the arm of chromosome 1B (Glu-B1c) are associated with good baking quality and that the replacement of these subunits by two others derived from *T. speltoides* (S1 and S2) led to poorer quality in the backcrossed lines examined. Radic *et al.* (1997) found differences in glutenin between spelt and common wheat using SDS polyacrylamide gel on 28 spelt samples, 16 spelt-wheat crosses and 10 winter wheat samples. Radic-Miehle *et al.* (1998) studied SDS soluble protein with and without a pre-extraction method, where typical bands could be identified for each species. Caballero *et al.* (2004) showed that variability in the LMW-glutenin subunits in spelt is higher than in other species. Alleles Glu-A3h and Glu-B3d coding LMW-glutenin subunits are present in spelt, but rare or absent in common wheat (Yan *et al.*, 2003).

Amino acids

Generally, cereal proteins are known for their low essential amino acid content, especially lysine (the first most deficient amino acid) (Kies and Fox, 1970) and threonine (the second most deficient amino acid), but they are rich in glutamic acid and proline, the major functional amino acid in dough formation (Abdel-Aal and Hucl, 2005). Spelt contains 38.2% of essential amino acids, as does wheat (Grela, 1996); the percentage of essential amino acids over total amino acids in protein is similar for

wheat and spelt, indicating equivalent protein quality (Grela, 1996; Jorgensen *et al.*, 1997; Abdel-Aal and Hucl, 2002; Ruibal-Mendieta, 2004) (Table 3.5) and equivalent biological value of proteins (indicated by the ratio of amino acid over protein) (Matuz *et al.*, 2000a).

The spelt amino acid composition of proteins differs slightly from that of wheat (Ranhotra *et al.*, 1995; Grela, 1996; Cubadda and Marconi, 1996; Bonafaccia *et al.*, 2000; Abdel-Aal and Hucl, 2002). Even if there is no statistical difference between spelt and common wheat in terms of amino acid content, there is evidence of higher values (except for isoleucine, leucine and glycine) in spelt than in common wheat (Dvoracek *et al.*, 2002). This was confirmed by Matuz *et al.* (2000a), who observed that wholemeal and flour from spelt had a higher content of most amino acids than some recently developed common wheat.

According to Grela (1996), the average lysine content is considerably higher in spelt (3.19 g/16gN) than in wheat (2.91 g/16gN), but most studies show that lysine content is lower in spelt than in wheat (Matuz *et al.*, 2000a; Abdel-Aal and Hucl, 2002), with a difference of up to 28% (Ranhotra *et al.*, 1995). Jorgensen *et al.* (1997) reported that mean lysine content was 2.72 g /16 g N (2.58-2.89) in spelt and 2.97 g/16 g N in wheat. Clamot (1984) found considerable genetic differences in protein and lysine content among 164 spelt samples, including 77 breeding lines from old Belgian landraces, 72 introductions from various countries and 15 induced mutants over a 3-year period.

Two studies found a higher methionine content in spelt than in common wheat (Ranhotra *et al.*, 1995; Bonafaccia *et al.*, 2000), but Matuz *et al.* (2000a) found the opposite. Jorgensen *et al.* (1997) found no significant differences for methionine content among the spelt varieties.

Within the group of essential amino acids, no significant difference was found for isoleucine, leucine, phenylalanine and valine content among the spelt varieties. In the group of non-essential amino acids, spelt had significantly more proline and less alanine and arginine than wheat (Jorgensen *et al.*, 1997). Spelt had also significantly more glutamic acid, significantly and negatively correlated with lysine (Jorgensen *et al.*, 1997; Abdel-Aal and Hucl, 2002) and more tyrosine than wheat (Ranhotra *et al.*, 1995; Jorgensen *et al.*, 1997). Aspartic acid was higher in spelt than in wheat (Ranhotra *et al.*, 1995).

3.2.5 Lipids

3.2.5.1 Fatty acid content and profiles

Lipids are minor grain constituents, accounting for about 3% of the wheat kernel. They are more concentrated in the germ (which contains 28.5% of lipids) and in the aleurone layer (8.0%) than in the endosperm (1.5%) (Delcour and Hoseney, 2010). Whole-wheat lipids are made up of about 70% non-polar lipids, 20% glycolipids and 10% phospholipids (Delcour and Hoseney, 2010), to which small percentages of sterols, tocopherols and other fat-soluble vitamins are added (Abdel-Aal and Hucl,

2005). In flour from the starchy endosperm, some lipids are associated with starch granules (1.0%), but others are not (1.4%). Among the non-starch lipids, free lipids (with non-polar and polar lipids) and bound lipids are distinct, and among the starch lipids the non-polar and polar lipids are separated (Chung *et al.,* 2009). Starch lipids are made up of 9% non-polar lipids, 5% glycolipids and 86% phospholipids (Delcour and Hoseney, 2010). Starch lipids are contained within starch granules as inclusion complexes and located between amylose and monoacyl lipids, such as lysophosphatidylcholines (Feillet, 2000). Non-starch lipids are made up of 60% non-polar lipids, 25% glycolipids and 15% phospholipids (Delcour and Hoseney, 2010). Non-starch lipids are dispersed inside the albumen and can react with other flour constituents. In the germ and the aleurone layer, lipids are assembled into spherosomes that are triglycerides surrounded by polar lipids and proteins (Feillet, 2000). In cereals, lipid content is usually determined by extraction with non-polar solvents. Bound lipids (to starch) are not taken into account because their extraction requires the use of polar solvents. Free lipid content is a practical estimate of total content and allows the comparison of data from different laboratories (Chung and Ohm, 2000).

Most studies on free lipids show that spelt is richer in lipids than wheat (Abdel-Aal *et al.*, 1995; Ranhotra *et al.*, 1995; Grela, 1996; Ranhotra *et al.*, 1996a; Piergiovanni *et al.*, 1996; Moudry et Dvoracek, 1999; Ruibal-Mendieta *et al.*, 2002; Ruibal-Mendieta *et al.*, 2005) (Table 3.6). Ruibal-Mendieta *et al.* (2002) found that total lipid content was also higher for spelt than wheat and true spelt might contain more lipids than hybrid spelt, although this difference was significant in two out of three harvest years. These observations suggest that germ is present in higher proportions in spelt kernels than in common wheat kernels (Marconi *et al.*, 1999).

With regard to fatty acids, studies show that the major fatty acids in spelt and wheat wholemeal are linoleic, palmitic, oleic and linolenic acids (Grela, 1996; Ruibal-Mendieta *et al.* 2004b; Ruibal-Mendieta *et al.*, 2005). The proportion of oleic acid in fatty acids is higher in spelt than in common wheat, but the proportion of linoleic and linolenic acids are lower in spelt than in common wheat. More saturated fatty acids have been observed in wheat than in spelt (averages of 19.8% and 18.9%, respectively; Table 3.7).

Most results have been obtained using extraction with ether or petrolether, so the variation in content can be attributed partly to the change of method. However, it is known that lipid content and composition are influenced by genetic variation (including wheat class and cultivar), environmental effects during growth (including location, year, weather and soils) and the effects of the genetic x environment interaction (Chung *et al.,* 2009). For fatty acids, Grela (1996) and Ruibal-Mendieta *et al.* (2004b, 2005) used different methods, respectively from Stoldt (1952) and from Folch (1957) but there was variability among studies that used the same method. Method, environment, genotype and their interaction therefore all contribute to the variability.

3.2.5.2 Sterols

Phytosterols are known to reduce serum cholesterol and could offer protection against several cancer types (Nurmi *et al.*, 2008). The range in phytosterol content in 16 spelt genotypes (nine cultivars and seven landraces) was broader in a study conducted by Ruibal-Mendieta *et al.* (2004a) than for five cultivars in a study by Nurmi *et al.* (2008). Ruibal-Mendieta (2004a) observed no difference in sterol content in spelt and winter wheat, but Nurmi *et al.* (2008) found higher average content in spelt than in wheat (928 [893-963] µg/g [d.m.] and 841 [670-959] µg/g [d.m.]). This latter finding was confirmed by Iafelice *et al.* (2009), who reported 717 (628-819) µg/g (d.m.) total sterol, on average, for spelt, and 634 (600-677) µg/g (d.m.) for wheat. The free sterol content, however, was higher in wheat 324 (288-387) µg/g (d.m.) than in spelt 252 (191-294) µg/g (d.m.), and esterified sterol content was slightly higher in spelt 267 (251-291) µg/g (d.m.) than in wheat 258 (219-330) µg/g (d.m.) (Iafelice *et al.*, 2009). This accords with the amount of free and esterified sterols measured by Ruibal-Mendieta *et al.* (2004a) of 527 µg/g (d.m.) in spelt and of 528 µg/g (d.m.) in wheat.

Ruibal-Mendieta *et al.* (2004a) found that spelt and wheat display a similar sterol profile and content. The Δ7- avenasterol content is an exception; it is 45% higher in spelt than in wheat (Ruibal-Mendieta *et al.*, 2004a). For spelt, Ruibal-Mendieta *et al.* (2004a) reported that sitosterol and campesterol accounted for about 70 and 20% of the total sterols, respectively, and stanols for 5%, which differed from the results reported by Nurmi *et al.* (2008) where sitosterol, campesterol and stanols accounted for 49%, 14% and 26%, respectively. In the Nurmi *et al.* (2008) study, the proportions for wheat were sitosterol 52%, campesterol 15% and stanols 24%. These differences in sterol profiles appear to have resulted from analytical differences (Nurmi *et al.*, 2008). The results reported by Iafelice *et al.* (2009) confirmed the earlier ones, with 59% sitosterol, 18% campesterol and 17% stanols in wheat and 56%, 16% and 19% in spelt, respectively, among the total sterols. For the free sterols, the proportions were similar in spelt and wheat, with slightly more sitosterol (by 2%) in wheat. For the esterified sterols, the proportion of sitosterol was higher in spelt than in wheat (59% and 56%, respectively), but that of campesterol and sitostanol was lower in spelt (16% and 15%, respectively) than in wheat (18% and 17%, respectively) (Iafelice *et al.*, 2009).

Wheat		Spelt		Growing conditions		Reference
n	Free lipids	n	Free lipids	Number of locations	Number of years	
1 (spring)	2.1	5	2.4 (2.2-2.5)	4 to 5	2	Abdel-Aal *et al.* (1995)
1	2.2	1	2.8	1*	1	Ranhotra *et al.* (1995)
1	2.3	4	3.9 (3.8-4.0)	1	1	Grela *et al.,* (1996)
2	3.2 (2.8-3.7)	3	2.4 (1.6-2.7)	5 spelt and 2-3 wheat	1	Ranhotra *et al.* (1996a)
3 (durum)	1.5 (1.1-2.1)	37	2 (1.4-2.8)	1	1	Piergiovanni *et al.* , (1996)
		5	4.4 (3.8-5.2)	4*	unknown	Marconi *et al.* , (1999)
1	1.6	10	1.9 (1.6-2.2)	1	3	Moudry and Dvoracek, (1999)
5	1.9 (1.7-2.0)	9	2.3 (1.9-2.6)	1	1	Ruibal-Mendieta *et al.,* (2005)
14	2.1 (1.1-3.7)	74	2.5 (1.4-5.2)			Weighted mean and range

* samples cultivated in different countries
n: number of genotypes

Table 3.6. Free lipid content (%, d.m.) of wheat and spelt whole grain

	Wheat								Growing conditions		Reference
n	Myristic acid	Palmitic acid	Palmitoleic acid	Stearic acid	Oleic acid	Linoleic acid	α-linolenic acid	Eikosenoic acid	Number of locations	Number of years	
	C14:0	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	C20:1			
1	0.5	16.7	0.2	0.8	11.3	62.8	7.1	0.6	1	3	Grela, (1996)
11		19.2		0.9	11.6	62.5	5.6		-	1	Ruibal-Mendieta *et al* ., (2004b)
5		19.1		0.5	11.6	63.5	5.1		-	1	
5		19.3			10.7	64.6	5.3		1	1	Ruibal-Mendieta *et al.* , (2005)
22	0.5	18.9 (16.7-19.3)	0.2	0.8 (0.5-0.9)	11.4 (10.7-11.6)	63.2 (62.5-64.6)	5.6 (5.1-7.1)	0.6			Weighted mean and range

	Spelt								Growing conditions		Reference
n	Myristic acid	Palmitic acid	Palmitoleic acid	Stearic acid	Oleic acid	Linoleic acid	α-linolenic acid	Eikosenoic acid	Number of locations	Number of years	
	C14:0	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	C20:1			
4	0.55	18.5	0.3	1.4	20.4	55.0	3.0	0.7	1	3	Grela, (1996)
11		16.8		1.0	18.2	59.4	4.5		-	1	Ruibal-Mendieta *et al* ., (2004b)
16		16.7		0.7	17.3	61	4		-	1	
9		16.8			16.1	63.2	3.9		1	1	Ruibal-Mendieta *et al.* , (2005)
40	0.6	17.2 (16.7-18.5)	0.3	1.0 (0.7-1.4)	18.1 (16.1-20.4)	59.6 (55.0-63.2)	3.8 (3.0-4.5)	0.7			Weighted mean and range

% of total identified fatty acids

n: number of genotypes

Table 3.7. Fatty acid distrubution (%) of wheat and spelt whole grain

A

Species	Wheat		Spelt	
Component	n	Weighted mean and range	n	Weighted mean and range
Starch	2	63.0 (62.4-68.1)	9	64.0 (60.9-67.6)
Sugars	2	3.3 (3.1-3.3)	9	2.2 (1.7-3.4)
Carbohydrate	5	66.5 (63.6-71.2)	18	66.1 (59.2-70.9)
Insouble Fiber	8	11.2 (10.7-13.2)	21	9.3 (8.0-12.9)
Soluble Fiber	8	1.7 (1.4-2.2)	21	1.7 (1.2-2.4)
Total Fiber	141	14.9 (12.3-15.4)	34	10.9 (7.7-14.9)
Lignin	4	0.9 (0.6-1.2)	8	0.9 (0.6-1.1)
Hemicellulose	4	8.2 (7.0-9.2)	8	9.3 (4.6-18.8)
Cellulose	4	2.3 (2.1-2.8)	8	2.0 (1.4-2.3)
Crude fibers	3	2.4 (1.8-3.3)	21	2.4 (1.7-3.3)
Crude proteins	17	14.9 (10.9-17.5)	84	15.6 (9.8-25.5)
% essential a.a./total a.a.	4	35.6 (34-39)	13	36.4 (34-41)
Free lipids	14	2.1 (1.1-3.7)	74	2.5 (1.4-5.2)

B

Component	n	Weighted mean and range	n	Weighted mean and range
Myristic acid	1	0.5	4	0.6
Palmitic acid	22	18.9 (16.7-19.3)	40	17.2 (16.7-18.5)
Palmitoleic acid	1	0.2	4	0.3
Stearic acid	17	0.8 (0.5-0.9)	31	1.0 (0.7-1.4)
Oleic acid	22	11.4 (10.7-11.6)	40	18.1 (16.1-20.4)
Linoleic acid	22	63.2 (62.5-64.6)	40	59.6 (55.0-63.2)
α-linolenic acid	22	5.6 (5.1-7.1)	40	3.8 (3.0-4.5)
Eikosenoic acid	1	0.6	4	0.7

n: number of genotypes

Table 3.8. Macronutrient content (weighted mean, minimum and maximum; %, d.m.) (A) and fatty acid distribution (%) (B) of wheat and spelt whole grain reported by different studies

3.2.6 Conclusion

This paper has highlighted differences in the macronutrient content and profiles of spelt and wheat. It was shown that spelt has a higher protein and lipid content and a lower insoluble and total fiber content than wheat, and that there is no significant difference between them in starch, sugar and soluble fiber content (Table 3.8, Appendix Fig. A.1 and A.2). The differences can affect the techno-functional properties of spelt. Bread-making and pasta making from spelt requires adapted techniques, and the evaluation procedures used for wheat and wheat products should not be directly applied to spelt and spelt products (Abdel-Aal and Hucl, 2005). This study should be followed by one on micronutrient content – ashes, minerals, vitamins and bioactive compounds – for which differences between spelt and wheat have also been reported. In addition, research on the applications of spelt should continue to look for new ways of using this crop in order to sustain its development. Exhaustive biochemical, nutritional and clinical research should be undertaken to assess claims for the pro-health properties of spelt grain and products that have not yet been scientifically proved.

3.2.7 References

AACC, 2001. The definition of dietary fiber. *Cereal Foods World*, 46,112-126.

Abdel-Aal E.S.M., Hucl P., Sosulski F.W., 1995. Compositional and nutritional characteristics of spring einkorn and spelt wheats. *Cereal Chemistry*, 72, 621-624.

Abdel-Aal E.S.M., Salama D.A., Hucl P., Solsulski F.W., Cao, W., 1996. Electrophoretic characterization of spring spelt wheat gliadins. *Journal of Agricultural and Food Chemistry*, 44, 2117-2123.

Abdel-Aal E., Solsulski F.W., Hucl P., 1998a. Origins, characteristics and potentials of ancient wheats. *Cereal Foods World*, 43, 708-715.

Abdel-Aal E.S.M., Hucl P., Sosulski F.W., 1998b. Food uses for ancient wheats. *Cereal Foods World*, 43, 763-767.

Abdel-Aal E.-S.M., Hucl P. and Sosulski F.W., 1999a. Optimizing the bread formulation for soft spelt wheat. *Cereal Foods World*, 44, 7, 480-483.

Abdel-Aal E.-S.M., Hucl P. and Sosulski F.W., 1999b. Starches from primitive wheats. II Thermal and structural properties. Annual meeting Abstract 225, AACC International St Paul MN.
http://www.aaccnet.org/meetings/99mtg/abstracts/acabc27.htm, consulted on 05/11/2010.

Abdel-Aal E., and Hucl P., 2002. Amino acid composition and in vitro protein digestibility of selected ancient wheats and their end products. *Journal of Food Composition and Analysis*, 15, 737-747.

Abdel-Aal E. and Hucl P. 2005. Spelt: a specialty wheat for emerging food uses. In Specialty grains for food and feed. Abdel-Aal E. and Wood P. American Association of Cereal Chemists. St-Paul, Minnesota. p 109-141.

An X.L., Li Q.Y., Yan Y.M., Xiao Y.H., Hsam S.L.K., Zeller F.J., 2005. Genetic diversity of European spelt wheat (*Triticum aestivum* ssp *spelta* L. em. Thell.) revealed by glutenin subunit variations at the Glu-A and Glu-3 loci. *Euphytica*, 146, 3, 193-201.

Andrews A.C., 1964. The genetic origin of spelt ad related wheats. Der Zuchter: *Zeitschrift fur theorische und angewandte genetic*, 34, 1, 17-22.

Belitz H.-D., Grosch W., 1999. Food chemistry. Second edition. Springer-Verlag, Berlin Heidelberg, p 631-636.

Bonafaccia G., Galli V., Francisci R., Mair V., Skrabanja V., Kreft I., 2000. Characteristics of spelt wheat products and nutritional value of spelt wheat-based bread. *Food Chemistry*, 68, 437-441.

Caballero L., Martin L.M., Alvarez J.B. 2004. Genetic variability of the low-molecular-weight glutenin subunits in spelt wheat (*Triticum aestivum* ssp. *spelta* L. em. Thell.). *Theoretical and Applied Genetics*, 108, 914-919.

Campbell K.G., 1997. Spelt: agronomy, genetics and breeding. *Plant Breeding Reviews*, 15, 187-213.

Chung O.K. and Ohm J.-B., 2000. Cereal lipids. In Handbook of cereal science and techonology. Second edition. Kulp K. et Ponte J.G.Jr., Marcel Dekker, NewYork, 417-477.

Chung O.K., Ohm J.-B., Ram M.S., Park S.-H., Howitt C.A., 2009. Wheat lipids in Wheat Chemistry and Technology. Fourth Edition. St Paul, Minnesota, AACC, p 363-399.

Clamot G., 1984. Genetic variability of the protein and lysine content of spelt (*Triticum spelta*). *Zeitschrift für Pflanzenzüchtung*, 93, 106-114.

Codianni P., Ronga G., Di Fonzo N., Troccoli A.,1996. Performance of selected strains of 'Farro' (*Triticum monococcum* L., *Triticum dicoccum* Schübler, *Triticum spelta* L.) and Durum Wheat (*Triticum durum* Desf. cv. Trinakria) in the difficult flat environment of southern Italy. *Journal of Agronomy and Crop Science*, 176, 15-21.

Cubadda R. and Marconi E., 1996. Technological and nutritional aspects in emmer and spelt. In Hulled wheats. Paludosi S., Hammer K., Heller J. Proceedings of the first international workshop on hulled wheats, 21-22 July 1995, Castelvechio Pascoli, Italy. IPGRI, Rome, 203-212.

Davin L.B., Lewis N.G., 2005. Lignin primary structures and dirigent sites. *Current Opinion in Biotechnology*, 16, 407-415.

Delcour J.A., Hoseney R.C., 2010. Principles of cereal science and technology. Third Edition. St Paul, Minnesota: AACC, 270 p.

Dupont F.M., Alltenbach S.B., 2003. Molecular and biochemical impacts of environmental factors on wheat grain development and protein synthesis. *Journal of Cereal Science*, 38, 133-146.

Dvoracek V., Curn V., Moudry J., 2002. Evaluation of amino acid content and composition in spelt wheat varieties. *Cereal Research Communications*, 30, 187-193.

Escarnot E., Agneessens R., Wathelet B., Paquot M., 2010. Quantitative and qualitative study of spelt and wheat fibres in varying milling fractions. *Food Chemistry*, 122, 857-863.

Escarnot E., Aguedo M., Agneessens R., Wathelet B., Paquot M., 2011a. Extraction and characterization of water-extractable and water-unextractable arabinoxylans from spelt bran: Study of the hydrolysis conditions for monosaccharides analysis. *Journal of Cereal Science*, 53, 45-52.

FAO, 1998. Carbohydrates in human nutrition. FAO Food and nutrition paper – 66. Report of a Joint FAO/WHO Expert Consultation. Rome, 14-18 April 1997. Reprinted 1998. Consulté le 11/01/07, http://www.fao.org/docrep/W8079E/W8079E00.htm.

Federmann G.R., Goecke E.U., Steiner A.M., 1992. Research note: detection of adulteration of flour spelt (*Triticum spelta* L.) with flour wheat (*Triticum aestivum* L. emend, Fiori and Paol.) by electrophoresis. *Plant Var. Seeds*, 5, 123-125.

Feillet P., 2000. Le grain de blé, composition et utilisation. Paris, France : Institut National de la Recherche Agronomique, 308 p.

Folch J., Lees M., Stanley G.H.S., 1957. A simple method for the isolation and purification of total lipids from animal tissues. *Journal of Biochemical Chemistry*, 226, 497-509.

Gräber S. and Kuhn, M., 1992. Evaluation of the baking quality of different varieties of spelt. *Getreide, Mehl u Brot* 46, 102-108.

Gray J., 2003. Carbohdyrates: nutritional and health aspects. ILSI Europe concise monograph series. Brussels, 30 p.

Gray J., 2006. Dietary fibre. Definition and analysis, physiology, health. ILSI Europe concise monograph series. Brussels, 36 p.

Gebruers K., Dornez E., Boros D., Fras A., Dynkowska W., Bedo Z., Rakszegi M., Delcour J.A., Courtin C.M., 2008. Variation in the content of dietary fiber and components thereof in wheats in the healthgrain diversity screen. *Journal of Agricultural and Food Chemistry*, 56, 9740-9749.

Gebruers K., Dornez E., Bedo Z., Rakszegi M., Courtin C.M, Delcour J.A., 2010. Variability in xylanase and xylanase inhibition activities in different creals in the healthgrain diversity screen and contribution of environnemnt and genotype to this variability in common wheat. *Journal of Agricultural and Food Chemistry*, 58, 9362-9371.

Grela E.R., 1996. Nutrient composition and content of antinutritional factors in spelt (*Triticum spelta* L.) cultivars. *Journal of the Science of Food and Agriculture*, 71, 399-404.

Harsch S., Günther T., Kling Ch.I., Rozynek B., Hesemann C.U., 1997. Characterization of spelt (*Triticum spelta* L.) forms by gel electrophoretic analyses of seed storage proteins. I The gliadins. *Theoretical and Applied Genetics*, 94, 52-60.

Hoseney C.R., 1994a. Proteins of cereals. In: Principles of cereal science and technology. Second edition. American Asociation of Cereal Chemists. St-Paul, Minnesota, p 65-79.

Hoseney C.R., 1994c. Gluten proteins. In: Principles of cereal science and technology. Second edition. American Association of Cereal Chemists. St-Paul, Minnesota, p 197-211.

Iafelice G., Verardo V., Marconi E., Caboni M.F., 2009. Characterization of total, free and esterified phytosterols in tetraploid and hexaploid wheats. *Journal of Agricultural and Food Chemistry*, 57, 2267-2273.

Jiang L.-N., Shao M., Liu L., Li C.-X., 2007. Effects of genotypes and environments on pentosan content and viscosity in wheat grains (in Chinese/English abstract). *Journal of Henan Agricutlural Science*, 1, 17-19

Jorgensen J.R., Olsen C.C., Christiansen S., 1997. Cultivation and quality assessment of spelt (*Triticum spelta* L.) compared with winter wheat (*Triticum aestivum* L.). In: Minutes from COST 814 programme, Copenhagen, Denmark, 31-37.

Kies C. and Fox H.M., 1970. Determination of the first limiting amino acid of wheat and triticale grain for humans. *Cereal Chemistry*, 47, 615-625.

Koenig A., Wieser H., Koehler P., 2009. Distinguishing wheat and spelt using typical protein markers. Proceedings of the 10th International Gluten Workshop 7-9 September 2009, Clermont-Ferrand, France, p 142-145.

Lecomte P., Boreux M., Agneessens R., Beckers Y., Dekeyser A., 1996. Caractérisation de la composition chimique et de la valeur de l'épeautre (*Triticum spelta*) en alimentation des ruminants. *Rencontres Recherche Ruminants*, 3, p112.

Li C.-X., Qiu Z.-B., Jiang L.-N., Zhang X., 2002. Research on content of pentosans in wheat grain in different ecological environment (in chinese/English abstract). Acta Agriculturae Boreali Sin, 17:1-4.

Loje H., Moller B., Laustsen A.M., Hansen A., 2003. Chemical composition, functional properties and sensory profiling of einkorn (*Triticum monococcum* L.). *Journal of Cereal Science*, 37, 231-240.

Marconi E., Carcea M., Graziano M., Cubadda R., 1999. Kernel properties and pasta-making quality of five European spelt wheat (*Triticum spelta* L.) cultivars. *Cereal Chemistry*, 76 (1), 25-29.

Marconi E., Carcea M., Schiavone M., Cubadda R., 2002. Spelt (*Triticum spelta* L.) pasta quality: combined effect of flour properties and drying conditions. *Cereal Chemistry*, 79 (5), 634-639.

Massaux C., Sindic M., Lenartz J., Sinnaeve G., Bodson B., Falisse A., Dardenne P., Deroanne C., 2008. Variations in physicochemical and functional properties of starches extracted from European soft wheat (*Triticum aestivum* L.): The importance to preserve the varietal identity. *Carbohydrate Polymers*, 71, 32-41.

Matuz J., Bartok T., Morocz-Salamon K., Bona L., 2000a. Structure and potential allergenic character of cereal proteins. I. Protein content and amino acid composition. *Cereal Research Communications*, 28 (3), 263-270.

Moonen J.H.E and Zeven A.C., 1985. Association between high molecular weight subunits of glutenin and bread-making quality in wheat Lines derived from backcrosses between *T. aestivum* and *T. speltoides*. *Journal of Cereal Science*, 3, 97-101.

Moore M.A., Beom Park C., Tsuda H., 1998. Soluble and insoluble fiber influences on cancer development. *Critical Review Oncology and Hematology*, 27, 229-242.

Moudry J., Dvoracek V., 1999. Chemical composition of grain of different spelt (*Triticum spelta* L.) varieties. *Rostlinna Vyroba*, 45 (12), 533-538.

Muramatsu M., 1963. Dosage effect of the spelta gene q of hexaploid wheat. *Genetics*, 48: 469-482.

Nesbitt M. and Samuel D., 1996. From staple crop to extinction? The archaeology and history of the hulled wheats. In: Hulled wheats: Proc. First. Int. Workshop on hulled wheats. Padulosi S. et Heller J. International Plant Genetic Resources Institute, Rome, p 41-100.

Nurmi T., Nyström L., Edelmann M., Lampi A.-M., Piironen V., 2008. Phytosterols in wheat genotypes in the healthgrain diversity screen. *Journal of Agricultural and Food Chemistry*, 56, 9710-9715.

Oliveira J.A., 2001. North Spanish emmer and spelt wheat landraces: agronomical and grain quality characteristics evaluation. *Plant Genetic Resources Newsletters,* 125, 16-20.

Percival J., 1921. The wheat plant. A monograph. Duckworth and Co. London, p 325-334.

Piergiovanni A.R., Laghetti G., Perrino P., 1996. Characteristics of meal from hulled wheats (*Triticum dicoccon* Schrank and *T. spelta* L.): an evaluation of selected accessions. *Cereal Chemistry*, 73 (6), 732-735.

Piergiovanni A.R., and Volpe, N., 2003. Capillary electrophoresis of gliadins as a tool in the discrimination and characterization of hulled wheats (*Triticum dicoccon* Schrank and *T. spelta* L.). *Cereal Chemistry*, 80, 269-273.

Pogna N.F., Autran J.C., Mellini F., Lafiandra D., Feillet P., 1990. Chromosome 1B-encoded gliadins and glutenins subunits in durum wheat: genetics and relationship to gluten strength. *Journal of Cereal Science*, 11, 15-34.

Pruska-Kedzior A., Kedzior Z., Klockiewicz-Kaminska E., 2008. Comparison of viscoelastic properties of gluten from spelt and common wheat. *European Food Research and Technology*, 227, 199-207.

Radic H., Günther T., Kling Ch.I., Hesemann C.U., 1997. Characterization of spelt (*Triticum spelta* L.) forms by gel electrophoretic analyses of seed storage proteins. II The glutenins. *Theoretical and Applied Genetics*, 94, 882-886.

Radic-Miehle H., Saam C., Huls R., Kling Ch.I., Hesemann C.U., 1998. Characterization of spelt (*Triticum spelta* L.) forms by gel-electrophoretic analyses of seed storage proteins. III. Comparative analyses of spelt and central European winter wheat (*Triticum aestivum* L.) cultivars by SDS-PAGE and acid-PAGE. *Theoretical and Applied Genetics*, 97, 1340-1346.

Ranhotra G.S., Gelroth J.A., Glaser B.K., Lorenz K.J., 1995. Baking and nutritional qualities of spelt wheat sample. *Lebensmittel-Wissenschaft und Technologie*, 28, 118-122.

Ranhotra G.S., Gelroth J.A., Glaser B.K., Stallknecht G.F., 1996a. Nutritional profile of three spelt wheat cultivars grown at five different locations. *Cereal Chemistry*, 73 (5), 533-535.

Ranhotra G.S., Gelroth J.A., Glaser B.K., Lorenz K.J., 1996b. Nutrient composition of spelt wheat. *Journal of Food Composition and Analysis*, 9,81-84

Ruibal-Mendieta N.L., 2004. Lipids and minerals in spelt (*Triticum aestivum* ssp. *spelta*) and common wheat (*T.aestivum* ssp. *vulgare*). Chemical and nutritional distinction between both cereals. Thèse de doctorat. Faculté d'ingénierie biologique, agronomique et environnementale. Université catholique de Louvain.

Ruibal-Mendieta N.L., Delacroix D.L., Meurens M., 2002. A comparative of analysis of free, bound and total lipid content on spelt and winter wheat wholemeal. *Journal of Cereal Science*, 35: 337-342.

Ruibal-Mendieta N.L., Rozenberg R., Delacroix D.L., Petitjean G., Dekeyser A., Baccelli C., Marques C., Delzenne N.M., Meurens M., Habib-Jiwan J.-L., Quetin-Leclercq J., 2004a. Spelt (*Triticum spelta* L.) and winter wheat (*Triticum aestivum* L.) wholemeals have similar sterols profiles, as determined by quantitative liquid chromatography and mass spectrometry analysis. *Journal of Agricultural and Food Chemistry*, 52, 4802-4807.

Ruibal-Mendieta N.L., Dekeyser, A., Delacroix D.L., Mignolet E., Larondelle Y., Meurens M., 2004b. The oleate/palmitate ratio allows the distinction between wholemeals of spelt (*Triticum spelta* L.) and winter wheat (*T. aestivum* L.). *Journal of Cereal Science*, 39, 413-415.

Ruibal-Mendieta N.L., Delacroix D.L., Mignolet E., Pycke J.-M., Marques C., Rozenberg R., Petitjean G., Habib-Jiwan J.-L., Meurens M., Quetin-Leclercq J., Delzenne N.M., Larondelle Y., 2005. Spelt (*Triticum aestivum* ssp. *spelta*) as a source of breadmaking flours and bran naturally enriched in oleic acid and minerals but not phytic acid. *Journal of Agricultural and Food Chemistry*, 53, 2571-2759.

Schober T.J., Clarke C.I., Kuhn M., 2002. Characterization of functional properties of gluten proteins in spelt cultivars using rheological and quality factor measurements. *Cereal Chemistry*, 79, 408-417.

Schmid J.E., Winzeler M. and Winzeler, H., 1994. Analysis of disease resistance and quality characters of F1 hybrids of crosses between wheat (*Triticum aestivum* L.) and spelt (*Triticum spelta* L.). *Euphytica*, 75, 105-110.

Shewry P.R., Tatham A.S., Barro F., Barcelo P., Lazzeri P., 1995. Biotechnology of breadmaking: unraveling and manipulating the multi-protein gluten complex. *Bio/Technology,* 13, 1185-1190.

Stoldt W., 1952. Vorschlag zur Vereinheitlichung der Fettbestimmung in *Lebensmittel Fette und Seifen*, 54, 206-207

Von Büren M., Stadler M., Lüthy J., 2001. Detection of wheat adulteration of spelt flour and products by PCR. *European Food Research Technology*, 212, 234-239.

Von Büren M., Lüthy J. and Hübner P., 2000. A spelt-specific γ-gliadin gene: discovery and detection. *Theoretical and Applied Genetics,* 100, 271-279.

Wieser H., 2000. Comparative investigations of gluten proteins from different wheat species. *European Food Research Technology*, 211, 262-268.

Wilson J.D., Bechtel D.B., Wilson G.W.T., Seib P.A. 2008. Bread quality of spelt wheat and its starch. *Cereal Chemistry*, 85, 5, 629-638.

Yan Y., Hsam S.L.K., Yu J.Z., Jiang Y., Ohtsuka I., Zeller F.J., 2003. HMW and LMW glutenins alleles among putative tetraploid and hexaploid European spelt wheat (*Triticum spelta* L.) progenitors. *Theoretical and Applied Genetics*, 107, 1321-1330.

Zorb C., Betsche T., Langenkamper G., Zapp J., Seifert M., 2007. Free sugars in spelt wholemeal and flour. *Journal of Applied Botany and Food Quality*, 81, 172-174.

4 CHAPTER 4

Quantitative and qualitative study of spelt and wheat fibers in varying milling fractions

Excerpt from Article 1:

Escarnot, E., Agneessens, R., Wathelet, B., Paquot, M., 2010. Quantitative and qualitative study of spelt and wheat fibres in varying milling fractions. *Food Chemistry*, 122, 857-863.

4.1 The context of the study

The previous chapter showed that spelt contained fibers in the same extent as wheat even if differences exist between both subspecies. If the previous chapter confirms the importance of fibers in the whole grain flour, few data are available for other parts of the grain such as the bran and the whole spikelet flour. As displayed in the bibliographic review, a lot of studies focused on insoluble and soluble fibers while much less concentrated on hemicelluloses, cellulose and lignin. Besides, the number of genotypes evaluated and their conditions of cultivation are not always strictly the same and comparison between spelt and wheat may be difficult.

Whole grain and fibers are very important in human nutrition and have several beneficial effects on human health. A high content of dietary fiber in whole-grain cereals, vegetables, legumes and fruits is associated with relative low energy density, promotion of satiety, and in observational studies with a lesser degree of weight gain than among those with lower intakes. Consumption of diet high in fruits, vegetables and whole grains is associated with reduced risk of type 2 diabetes, coronary heart disease and stroke and should therefore be included in diets for long-term weight management (Van Dam and Seidell, 2007). Many of these foods especially those that are high in dietary fiber, will reduce total and low-density lipoprotein cholesterol and help to improve glycaemic control in those with diabetes. Concerning cancer risk, the results of studies have varied and no firm conclusion can be drawn but the available data suggest that high intakes of dietary fiber possibly reduce the risk for colorectal cancer (Key and Spencer, 2007).

As there is a lack of knowledge on spelt whole grain and dietary fiber in spite of their importance in human nutrition, the whole grain flour from spelt and wheat will be compared regarding the composition in fibers: insoluble, soluble, hemicelluloses, cellulose and lignin. In spite of advises to consume whole grain food, in western countries, average intakes of dietary fiber are below the recommendation (Mann, 2007). In order to remedy to this state, the incorporation of high-fiber ingredient in food has been evaluated. For instance, wheat bran has been experimented as a supplement for producing high-fiber products, such as bread and cake (Vetter, 1984; Lebesi and Tzia, 2011). In this objective, it was interesting to evaluate the potential of spelt bran. Consequently, spelt and wheat bran will be analysed for their content in insoluble, soluble fibers and hemicelluloses, cellulose and lignin. Besides, this study will enable to provide data on the fibers of spelt bran for which none is available. As already mentioned, main use of spelt is feed, and fibers are very important in the feed equilibrium of ruminants. Only one study deals with the fiber composition of the whole spikelet flour (Lecomte *et al.,* 1996), consequently it seems important to analyse the content of the different fibers.

The following chapter will bring information for the milling fractions and the type of fibers mentioned above. The genotypes chosen will be registered varieties and land races which are different regarding

the breeding origin, the genetic background and the use (baking and forage varieties will be present in the panel). The grains in the study will originate from the same trial, which is an essential condition to compare the samples as environmental and cultivation conditions can modify the content in fibers.

4.2 Quantitative and qualitative study of spelt and wheat fibers in varying milling fractions

Abstract

The fiber composition of four spelt genotypes and of three wheat genotypes was studied on three grindings: bran, whole bare grains flour for all genotypes, and whole hulled grains flour for spelt only. Insoluble fiber and soluble fiber contents were measured after removal of proteins, starch and ashes from the sample (Lee *et al.,* 1992). Cellulose, hemicelluloses and lignin contents were measured according to the same principles with different chemical degradations of the sample (Van Soest and Wine, 1967). Spelt and wheat bran and whole grain flour displayed significant statistical differences for hemicelluloses and cellulose contents. Variability among the spelt genotypes was much higher than among the wheat genotypes. The study also highlighted the special profile of the true baking variety Ressac and the richness in fibers of the landrace 140. Finally, various methods of measurement were compared and a combination of these methods was proposed for cereal grains dietary fiber analysis.

4.2.1 Introduction

Spelt (*Triticum aestivum* ssp. *spelta*) is an ancient subspecies of common wheat (*Triticum aestivum* ssp. *aestivum*). Until the beginning of the 20th century, spelt was the main grain used for bread production in south-western Germany and parts of Switzerland and Austria. Since then, however, modern wheat has largely replaced spelt. This is due to spelt's lower yield, to its sensitivity to lodging and to the hulls which account for 21-32% of the spikelet (Percival, 1921). In agronomic terms, spelt might be more resistant to disease, and perform better than wheat under less advantageous growing conditions, such as wet, cold soils and high altitudes (Campbell, 1997). Besides, with the hull covering the seed, chemical treatment before sowing might not be necessary and due to its long straw, it does not support a high level of nitrogen fertilisation (Bonafaccia *et al.,* 2000). In addition, it contributes to agrobiodiversity. Spelt meets the European Union guidelines on growing practices for more environmentally friendly cereal production and is also suitable for organic farming. Therefore, it continues to be cultivated in several central and middle European countries. In Belgium, it covers 10,000 ha, in Germany 23,000 ha (Statistisches Bundesamt Deutschland, 2008), while in Switzerland it represents 10,000 T of grains (Office fédéral de la statistique Suisse, 2008).

Spelt is believed to possess valuable nutritional qualities, differing from those of wheat. It has a higher protein content than wheat (Abdel-Aal *et al.,* 1995; Ranhotra *et al.,*1995; Grela, 1996; Ranhotra, *et al.,* 1996a; Marconi *et al.,* 1999; Bonafaccia *et al.*, 2000), a higher lipid content, especially in Δ^7-avenasterol (Abdel-Aal *et al.*, 1995; Ranhotra *et al.*, 1995; Grela, 1996; Ranhotra *et al.*, 1996a; Marconi *et al.*, 1999; Ruibal-Mendieta *et al.,* 2002) and higher magnesium, phosphorus, iron, copper and zinc contents (Ruibal-Mendieta *et al.*, 2005). Spelt has long been used mainly in cattle feed especially for calves. It also suits growing and fattening animals and can be used to supplement forage for rearing animals (Lecomte *et al.,* 1996). Over the past few decades spelt has attracted renewed and

increasing interest as human food due to its image as a “healthier, more natural, less over-bred” cereal than modern wheat (Schober *et al.,* 2006). It falls into the niche product and is important in specialty breads, organic food and food products with characteristics that differ from regular wheat products (Ranhotra *et al*., 1995). Making bread from spelt flour requires adapted baking methods. Spelt dough is less stable and has less elasticity and a higher extensibility than common wheat dough. It is very soft and sticky after kneading; handling it is therefore more difficult and the loaf volume is generally lower than with modern wheat cultivars (Schober *et al*., 2002). However, the technological potential of spelt for milling, bread making and pasta production seems promising (Ranhotra *et al*., 1995; Bonafaccia *et al*., 2000; Schober *et al*., 2002; Schober *et al*., 2006). From the health point of view, spelt is recommended for the treatment of colitis ulcerosa, neurodermitis and other allergies, as well as for high blood cholesterol (Strechlow *et al.,* 1991), but it is forbidden for people suffering from celiac disease, and it can provoke wheat allergy and gluten enteropathy (Kasadra and D’Ovidio, 1999).

Cereal foods constitute a major part of the daily diet in Europe and are one of the main dietary sources of fiber. The recommended current fiber consumption is 25 g/day/person (Poutanen, 2006). There is increasing agreement that a sufficient amount of whole grain consumption protects against the development of diet-related disorders such as cardiovascular disease and type 2 diabetes (Jones, 2006), as well as colorectal cancer (Larsson *et al.,* 2005). A greater intake of whole grain foods is also associated with less obesity (Koh-Banerjee *et al.,* 2004). Concerning colon cancer risk, whole grains and wheat bran might be the most protective fiber sources (Kritchevsly, 2001). Wheat bran is very effective in increasing laxation and therefore is used in the treatment of constipation and might prevent colorectal cancer (Ferguson and Harris, 1999). The addition of large amounts of wheat bran to the diet will also significantly reduce cholesterol saturation (McDougall *et al.,* 1978).

Several studies (Abdel-Aal *et al*., 1995, Ranhotra *et al.*, 1995; Ranhotra *et al.*, 1996a; Marconi *et al.*, 1999; Bonafaccia *et al.*, 2000) have analysed IF and SF from spelt whole grain flour in comparison to wheat. No clear difference was found except for a slightly higher SF/IF ratio for spelt (Abdel-Aal *et al*., 1995, Ranhotra *et al.*, 1995; Ranhotra *et al.*, 1996a; Marconi *et al.*, 1999; Bonafaccia *et al.*, 2000). However, most of the studies compared one wheat variety against 1 to 5 spelt varieties; often from different locations and/or years; and without reference to the growing conditions. Only one study concentrated on L, H and C without reference to the growing locations and conditions (Grela, 1996). As noted earlier, bran holds great interest for human nutrition and is regarded as a ‘high-fiber ingredient’ in the food industry. However, there is a gap of knowledge regarding spelt bran composition. As the first outlet of spelt production, whole spikelet flour should not be neglected. One thorough study in this area analyses 24 spelt varieties for C, H and L but not SF (Lecomte *et al.*, 1996). Consequently these three milling fractions were analysed for insoluble (IF) and soluble fibers (SF), lignin (L), hemicelluloses (H) and cellulose (C) for three wheat varieties and four spelt genotypes grown and stored in one location under the same conditions.

4.2.2 Experimental conditions

4.2.2.1 Materials

The genotypes were grown in one replicate in a single trial at Gembloux, Belgium. The crop was conducted to obtain safe grains free of disease. The plots were 4.5 m long and 1.5 m wide, and were sown in October 2005 (density: 300 seeds/m²). At the end of the winter, a weed-killer was spread on the trial and during spring nitrogen (2 times), one growth regulator and one fungicide (2 times) were applied. Plots were harvested in August 2006. An auto-blowing combine harvester was used to prevent the blending of samples. Grain sacks were dried at 30°C for 1 day and stored for 1 month at room temperature in a dry place. Harvest per plot yielded about 4 kg. After homogenisation, 500 g were sampled for milling. Two milling fractions were chosen for wheat: whole grain flour (Cyclotec) and bran (Chopin CD1 Mill). Three milling fractions were chosen for spelt: whole grain flour (Cyclotec), bran (Chopin CD1 Mill) and whole spikelet flour (Cyclotec). All the milling fractions were ground with a 0.5 mm grid. After milling, the samples were stored at 4°C to prevent mould formation and enzyme activity. Prior to analysis, the samples were set at room temperature and homogenised.

Four spelt genotypes were selected for analysis: Cosmos, a Belgian variety with high yield potential used for feeding and secondly baking; Landrace (LR) 140, a Belgian landrace; Alkor, a new Swiss variety introduced in Belgium used for feeding; and Ressac, the only true baking Belgian variety. LR140 has never been bred and should be a pure spelt; Ressac and Cosmos were descendants from the Belgian breeding and contained respectively 9.5 and 29.7% of winter wheat in their genetic background. Indeed several ten years ago due to the lack of spelt genetic resources, Ardenne, a cross between Swedish winter wheat and Belgian spelt, and Castell a German winter wheat, were crossed with spelt (Clamot, 1978; Herman; 2007a). Three winter wheat varieties were chosen: Centenaire, a Belgian feed wheat cultivated mainly in Belgium; Apache, the variety the most cultivated in Europe between 2000 and 2004; and Soissons, a successful European variety registered in 1987. These last two varieties are both baking cultivars.

4.2.2.2 Methods

The fiber content was measured using non-enzymatic and enzymatic gravimetric methods.

Non-enzymatic gravimetric methods

Two non-enzymatic gravimetric methods - Acid Detergent Fiber (ADF) analysis and Acid Detergent Lignin (ADL) analysis as described by Van Soest (1963) - were used to measure the cellulose and lignin content by removal; ADF includes cellulose and lignin. The samples (1 g = P) were boiled for 1 h with 100 mL acid detergent solution (sulphuric acid 0.2 N and 20 g/L of N-cetyl-N,N,N-trimethyl ammonium bromide). The residue was washed with distilled water and acetone. The crucibles were dried in drying oven at 103°C overnight, cooled in a dessicator and weighted (P_1). The remaining ADF

residues were submerged with 72% sulphuric acid and then washed with hot distilled water. The crucibles are dried again, weighed (P_3) and calcinated (P_2) in a muffle furnace at 550°C during 3 h or until ashes were white.

The fiber percentage formulae used were:

$$ADF\% = \frac{P_1 - P_2}{P} \text{ x } 100 \text{ x } \frac{100}{DM}$$

$$ADL\% = \frac{P_3 - P_2}{P} \text{ x } 100 \text{ x } \frac{100}{DM}$$

DM = sample dry matter

Enzymatic gravimetric method

Two enzymatic gravimetric methods were used. One was the Van Soest and Wine (1967) Neutral Detergent Fiber (NDF) method, which consists of an α-amylase treatment (Schaller, 1977) and provides data on L, H and C content by subtraction with ADF and ADL. The samples (1 g = P) were treated with α-amylase (Termamyl, Novozymes) (0.1 mL) and were boiled for 1 h with 100 mL NDF solution, ~ 2 g sodium sulphite (Merck) and 50 µL octanol (Merck). The residue was rinsed with hot water and acetone. The crucibles were dried (P_1) and calcinated (P_2).

ND solution was prepared with three solutions of 1 L each: 1) 150 g of dodecyl sulphate sodium salt ($C_{12}H_{25}NaO_4S$) and 50 mL ethylene glycol monoethyl ether ($C_4H_{10}O_2$); 2) 93.05 g of ethylenedinitrilotetraacetic acid disodium salt ($C_{10}H_{14}N_2Na_2O_8.2H_2O$) and 34.05 g of sodium tetraborate ($NaB_4O_710H_2O$); 3) 22.8 g of disodium hydrogen phosphate (Na_2HPO_4). Solutions 2 and 3 were successively added to solution 1.

The NDF percentage formula used was:

$$NDF\% = \frac{P_1 - P_2}{P} \text{ x } 100 \text{ x } \frac{100}{DM}$$

DM = dry matter of the sample

The second enzymatic gravimetric method, described by Lee, Prosky and De Vries (1992), measures both the SF and IF. The samples (1 g) were dissolved in a MES-TRIS buffer 0.05 M, pH 8.2. The solution, with 50 µL alpha-amylase A3306 (Sigma Chemical Co., St Louis, MO63178), was heated in

a 95-100°C bath for 15 min with continuous stirring. The beakers were cooled until 60°C and maintained to this temperature and incubed for 30 min with continuous stirring with 100 µL protease (at 50 mg/mL) P3910 (Sigma Chemical Co.). The pH was adjusted between 4.0 and 4.7 at 60°C; the solution, with 300 µL Amyloglucosidase A9913 (Sigma Chemical Co.), was then put in a 60°C bath for 30 min. Two filtrations were carried out, successively. The first was the IF filtration: digestate was filtered onto an acid-washed celite bed and the residue was rinsed with 2 x 10 mL 70°C H_2O. The filtrate was set apart for the second filtration. The residue was washed with 2 x 15 mL 78% and 2 x 15 mL 95% ethanol. The second filtration was the SF filtration: the previous filtrate was precipitated for 1 h with 4 volumes of 95% ethanol at 60°C. The washings were applied as previously described. The residues from SF and IF are dried overnight at 105°C, cooled in a dessicator and then weighed. The extraction was made on two samples (duplicate), with the protein content being determined for one residue and the ash content for the other. For mineralisation, the crucibles were left for 5 h in a 525°C oven.

For the fiber percentage, the formula used was:

$$DF1 = (R1 - P - A - B) / M1 \times 100$$

$$DF2 = (R2 - P - A - B) / M2 \times 100$$

DF = % dietary fiber; R_1 and R_2 = weight of sample residues (g); P = weight of sample proteins (g); A = weight of sample ash (g); B = blank weight (g); M_1 and M_2 = weight of samples (g)

The formula used for the blank weight was:

$$B = \left[\left(R_{B1} + R_{B2}\right)\right] - P_B - A_B$$

B = blank weight (g); R_{B1} and R_{B2}= weight of blank residue (g); P_B = weight of blank proteins (g); A_B = weight of blank ash (g)

4.2.2.3 Data analysis

The data were checked using analysis of variance, diagram of probability and coefficients of variation (Planchon, 2005). The limits chosen for each method were:

- the coefficient of variation of the ratio residue/sample did not exceed 20% for SF or 5% for IF;

- the residues from the analysis of variance were not >2 or <-2;

- on the diagram of probability the data were in the 95% interval confidence of the normal Gaussian law.

To check the extraction efficiency, the residue/sample ratio and the corresponding coefficients of variation were calculated and averaged 1% for IF and 7.8% for SF.

Since data were proportions, they were transformed in Arc sinus. The p-values, T-test, Student-Neuwman-Keuls' test and Duncan's test were performed on these values.

4.2.2.4 Method's precision

Average coefficients of variation were 2.1% for IF, 4.7% for TF, 4.8% for L, 1.5% for H, and 1.9% for C. In spite of the screening of the data, the average coefficient of variation for SF was 59.3%. This is due to the combination of the very low SF content of the samples and to the technique (vacuum filtration, ethanol precipitation). The SF data were presented as they provided an idea of the SF content but they should not enable to conclude on differences between subspecies and between varieties. For high fiber cereal, Lee *et al.* (1992) obtained an average coefficient of variation of 1.44% and 15.8% for IF and SF, respectively. In the studies conducted by Prosky *et al.* (1992) and Da Silva and Santorio Ciocca (2005), the method's lack of sensitivity to samples with low SF content was also evident.

With regard to ashes, negative values were sometimes obtained (data not shown). Prosky *et al.* (1992) observed the same phenomenon and explained it by a loss of celite during filtration, as did Da Silva *et al.* (2005). The current study showed that a loss of celite happened during the calcination. About 1 mg for 1g of celite was lost and was taken into account for dietary fiber calculations.

4.2.3 Results and discussion

4.2.3.1 Milling fractions analysis

For spelt, milling showed a significant effect for each fiber fraction. Variety also had a significant effect except for SF. There was also an interaction variety*milling for all fiber fractions of spelt except again for SF. Concerning wheat, this interaction was only significant for hemicelluloses. The variety effect was statistically significant for H, C and L and the milling effect remained the strongest except for SF where no difference appeared between bran and whole grain flour. The existence of an interaction brought question about the way that varieties reacted when milled. Normally, there should not be any interaction between these two parameters.

Among the spelt milling fractions, bran was the richest in SF, IF, H and L and the whole spikelet flour was from far the richest in cellulose 10.4%. The three milling fractions of spelt and two milling fractions of wheat belonged to different statistical groups for each fiber fraction except for SF. Concerning wheat, bran was the richest fraction in all fiber type except SF. For all milling fractions from wheat and spelt, hemicelluloses and cellulose were the predominant IF (Table 4.1, Appendix Fig. A.3).

4.2.3.2 Subspecies and cultivar analysis

Whole grain flour

A statistical difference between wheat and spelt was observed for IF, hemicelluloses and cellulose.

Wheat whole grain flour was statistically richer in IF than spelt, respectively 10.1 and 8.8%. IF content ranged from 7.8 to 10.1% for spelt and from 9.8 to 10.5% for wheat (Table 4.2). The literature had previously reported values between 8.0 and 12.9% for spelt and between 10.7 and 13.2% for wheat (Abdel-Aal *et al.*, 1995; Ranhotra *et al.,* 1995; Ranhotra *et al.,* 1996a; Marconi *et al.*, 1999; Bonafaccia *et al.*, 2000). Higher IF content for wheat was due to hemicelluloses and cellulose. On average, the wheat varieties were also statistically richer in hemicelluloses (7.3%) than the spelt genotypes (5.4%) however Grela (1996) reported contradictory values: 9% for wheat and 10.5% for spelt. Concerning cellulose, in the current study there was a significant statistical difference of 0.7% between wheat and spelt (2.4 for wheat and 1.7 for spelt) while Grela (1996) found similar content for both subspecies (about 2%). For lignin, Grela (1996) found higher absolute values, 1% against 0.7% in the current study and in both studies the lignin content of both subspecies did not differ.

The SF content varied from 0.8 to 2.0% for spelt and from 1.4 to 1.9% for wheat and no significant statistical difference was observed between subspecies neither among the varieties within each subspecies. The literature reported values from 1.2 to 2.4% for spelt and from 1.4 to 2.2% for wheat (Abdel-Aal *et al.,* 1995; Ranhotra *et al.*, 1995; Ranhotra *et al.*, 1996a; Marconi *et al.*, 1999; Bonafaccia *et al.*, 2000). Detailed comparisons with the absolute values given in the literature, especially for SF, were difficult because of the sensitivity of the method.

The wheat varieties displayed a significant statistical difference for hemicelluloses and cellulose, where two groups were made up: one including Apache and Soissons, and the other with Centenaire. This last, which was the richest in H and C, is a feed wheat contrary to the others which are baking varieties.

With regard to spelt, there were significant statistical differences among the varieties except for SF. Hemicelluloses contents separated the four varieties in four distinct groups and cellulose contents also created three groups, one containing LR140 and Alkor. Cosmos was the richest in TF 11.9%, unlike Ressac, the only spelt true baking variety, which displayed the lowest content in TF 8.5% and they belonged to different groups according to the T-test. Fibers are known for their detrimental effect on dough rheology and bread quality. In baked goods, they reduce loaf volume, increase the crumb hardness and the crust's darkness, and sometimes have an effect on taste (Rosell *et al.,* 2006). The special characteristics of Ressac could account for its suitability for baking, as Gomez *et al.* (2003) found that low dietary fiber content favoured dough rheological properties, quality and sensory properties of bread.

Bran

Spelt and wheat bran displayed significant statistical differences for SF, hemicelluloses and cellulose contents (Table 4.2). Spelt bran contained less hemicelluloses and cellulose than wheat bran, respectively 18.6 and 5.7% against 22.6 and 7.5%. However spelt bran is richer in SF than wheat, respectively 2.9 and 1.2%.

Among the wheat varieties, all the genotypes were very similar except for Centenaire which was different from the other varieties for its higher cellulose content 8.2% and lower lignin content 2.0% against respectively in average 7.5 and 2.1%. Hemicelluloses content made up three different groups corresponding to the three wheat varieties.

In contrast, diversity was generally higher among spelt genotypes except for SF for which no statistical difference was observed among spelt varieties. The landrace 140 showed a remarkably high IF content 34.4%, deriving from the hemicelluloses and cellulose. This had already been observed in whole grain flour for hemicelluloses. LR140 belonged to the first statistical group (A), whatever the type of fiber, which illustrated its richness in fibers. As for the whole grain flour, Ressac differed from the other genotypes, having lower IF, TF, L, H, C contents.

In terms of providing healthy products to the consumer, scientists and the food industry aim to produce high-fiber foods. Bran, as a by-product of cereal milling and a good source of fiber, has been used for this purpose, for instance wheat bran in high-fiber bread and biscuits. Spelt bran could easily replace wheat bran in this area. The diversity of its fiber composition facilitates choosing a genotype bran that meets the quality requirements. Spelt is more often cultivated under organic farming conditions than wheat, with the hull protecting the grain from residues of pesticides and diseases. At the very least, the special taste and image of spelt needs to be rehabilitated.

Subspecies	Spelt									Winter wheat						
Milling fraction	Bran		Whole grain flour		Whole spikelet flour		p-value			Bran		Whole grain flour		p-value		
Fibre fraction	%	Group	%	Group	%	Group	Milling	Variety	Milling*Variety	%	Group	%	Group	Milling	Variety	Milling*Variety
Cellulose	5,7	B	1,7	C	10,4	A	0,0001	0,0001	0,0001	7,5	A	2,4	B	0,0001	0,0001	0,6284
Hemicellulose	18,6	A	5,4	C	12,3	B	0,0001	0,0001	0,0001	22,6	A	7,3	B	0,0001	0,0063	0,0064
Lignin	2,3	A	0,7	C	1,3	B	0,0001	0,0027	0,0006	2,1	A	0,7	B	0,0001	0,0258	0,2644
Total fibre	32,5	A	10,3	C	25,6	B	0,0001	0,0001	0,0001	33,3	A	11,7	B	0,0001	0,6950	0,3750
Soluble fibre	2,9	A	1,4	B	1,0	B	0,0010	0,0950	0,9140	1,2	A	1,6	A	0,5550	0,5320	0,2130
Insoluble fibre	29,4	A	8,8	C	24,7	B	0,0001	0,0001	0,0001	32,1	A	10,1	B	0,0001	0,1885	0,0740

Table 4.1. Spelt and wheat fiber content (%, d.m.) by milling fractions; p-values for variety, milling and interaction variety*milling; statistical groups from T-test Student-Newman-Keuls' test and Duncan's test

	Subspecies	Spelt											Winter wheat									p-value
	Variety	Alkor		Cosmos		Ressac		LR140		p-value	Mean		Apache		Centenaire		Soissons		p-value	Mean		p-value
Milling fraction	Fibre fraction	%	Group	%	Group	%	Group	%	Group	Variety	%	Group	%	Group	%	Group	%	Group	variety	%	Group	Subspecies
Whole grain flour	Insoluble fibre	8,0	B	10,1	A	7,8	B	9,4	A	0,0209	8,8	B	10,0	A	10,5	A	9,8	A	0,1332	10,1	A	0,0231
	Soluble fibre	2,0	A	1,8	A	0,8	A	1,2	A	0,2320	1,4	A	1,9	A	1,4	A	1,5	A	0,8260	1,6	A	0,0681
	Total fibre	10,0	AB	11,9	A	8,5	B	10,5	AB	0,0611	10,3	A	11,9	A	11,8	A	11,3	A	0,8950	11,7	A	0,9130
	Lignin	0,6	B	0,8	A	0,7	AB	0,6	B	0,0390	0,7	A	0,6	A	0,6	A	0,7	A	0,2812	0,7	A	0,6978
	Hemicellulose	5,6	B	4,6	D	5,1	C	6,4	A	0,0003	5,4	B	7,0	B	8,1	A	7,0	B	0,0344	7,3	A	0,0003
	Cellulose	1,9	A	1,5	B	1,4	C	1,9	A	0,0001	1,7	B	2,1	B	2,8	A	2,2	B	0,0082	2,4	A	0,0010
Bran	Insoluble fibre	28,5	B	29,4	B	21,2	C	34,4	A	0,0001	29,4	A	32,2	A	32,0	A	32,1	A	0,7100	32,1	A	0,1499
	Soluble fibre	3,5	A	3,1	A	2,5	A	2,6	A	0,8940	2,9	A	0,6	A	0,3	A	2,6	A	0,1122	1,2	B	0,0070
	Total fibre	32,5	B	32,5	B	23,7	C	37,1	A	0,0000	32,6	A	32,8	A	32,3	A	34,7	A	0,1401	33,3	A	0,6870
	Lignin	2,3	A	2,6	A	1,8	B	2,3	A	0,0192	2,3	A	2,2	A	2,0	B	2,2	A	0,0193	2,1	A	0,3584
	Hemicellulose	20,5	B	16,6	C	12,2	D	23,3	A	0,0001	18,6	B	22,9	A	22,6	B	22,2	C	0,0160	22,6	A	0,0399
	Cellulose	6,0	B	5,1	C	3,3	D	7,9	A	0,0001	5,7	B	7,2	B	8,2	A	7,2	B	0,0145	7,5	A	0,0181

Table 4.2. Fiber content in whole grain flour and bran by variety and subspecies (%, d.m.); p-values for subspecie and variety; statistical groups from T-test

Whole spikelet flour

Variety	Alkor		Cosmos		Ressac		LR140		Mean	p-value
Fibre fraction	%	Group	%	Group	%	Group	%	Group	%	Variety
Insoluble fibre	26,5	A	24,8	B	21,9	C	23,7	B	24,7	0,0012
Soluble fibre	1,5	AB	2,0	A	0,7	AB	0,3	B	1,0	0,0811
Total fibre	28,0	A	25,9	AB	22,6	C	24,1	BC	25,6	0,0040
Lignin	1,3	A	1,2	C	1,2	B	1,2	B	1,3	0,0005
Hemicellulose	12,9	A	13,2	A	10,0	B	12,6	A	12,3	0,0001
Cellulose	11,1	B	11,3	A	8,1	D	10,6	C	10,4	0,0001

Table 4.3. Fiber content in whole spikelet flour by variety (%, d.m.); p-values for variety; statistical groups from T-test.

On average, whole spikelet flour was composed of 10.4% cellulose, 12.3% hemicelluloses and 1.3% lignin (Table 4.3). Lecomte *et al.* (1996) found in whole spikelet flour of 24 spelt varieties, 13% of cellulose, 13.2% of hemicelllulose and 2.7% of lignin, representing higher absolute values.

The variety effect is statistically significant for all fibers fractions except SF. There was a noticeable variability between genotypes for IF, from 21.9 to 26.5%, and for TF, from 22.6 to 28.0%, where Alkor was the richest variety while Ressac was the poorest. Lignin contents were distributed in a narrow range between 1.2 and 1.3% however three significant statistical groups were formed. Cellulose contents enabled to separate the four varieties in four distinct groups with values ranging from 8.1 to 11.3%. Concerning hemicelluloses, Ressac differed significantly from the three other varieties for its low content 10% against 12.3% in average for spelt.

As noted earlier, the current study confirmed the usefulness of the spikelet for feeding. The comparison of the spikelet with wheat straw, grain and bran showed that it belonged to the concentrate group. However, it was poorer in starch and richer in cellulose than grain, reducing the risk of acidose for ruminants. It contained more starch and less hemicelluloses than bran and so would be more suited for animals with high energy requirements. As noted earlier, Cosmos which contained more hemicelluloses and cellulose than the other spelt varieties was highly suitable as animal feed. It is noteworthy to add that Cosmos is the spelt variety which contained the highest proportion of wheat in its genetic background 30%.

Feed	Cellulose	Hemicellulose	Lignin	Protein	Starch
Wheat straw[a]	42	29,4	8,4	3,5	-
Wheat grain[a]	2,5	10,7	1,1	12,1	69,8
Wheat bran[a]	9,7	31,9	3,9	17	22,7
Spelt spikelet	10,4	12,3	1,3	12,9	47,4

[a] adapted from INRA, 2007

4.2.3.3 Comparison NDF-IF methods

Scientists in animal feeding usually use Van Soest's method to analyse feed. Scientists working on human nutrition use Prosky's method because of their interest in SF. However, it could be useful in human nutrition to know the composition of IF, as it is possible that IF do not all have the same effect on human health. SF also play an important role in poultry feed and might also do so in cattle feed.

The choice of the method for fiber analysis remains controversial. The NDF and IF methods were applied to the same samples. IF includes hemicelluloses, cellulose, lignin, cutin, suberin, chitin, chitosan, waxes and resistant starch. NDF includes hemicelluloses, cellulose and lignin. The correlation between the two methods is very high, $r^2= 0.99$, showing that NDF and IF cover the same types of fiber for cereal grains such as wheat and spelt. It also shows that there is no or very little cutin, suberin, chitin, chitosan, waxes or resistant starch in cereal grains (Fig. 4.1). For IF analysis, the NDF method is faster and more thorough.

The aim was to measure only NDF, ADF, ADL and TF and therefore detailed data on IF would be available while SF content could be obtained by removal. This approach could be criticised in terms of its precision, but it does produce a reasonably accurate idea of fiber composition and content. It is also important to bear in mind that, in the current study, the NDF, ADF, ADL and TF methods were more sensitive than the SF method.

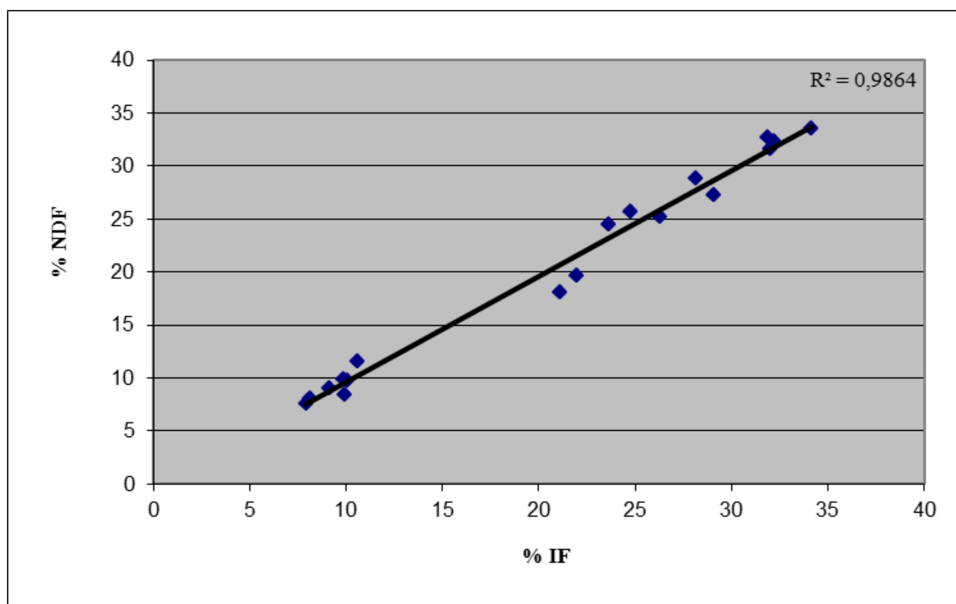

Figure 4.1. Correlation between IF and NDF measures

4.2.4 Conclusion

This study provided results on samples where the growing and storage conditions were strictly controlled. Wheat and spelt whole grain flour differed significantly for IF, hemicelluloses and cellulose contents and bran differed significantly for SF, hemicelluloses and cellulose contents. Wheat had higher contents for these fiber fractions than spelt. Variability within the varieties was higher among spelt than wheat. Ressac, the only true baking variety, differed from the other spelt varieties while LR140 was remarkable for its high fiber contents. Finally, the study showed that a combination of two methods of analysis could replace IF and SF measurements. This type of study should be extended to a greater number of spelt and wheat genotypes in order to obtain more representative data.

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4.2.6 References

Abdel-Aal E., Hucl P., Solsulski F.W., 1995. Compositional and nutritional characteristics of spring einkorn and spelt wheats. *Cereal Chemistry*, 72 (6), 621-624.

Bonafaccia G., Galli V., Francisci R., Mair, V., Skrabanja, V., Kreft I., 2000. Characteristics of spelt wheat products and nutritional value of spelt wheat-based bread. *Food Chemistry*, 68, 437-441.

Campbell K.G., 1997. Spelt: agronomy, genetics, and breeding. *Plant Breeding Reviews*, 15, 187-213.

Clamot G., 1978. Disponibilités et perspectives variétales en épeautre. In *Colloque d'information scientifique, L'épeautre,* Libramont-Chevigny, le 10 mars 1978 (pp 14-24). Royaume de Belgique, Centre de Recherches Agronomiques de l'Etat.

Da Silva L.P., Santorio Ciocca M.L., 2005. Total, insoluble and soluble dietary fiber values measured by enzymatic-gravimetric method in cereal grains. *Journal of Food Composition and Analysis*, 18, 113-120.

Ferguson L.R. and Harris P.J., 1999. Protection against cancer by wheat bran: role of dietary fibre and phytochemicals. *European Journal of Cancer Prevention*, 8, 17-25.

Gomez M., Ronda F., Blano C.A., Caballero P.A., Apesteguia A., 2003. Effect of dietary fibre on dough rheology and bread quality. *European Food Research and Technology*, 216, 51-56.

Grela E.R., 1996. Nutrient composition and content of anti-nutritional factors in spelt (*Triticum spelta* L.) cultivars. *Journal of the Science of Food and Agriculture*, 71, 399-404.

Herman J.L., 2007a. Essais variétaux épeautre. Essais valeur culturale et d'utilisation. *Comité pour l'élaboration du catalogue national des variétés des espèces de plantes agricoles,* (8p). Gembloux, Centre Wallon de Recherches Agronomiques.

INRA, 2007. Alimentation des bovins, ovins et caprins. Besoins des animaux - Valeur des aliments. *Tables INRA 2007*. Editions Quae, Versailles, 307p.

Jones J.A., 2006. Grain-based foods and health. *Cereal Foods World*, 51, 108-113.

Kasarda D.D. and D'Ovidio P., 1999. Deduced amino acid sequence of an α-gliadin gene from spelt wheat (spelta) includes sequences active in celiac disease. *Cereal Chemistry*, 76 (4), 548-551.

Koh-Banerjee P., Franz M., Sampson L., Liu S., Jacobs Jr D.R., Spiegelman D., Willett W., Rimm E., 2004. Changes in whole-grain, bran, and cereal fibre consumption in relation to 8-year weight gain among men. *American Journal of Clinical Nutrition*, 80, 1237-1245.

Kritchevsky D., 2001. Dietary fibre in health and disease. In McCleary B.V. and Prosky L., *Advanced dietary Fibre Technology*, (pp 149-161). Oxford: Blackwell Science.

Larsson S.C., Giovanucci E., Bergkvist L., Wolk A., 2005. Whole grain consumption and risk of colorectal cancer: a population-based cohort of 60000 women. *British Journal of Cancer*, 92, 1803-1807.

Lecomte P., Boreux M., Agneessens R., Beckers Y., De Keyser A., 1996. Characterization of the chemical composition and feeding value for spelt (Triticum spelta) as a feedstuff for ruminants. *Rencontres Recherche Ruminants*, 3, 112.

Lee S.C., Prosky L., De Vries J.W., 1992. Determination of total, soluble, and insoluble dietary fiber in foods – Enzymatic-gravimetric method, MES-TRIS buffer : collaborative study. *Journal of AOAC International*, 75 (3), 395-416.

Marconi E., Carcea M., Graziano M., Cubadda, R., 1999. Kernel properties and pasta-making quality of five European spelt wheat (*Triticum spelta* L.) cultivars. *Cereal Chemistry*, 76 (1), 25-29.

McDougall R.M., Yakymyshyn L. Walker K., Thursten O.G., 1978. The effect of wheat bran on serum lipoproteins and biliary lipids. *Canadian Journal of Surgery*, 21, 433-435.

Office fédéral de la statistique Suisse, 2008. http://www.bfs.admin.ch/bfs/portal/fr/index.html

Percival J., 1921. *The wheat plant. A monograph*. Duckworth and Co. London, p 325-334.

Planchon V., 2005. Traitement des valeurs aberrantes : concepts actuels et tendances générales. *Biotechnologie Agronomie Société et Environnement*, 9 (1), 19-34.

Poutanen K., 2006. Grain fibre: how to deliver the health benefits. L42. *Dietary fibre 2006 Conference*, June 12-14, 2006, Helsinki, Finland.

Prosky L., Asp G.N., Scheweizer T.F., de Vries J.W., Furda I., 1992. Determination of insoluble and soluble dietary fiber in foods and food products: collaborative study. *Journal of the Association of Analytical Chemistry International*, 75, 360-367.

Ranhotra G.S., Gelroth J.A., Glaser B.K., Lorenz K.J., 1995. Baking and nutritional qualities of spelt wheat sample. *Lebensmittel-Wissenschaft und Technologie,* 28, 118-122.

Ranhotra G.S., Gelroth J.A., Glaser B.K., Stallknecht G.F., 1996a. Nutritional profile of three spelt wheat cultivars grown at five different locations. *Cereal Chemistry*, 73 (5), 533-535.

Rosell C.M., Santos E., Collar C., 2006. Mixing properties of fibre-enriched wheat bread doughs: a response surface methodology study. *European Food Research and Technology*, 223, 333-340.

Ruibal-Mendieta N.L., Delacroix D.L., Meurens M., 2002. A comparative of analysis of free, bound and total lipid content on spelt and winter wheat wholemeal. *Journal of Cereal Science*, 35, 337-342.

Ruibal-Mendieta N.L., Delacroix D.L., Mignolet E., Pycke J.-M., Marques C., Rozenberg R., Petitjean G., Habib-Jiwan J.-L., Meurens M., Quetin-Leclercq J., Delzenne N.M., Larondelle Y., 2005. Spelt (*Triticum aestivum* ssp. *spelta*) as a source of breadmaking flours and bran naturally enriched in oleic acid and minerals but not phytic acid. *Journal of Agricultural and Food Chemistry*, 53, 2571-2759.

Schaller D., 1977. Analysis of dietary fiber. *Food Product Development*, 11, 70-72.

Schober T.J., Bean S.R., Kuhn M., 2006. Gluten proteins from spelt (*Triticum aestivum* ssp. *spelta*) cultivars: a rheological and size-exclusion high-performance liquid chromatography study. *Journal of Cereal Science*, 44, 161-173.

Schober T.J, Clarke C.I., Kuhn M., 2002. Characterization of functional properties of gluten proteins in spelt cultivars using rheological and quality factor measurements. *Cereal Chemistry*, 79, 408-417.

Statistisches Bundesamt Deutschland, 2008. http://www.destatis.de

Strechlow W., Hertzka G., Wueffen W, 1991. The dietary properties of spelt in the treatment of chronical diseases. *Proceeding, 2,* Hohenheimer Dinkelkolooquium Verlag, Stuttgart, 243-259.

Van Soest P.J., 1963. Use of detergents in the analysis of fibrous feeds. II. A rapid method for the determination of fiber and lignin. *Journal of the Association of Official Analytical Chemists*, 46 (5), 829-835.

Van Soest P.J. and Wine R.H., 1967. Use of detergents in the analysis of fibrous feeds. IV. Determination of plant cell-wall constituents. *Journal of the Association of Analytical Chemists*, 50, 50-55.

5 CHAPTER 5

Extraction and characterization of water-extractable and water-unextractable arabinoxylans from spelt bran: Study of the hydrolysis conditions for monosaccharides analysis.

Excerpt from Article 2:

Escarnot, E., Aguedo, M., Agneessens, R., Wathelet, B., Paquot, M., 2011a. Extraction and characterization of water-extractable and water-unextractable arabinoxylans from spelt bran: Study of the hydrolysis conditions formonosaccharides analysis. *Journal of Cereal Science*, 53, 45-52.

5.2 The context of the study

The previous chapter confirmed that whole grain flour, whole spikelet flour and bran contain dietary fibers. It also detailed the proportion of the different fiber components in each milling fraction and highlighted the high content in fiber of the bran which is especially rich in hemicelluloses and cellulose.

Bran is not a homogeneous product, it has a heterogeneous structure as it contains several distinct layers from the grain and each layer has its own distinct composition (Pomeranz, 1988). As already mentioned, hemicelluloses are a heterogeneous group of non-starch polysaccharides with diverse structures (Ebringerova *et al.*, 2005). In wheat bran, it is known that hemicelluloses are mainly AXs (Brillouet and Mercier, 1981 ; Carré and Brillouet, 1986). AX content is high but can vary a lot, from 17 to 26% (Beaugrand *et al.*, 2004a). Two types of AX are distinguished: water-extractable and water-unextractable. The structure of the arabinoxylans has been widely studied: a backbone of xylose with substitution by arabinose residues; other components such as glucuronic acid and phenolic acids can also be branched. The A/X ratio, a key feature of the AX, is most of the time used to describe the molecule as it is handy and easily calculated. Some studies also describe the structure of the molecule and provide the position of the branching and the number of branching on the xylose backbone. Indeed, AXs have strong interactions with other components from the cell wall such as cellulose, lignin and protein through different kinds of linkages. Besides, phenolic acids create bonds between polysaccharides (Saulnier *et al.*, 2007).

AXs arouse an interest as they have various applications. As AXs are gellable they can be used as food additives where texturization and stabilization are required or in non-food where high water absorption, protection and stabilization are required. However, the only quantitative extraction method of AX from cereal, alkaline, makes the AX ungellable. In baking, while WU-AXs seem to have negative impact, WE-AXs have positive influence on dough characteristics. AX soluble fiber may also have the potential to have beneficial effects on human health. Besides, AX extracted by alkaline or enzymic aided-procedures could be explored as good source of natural immunomodulators (Saulnier *et al.*, 2007).

The location and the degree of interaction in the different tissues of the grain make AX extraction difficult. Only a part of them is extractable with water while the majority is water-unextractable (Saulnier *et al.*, 2007). In order to obtain pure AX, fat, protein, starch, lignin and ashes from bran are enzymically, chemically or physically removed (Brillouet and Mercier, 1981; Dupont and Selvendran, 1987; Bataillon, 1998; Maes and Delcour, 2002). Delignification has also been thought about in order to access more easily the AX and to obtain pure fraction however it did not seem to increase the yield of extraction of AX (Bataillon, 1998). Generally, alkaline solutions have been used to extract the water-unextractable AXs (Persson *et al.*, 2009). The nature of the alkaline solution is the first criteria

which influences the result of the extraction. Calcium hydroxide, barium hydroxide, hydrogen peroxide have been tested on wheat bran (Schooneveld-Bergmans, 1998; Maes and Delcour, 2001). Temperature, extraction time and concentration of alkaline solution are other parameters which influence the extraction. These extractions work well with different results regarding the yield of extraction and the type of AX extracted which have different properties. Ethanol precipitation has also been used in order to separate the different AXs extracted (Dupont and Selvendran, 1987; Izydorczyk and Biliaderis, 1995; Maes and Delcour, 2002).

As AX from spelt bran are unknown and as AX have several applications, the following chapter will aim at extracting and characterizing them. Prior to any extraction, the methods of quantification and characterization must be validated for the present materials. Characterization of the fraction is based on the content in sugars and enable to establish a profile of the fraction and to calculate the yield of extraction. The conditions of hydrolysis can influence the results; it will be thus necessary to evaluate them and to choose the most adapted and compatible with material conditions. The first step of the extraction will be the removing of the starch and the proteins. As already mentioned, the second step will be the alkaline treatment which is efficient in solubilizing the hemicelluloses. The method with hydrogen peroxide at low concentration will be elected as it is reputed as a soft treatment. The conditions will be based on the method that Maes and Delcour (2001) applied to wheat bran and which provided satisfactory results.

5.3 Extraction and characterization of water-extractable and water-unextractable arabinoxylans from spelt bran: Study of the hydrolysis conditions for monosaccharides analysis.

Abstract

Monosaccharides analysis were performed, statistically treated and adopted for spelt bran fractions; with regard to the arabinoxylan content. The AX content of the initial spelt bran reached 9.2% with an A/X ratio of 0.39. The initial spelt bran was rich in starch (41.2%) and protein (18.9%). WE- and WU-AXs from micronized spelt bran were extracted after improved enzymic destarching and deproteinisation treatments. WU-AXs were obtained by two successive extractions with 2% alkaline hydrogen peroxide at 60°C during 4h. 55% of the AX present in spelt bran was extracted by using the three extraction steps (WE- and WU-AXs), among AX, 13% were WE and 87% were WU. A/X ratios were different depending on the extraction process. WE-AXs were less rich in arabinose than WU-AXs. Each fraction contained two populations of AX. The first one consisted of low MW AX (7-8 kDa). The second population had a higher MW, 310-415 kDa for WU-AXs and 28 kDa for WE-AXs. The extracts had to be purified in order to improve the AX content. Results were compared to those obtained with wheat bran in the literature. This research was, to the best of our knowledge, the first study on AX extraction from spelt bran.

5.3.1 Introduction

Spelt (Triticum aestivum ssp. spelta) is an ancient subspecies of common wheat (Triticum aestivum ssp. aestivum) which main characteristic is the hull attached to the grain. Namely, both wheat and spelt have a hull, but in wheat it is free, and in spelt it is attached to the grain. In agronomic terms, spelt shows a high resistance to environmental factors (diseases...) and might perform better under less advantageous growing conditions (Campbell, 1997). Spelt is suitable for organic farming and contributes to agro-biodiversity, meeting consequently the objectives of the European Union on growing practices. Moreover, spelt has a higher protein content than wheat, a higher lipid content, especially Δ7-avenasterol (Bonafaccia et al., 2000; Ruibal-Mendieta et al., 2004a) and higher magnesium, phosphorus, iron, copper and zinc contents (Ruibal-Mendieta et al., 2005). It has been mainly used in specialty breads, organic food and products with characteristics that differ from regular wheat products (Ranhotra et al., 1995).

Bran is a by-product of the milling industry which accounts for 14 to 19% of the wheat grain. It comprises the outer layers, the aleurone layer and remnants of the starchy endosperm (Pomeranz, 1988). Contrary to spelt bran, wheat bran has been much studied. It is one rich source of dietary fibers including 46% of non-starch polysaccharides (NSPs) (Ralet et al., 1990), 15 to 25% of starch (Beaugrand et al., 2004a), 3 to 10% of lignin (Ralet et al., 1990; Bergmans et al., 1996), 11 to 25% of proteins (Brillouet and Mercier, 1981; Dupont and Selvendran, 1987; Beaugrand, 2004), 4 to 6% of

ashes (Bataillon et al., 1998; Maes and Delcour, 2001), 6% lipids (Bataillon et al., 1998), pectins and some minor components (e.g. 5% uronic acids) (Brillouet and Mercier, 1981). In destarched bran, ferulic acid amounts in average 5000 µg/g, p-coumaric acid 150 µg/g, and sinapic acid 200 µg/g (Beaugrand et al., 2004a). The main NSPs are arabinoxylans (AX), cellulose and (1->3), (1->4)-β-D-glucans which represent respectively 73%, 24% and 6% of the NSP of the bran (Selvendran et al., 1980; Brillouet and Mercier, 1981; Ralet et al., 1990). Small amounts of xyloglucan are also present in the pericarp (Dupont and Selvendran, 1987). Glucomannan (Mares and Stone, 1973) and arabinogalactan (Fincher et al., 1974) were reported in aleurone and endosperm cells. Wheat bran carbohydrates contain 7.1-9.9% arabinose, 1-1.8% galactose, 24.4-35.7% glucose, 10.8-16.6% xylose; the whole represents 50.6-59.8% of total dry weight (DW). Regarding free sugars, 7.2% was reported, from which 2.4% of sucrose and 1.3% of raffinose (Beaugrand et al., 2004a).

Recent research has been focused on the major component of wheat bran: AX. AX are constituted by a main chain of β-linked (1->4)-3-D-xylopyranose and are substituted mainly by side chains of α-L-arabinofuranose (Bacic et al., 1988). The substituent arabinoses are in position α-(O-2) and/or α-(O-3) of the xylose residues (Bacic and Stone, 1981b). The substitution rate arabinose/xylose of the AX increases from the centre towards the outer layers of the wheat grain (Lempereur et al., 1997), the higher rate is observed in the pericarp where in average one xylose carries one arabinose (Ring and Selvendran, 1980; Brillouet and Mercier, 1981). For wheat bran, 31% of the xylose is unsubstitued, 24% is monosubstitued and 39% is disubstitued. The presence of 3,5-dimethyl-arabinose and 5-methyl-arabinose suggests that around 25% of arabinose residues are present under oligomeric side chains (Edwards et al., 2003). Uronic acids are associated with AX (such as glucuronic acid) (Brillouet and Joseleau, 1987).

Some AXs in wheat bran are physically and chemically inter-linked and linked also to lignin and cellulose through diferulic acid bridges and hydrogen bonds (Iiyama et al., 1994). However, Mares and Stone (1973) found evidence that WE-AX are not bound to the other cell wall polymers and that they are located at the surface of the cell wall. Concerning the WU-AX, they are maintained in the cell wall structure by ester linkages (Delcour et al., 1999). Consequently most of the AX can only be extracted with alkaline media (Shiiba et al., 1993; Dupont and Selvendran, 1987; Bergmans et al., 1996). The procedure with peroxide hydrogen uses cheap chemicals with inherent low toxicity (Hollmann and Lindhauer, 2005) contrary to barium hydroxide used for example by Bergmans et al. (1996).

During the last decades a renewed interest rose for spelt as human food due to its image as a "healthier, more natural, less over-bred" cereal than modern wheat (Schober et al., 2006). To the best of our knowledge the only application of spelt bran is as a feeding ingredient and no study deals with other potential uses. Consequently, development of spelt bran as high value-added products is an

important objective to support spelt development. From a technological point of view, AXs have a role in bread making performance and have functional properties interesting for use as food additives. They can be used as viscosity enhancers or thickeners because they have a high water-holding capacity and are able to stabilise protein foams (Courtin and Delcour, 2002). They can also be used as texturizers, more especially WE-AXs (Saulnier *et al.,* 2007). Besides, they can be used for the production of oligosaccharides with physiological functions (Kabel *et al.,* 2002a) and AX soluble fibers may have the potential to reduce glucose and lipid absorption (Saulnier *et al.* 2007). While a lot of works concern wheat AXs, few or none are carried out on spelt. The objectives of this study are to extract and describe spelt bran AXs in order to open new ways for spelt bran applications as it is already the case for wheat bran. In the present study, AXs from spelt bran were extracted with water and with alkali peroxide hydrogen. Different AX fractions were characterized and results obtained here were compared to those of the literature on wheat bran. Besides, different conditions of hydrolysis were tested on the spelt extraction fractions (Englyst and Cummings, 1984; Southgate, 1995; Maes and Delcour, 2001).

5.3.2 Material and methods

5.3.2.1 Materials

The bran came from the spelt cv. Ressac, harvested in august 2007. The spikelets were hulled and the grains milled at “Le Moulin de Hollange” in November 2007. The milled fractions: white flour, bran and hulls were stored at 4°C. Bran was screened at 1mm to remove parts of the hulls, and was then micronized by jet milling (Alpine 100 AFG, Augsburg, Germany) with 6-7 bars of pressure and turbine at 2000 rpm. The granulometry was measured in triplicate with a Mastersizer 2000 (Malvern Instruments Ltd, Worcestershire, UK).

All reagents were of analytical grade. All enzymes were supplied by Novozymes (Bagsvaerd, Denmark).

5.3.2.2 Methods

Monosaccharides composition

The monosaccharides analysis was based on the method of Englyst and Cummings (1984). A sample (in triplicate) was accurately weighted and the acid was added. The hydrolyses were performed with two acids: H_2SO_4 or TFA, at three concentrations: 0,5 M; 1 M and 2 M, during 3 h at 100°C and the trial was completed with TFA 2 M at 110°C during 2 h. The solution was then neutralized with concentrated NaOH and the alkalinity of the solution was checked. Sugars (0.4 mL of supernatant) were reduced to their corresponding alditols with 2 mL of DMSO containing 2 mL NaBH4. Reduction was performed for 90 min at 40°C. The excess of NaBH4 was eliminated by adding 6 mL glacial

acetic acid. Acetylation was then performed with acetic anhydride (4 mL, 10 min at room temperature) in the presence of 1-methyl-imidazole (0.4 mL) as a catalyst. Acetylation was stopped with 10 mL deionized water and the acetylated alditols were partitioned between dichloromethane (4 mL) and water. After the phases separation, 1 mL of the lower one was transferred in a septum-cap vial. 2-Deoxy-D-glucose was used as internal standard and standards of D-arabinose, D(+)-xylose, D(+)-mannose, D(+)-glucose, D(+)-galactose (purity > 99.5%, Sigma Chemical Co., St-Louis MO, USA) were used. The analyses were performed with a Hewlett-Packard Agilent 6890 series gas chromatograph equipped with a high-performance capillary column, HP1-methylsiloxane (30 m x 0.32 mm, 0.25 µm film thickness) (Scientific Glass Engineering, Melbourne, Australia). 0.2 µL of derivatized sugars in dichloromethane was injected. Helium was the carrier gas with a flow of 1.6 mL/min. The injection temperature was 290°C, and the temperature program was 1 min at 120°C, followed by a linear increase in 4 min to 220°C and then in 35 min to 290°C which was maintained for 4 min. Compounds were detected using a flame ionization detector at 320°C.

Preparation of destarched deproteinised bran

Spelt bran in 0.05 M pH 6.5 phosphate buffer (1:7, w/v) was heated under continuous stirring until 75°C and α-amylase Termamyl 120L (Novozymes) was added (10 µL/g bran). The suspension was then heated until 90°C and maintained 1 hour. It was then cooled to 50°C and Fungamyl 800L (Novozymes) was added (0.4 µL/ g bran). The suspension was kept 30 min at 50°C. Three proteases were successively added: Neutrase (50 µL/g bran), Alcalase (50 µL/g bran) and Flavourzyme 1000L (2 µL/g bran) (all three from Novozymes). The suspension was heated to 55°C and left 4 hours under continuous stirring. The solution was heated at 100°C during 15 min. The mixture was vacuum filtered through a 20 µm nylon filter. The residue, i.e. destarched and deproteinised spelt bran or water-unextractable material (WUM), was washed several times with distilled water and dried at 50°C in an oven for 24 h. The filtrate and the residue contain different AXs whose extraction is detailed in the following paragraphs and presented on Fig. 5.1.

Extraction of WE-AX

The pH of the previous filtrate was set to 4 with HCl 4 M. Amyloglucosidase AMG 300L (Novozymes) (10 µL/g bran) was added and the suspension was heated to 73°C during 28 h under continuous stirring. It was then heated at 100°C during 15 min and centrifuged (4750 rpm, 4°C, 30 min). The supernatant was carefully collected and dialysed during 48 h at 4°C against distilled water which was replaced every 12 h (Spectra Por, cut-off 1000 Da). The solution was again centrifuged and the water-extractable material (WEM) was frozen.

Purification of WEM

The pH of WEM was maintained at 4 with HCl 1 M. Amyloglucosidase AMG 300L (20µL/g WEM d.m.) was added to remove dextrins (Maes and Delcour, 2002) and the suspension was heated at 73°C during 15 h under continuous stirring. The enzymes were then inactivated at 100°C during 15 min. In order to lower the protein content, silicagel gel (SI 1721, Rocc) was added to the suspension (40 mg/mL of solution) which was left under stirring at room temperature during 30 min. The solution was then centrifuged (4750 rpm, 4°C, 30 min). The supernatant was dialysed against distilled water during 48h and centrifuged in the same conditions as above described. The purified WEM (PWEM) was then frozen.

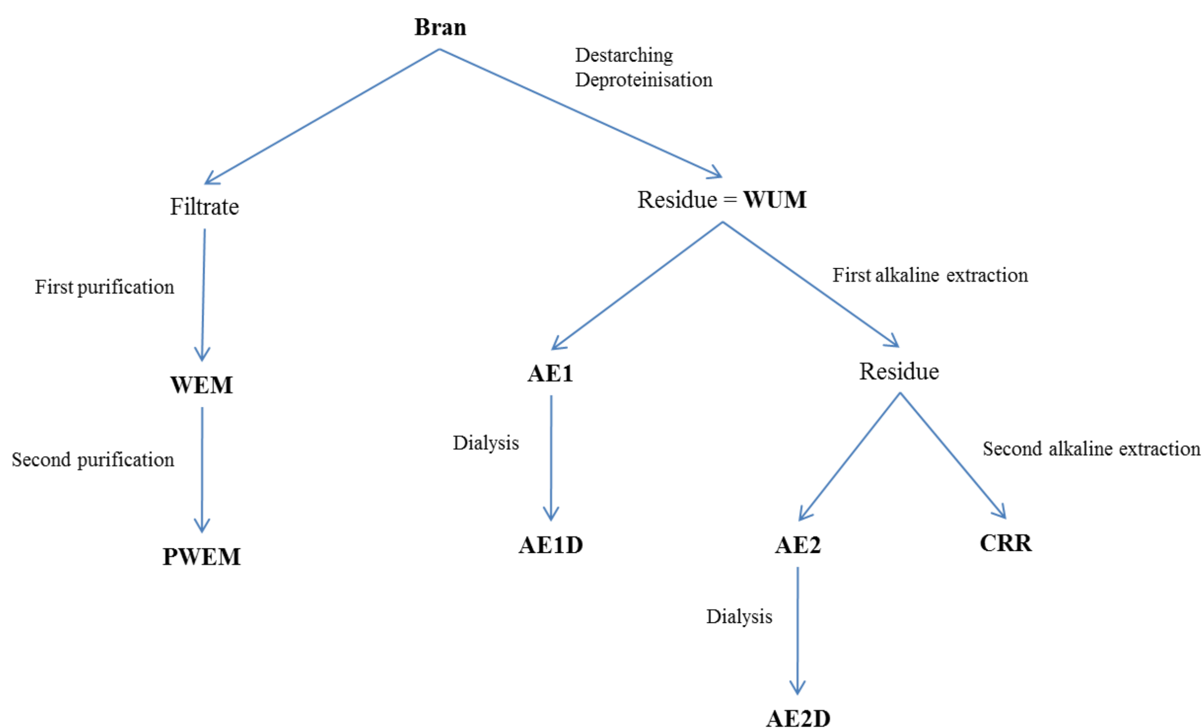


Figure 5.1. The different steps of the procedure for the extraction of WE-AXs and WU-AXs.

Extraction of WU-AX

A solution of H_2O_2 2% was freshly prepared and its pH was adjusted to 11.5 with NaOH 4 M. The WUM was dispersed in this solution at a ratio 1:10 (w/v). It was maintained 4 h at 60ºC under continuous stirring. The solution was neutralized with HCl 4 M and centrifuged (4750 rpm, 4°C, 45 min). The supernatant which was the first alkali extractable material (= AE1) was frozen. The whole residue underwent the same treatment and a second supernatant was obtained, the second alkali extractable material (= AE2). The final residue, presumed rich in cellulose (= CRR), was dried at 50°C during 24 h.

Purification of the alkali extractable materials

The alkali extractable materials, AE1 and AE2, were dialysed 72 h against distilled water in the same conditions as above described. The solutions AE1D and AE2D were centrifuged (4750 rpm, 4°C, 45 min) and the supernatants were then frozen while the pellets were discarded.

Analyses

Protein content was measured (in duplicate) by the Kjeldahl method (Kjeltec 2300, Foss) and by multiplying the N content by 5.7.

For the ashes content, samples were combusted at 600°C during 5 h and were then cooled in a dessicator.

Monosaccharides analysis was performed as above explained (in duplicate).

Starch content was measured enzymatically with total starch test kit (Megazyme, Bray, Wicklow, Ireland).

Molecular weights were determined in triplicate by High Performance Size Exclusion Chromatography (HPSEC) on a Waters 2690-HPLC system (Waters INC., Milford MA, USA), equipped with a TSKgel GMPWxl column (300 x 7.8 mm) (TosoHaas Co. Ltd., Tokyo, Japan) and coupled on-line with a single detector system: a Waters 2410 differential Refractometer Index (RI). Arabinoxylan solutions (2 mg/mL) were filtered through a 0.45 µm membrane filter (Millipore Co., Milford MA, USA) and 100 µL was injected in HPSEC. Elution was carried out at a flow rate of 0.7mL/min with 50 mM sodium nitrate ($NaNO_3$) solution containing 0.05% sodium azide (NaN_3) as preservative. Dextrans of 1, 5, 12, 25, 50, 150, 270, 410, 610 kDa were used to establish a calibration curve to calculate the MWs.

5.3.3 Results and discussion

5.3.3.1 Hydrolysis method for monosaccharides analysis

Large variations in the acid hydrolyses results have been reported in literature (Mankarios *et al.,* 1979; Chen and Anderson, 1981; Blakeney *et al.,* 1983). So, it was decided to test different acid hydrolysis methods for non-cellulosic polysaccharides in order to determine the most adequate for the different substrates and a statistic process of the data was applied. The Student-Newman-Keuls (SNK) and Duncan tests were applied on arabinose and xylose (content in percent transformed in Arc sinus) to the different groups of fractions: WE-AX group: WEM and PWEM; WU-AX group: AE1, AE2, AE1D and AE2D; solid group: bran, WUM and CRR (Table 5.1, Appendix Fig. A.4). In order to measure the arabinose and xylose contents, a quantitative method was sought, the highest percentage of extraction of both sugars determined the choice of the method. Concerning the A/X ratio, it brought a qualitative

information on the characteristics of the polysaccharides and should not be taken into account in the choice of the hydrolysis method. However, it was interesting to study the variation of the A/X ratio according to the hydrolysis method, for this reason the same statistical tests as for AX content were applied.

Concerning the solids' group, the best method was the TFA 2 M, as shown by Duncan statistical test however the SNK test didn't differentiate any method due to close averages. Concerning WE-AX group, the best method was the H_2SO_4 1 M. According to the statistical tests, all methods were equivalent except H_2SO_4 2 M, which gave the lowest percentage of arabinose plus xylose, probably due to a degradation of the sugars. Concerning the WU-AX group, the best method was TFA 2 M, 2h at 110°C which was in a separate group according to Duncan's test but not according to SNK test. TFA 0.5 M was the second best method after TFA 2 M, 2 h at 110°C with respective arc sinus averages of 1.71 and 1.74. Again, the small difference between averages did not imply a clear statistical difference between methods.

The statistical study on A/X ratio indicates that whatever the group of fractions, SNK and Duncan' tests are in accordance. For WE-AX fractions, three statistical groups are formed with the highest A/X ratio obtained with TFA 2M 2h at 110°C and H_2SO_4 2 M 3h at 100°C. For the WU-AX fractions, only TFA 2M 2h at 110°C provided a statistically higher A/X ratio than the other methods. Thus, liquid fractions hydrolysed with TFA 2M 2h at 110°C had a higher A/X ratio than with the other methods. No statistical difference arose in the solid group among the methods, however it was clear that TFA degraded arabinose less than H_2SO_4 did (Table 5.2, Appendix Fig.A.5).

Generally, variations in results obtained with the different methods were not clearly distinct. However, this study enabled us to make a choice among the different methods for the spelt extraction fractions. Finally, the method with TFA 2 M 3h at 100°C was applied on the solid group, the H_2SO_4 1 M 3h at 100°C for WE-AX fractions and for practical reasons, the method for the WUAX fractions should operate at 100°C, so the TFA 0,5 M was preferred to TFA 2 M 2h at 110°.

WE-AX: WEM and PWEM, A+X				
SNK group	Duncan group	Mean	Number	Method
A	A	19,9	6	H_2SO_4 1M
A	A	18,9	6	TFA 1M
A	A	18,8	6	H_2SO_4 0.5M
A	A	18,6	6	TFA 0.5M
A	A	18,3	6	TFA 2M 110
A	A	18,2	6	TFA 2M
B	B	14,0	6	H_2SO_4 2M
WU-AX: AE1, AE2, AE1D, AE2D, A+X				
SNK group	Duncan group	Mean	Number	Method
A	A	37,4	12	TFA 2M 110
A	AB	36,0	12	TFA 0.5M
A	AB	35,5	12	TFA 1M
A	AB	35,4	12	H_2SO_4 0.5M
A	AB	35,0	12	H_2SO_4 1M
A	B	32,1	12	TFA 2M
B	C	26,1	12	H_2SO_4 2M
SOLIDS: BRAN, WUM, CRR, A+X				
SNK group	Duncan group	Mean	Number	Method
A	A	28,3	12	TFA 2M
A	AB	27,0	14	H_2SO_4 1M
A	AB	26,8	11	TFA 1M
A	AB	25,7	9	H_2SO_4 0.5M
A	B	25,2	9	H_2SO_4 2M
A	B	25,0	9	TFA 2M 110
A	B	24,7	9	TFA 0.5M

Table 5.1. Statistical analysis on A+X (%, d.m.) through SNK and Duncan's tests on three groups of fraction, WU-AX, WE-AX and solids, according to different hydrolysis methods.

WE-AX: WEM and PWEM, A/X				
SNK group	Duncan group	Mean	Number	Method
A	A	0,84	6	TFA 2M 110
B	B	0,73	6	H_2SO_4 2M
C	C	0,67	6	TFA 2M
C	C	0,67	6	H_2SO_4 1M
C	C	0,64	6	TFA 1M
C	C	0,63	6	H_2SO_4 0.5M
C	C	0,63	6	TFA 0.5M
WU-AX: AE1, AE2, AE1D, AE2D, A/X				
SNK group	Duncan group	Mean	Number	Method
A	A	0,87	9	TFA 2M 110
B	B	0,72	12	H_2SO_4 1M
B	B	0,72	12	H_2SO_4 2M
B	B	0,72	12	TFA 2M
B	B	0,71	12	TFA 0.5M
B	B	0,70	12	TFA 1M
B	B	0,69	12	H_2SO_4 0.5M
SOLIDS: BRAN, WUM, CRR, A/X				
SNK group	Duncan group	Mean	Number	Method
A	A	0,44	9	TFA 0.5M
A	A	0,42	9	TFA 2M 110
A	A	0,41	11	TFA 1M
A	A	0,42	12	TFA 2M
A	A	0,39	9	H_2SO_4 0.5M
A	A	0,37	9	H_2SO_4 2M
A	A	0,37	14	H_2SO_4 1M

Table 5.2. Statistical analysis on A/X through SNK and Duncan's tests on three groups of fraction, WU-AX, WE-AX and solids, according to different hydrolysis methods.

5.3.3.2 Characterization of spelt bran

The initial spelt bran mainly contained starch (41.2%) and proteins (18.9%). The main sugars were glucose (49.9%), xylose (7.5%) and arabinose (3.7%) (Table 5.3, Appendix Fig.A.6). Thus the AX content reached 9.2% for spelt bran with an A/X ratio of 0.39. In Beaugrand *et al.*'s study (2004a) on 11 wheat brans, arabinose plus xylose content varied from 17.9 to 25.7% and the A/X ratio from 0.53 to 0.71. Here, the high starch content of spelt bran lowered the AX content value so the destarched bran was considered. Reported values for arabinose plus xylose content of destarched wheat bran vary from 36.8 to 45.9% (Beaugrand *et al.,* 2004a) which is higher than the arabinose plus xylose content of the present spelt destarched and deproteinised bran (30.7%). Wheat A/X ratios vary from 0.55 to 0.68 in destarched bran (Beaugrand *et al.,* 2004a) and here it was 0.46 for destarched and deproteinised spelt bran. These values demonstrate that spelt bran is less rich in AX and that the A/X ratio is lower than for wheat, suggesting a low substitution rate for xylose in spelt.

Fraction	Bran* (%)	WUM (%)	WEM (%)	PWEM (%)
Yield/bran	100,0	29,6	10,4	1,9
Ashes	4,9	5,6	8,3	9,2
Proteins	18,9 ± 0,1	10,9 ± 0,1	24,5 ± 0,1	20,8 ± 1,0
Total non cellulosic sugars	63,3	40,1	46,4	60,1
Arabinose	3,6 ± 0,2	9,6 ± 1,2	3,8 ± 0,2	16,7 ± 0,5
Xylose	7,5 ± 0,3	21,1 ± 1,50	5,1 ± 0,3	26,7 ± 0,5
Mannose	1,1 ± 0,1	0,4 ± 0,0	0,4 ± 0,1	0,8 ± 0,0
Glucose	49,9 ± 3,2	7,7 ± 0,4	35,5 ± 3,1	10,0 ± 0,1
Galactose	1,0 ± 0,2	1,2 ± 0,1	1,6 ± 0,1	5,9 ± 0,2
Starch	41,2	0,8	0,2	0,0
Total	87,1	56,7	79,2	90,1
AX	9,2	27,0	6,8	34,5
A/X	0,39	0,46	0,51	0,47

*Granulometry

Distribution	d(0,1)	d(0,5)	d(0,9)
Particle size µm	13	109	600

AX for WE-AX = (0,88(%ara+%xyl-0,7%gal)
A/X for WE-AX= (%ara-0,7%gal)/%xyl
AX for WU-AX=(0,88(%ara+%xyl))

Table 5.3. Composition of the bran, WUM and WE-AX fractions in percent (d.m.) from spelt

5.3.3.3 Destarching and deproteinisation

Spelt bran used in this study had a high content in protein and starch and consequently it became necessary to modify the procedure described by Maes and Delcour (2002) for wheat bran. The starch content of the original micronized spelt bran was 41.2%. A combination of the enzymes Termamyl and Fungamyl in phosphate buffer pH 6.5, enabled to get bran with 0.8% starch. Concurrently, WEM contained 0.2% starch and the purified water-extractable material (PWEM) 0%.

The protein content of the spelt bran was 18.9%. A mix of enzymes was applied: Neutrase (50 µL/g bran), Alcalase (50 µL/g bran) and Flavourzyme (2 µL/g bran) which enabled to get 24.5% proteins in WEM, 14.5% in AE1D, 13.6% in AE2D and 2.3% in CRR. Even if in these conditions the protein content is still important, it represents an efficient removal of proteins.

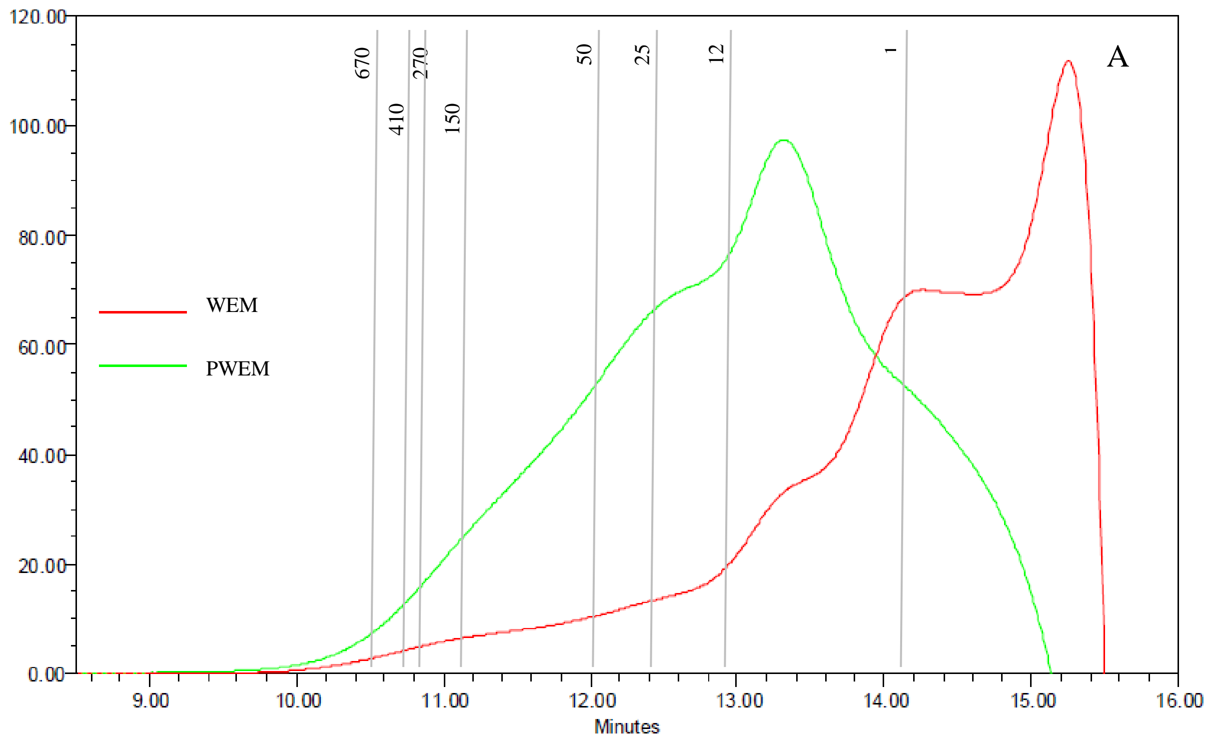
5.3.3.4 Characterization of WE-AX

Two fractions containing WE-AX (WEM and PWEM) were successively obtained. The yield of WEM regarding bran was 10.4% (Table 5.3). WEM contained 6.8% of AX and still 35.5% of glucose even after a dialysis of 48 h. The following purification of WEM managed to eliminate dextrins and proteins and to concentrate the AX, rising their content to 34.5% in PWEM. Besides, PWEM contained only 10% of glucose while WEM contained 35.5% of glucose. Dialysis enabled to remove glucose, a highly digestible monomeric sugar, which lowered the AX purity of the fraction and thus its potential commercial value. The yield of PWEM regarding bran was 1.9%. The purification of WEM

led to 82% of material loss but enabled an improvement in purity and AX concentration. To the best of our knowledge, none information exists in the literature concerning spelt AX. Regarding the WE-AX of wheat, Mandalari *et al.* (2005) obtained WE-AX fractions with 9.7-13.6% arabinose and 12.2-21.5% xylose, which are in the ranges of what was determined here for spelt. The yield in WE-AX was only 0.65% starting from spelt bran while in wheat it reached 1% (Hollmann and Lindhauer, 2005) or 1.45% (Maes and Delcour, 2002). Regarding A/X ratios, it was 0.47 for spelt while for wheat a broad range of 0.2-1.2 is reported in literature (Schooneveld-Bergmans *et al.,* 1999; Maes and Delcour, 2002; Hollmann and Lindhauer, 2005). According to Delcour *et al.* (1999), there exists a pentosan gradient from the centre toward the outer layers of the wheat kernel. This is more pronounced for the total pentosans content and only to a minor extent for the WE-pentosans. WE-AX from the outer layers are less substituted with arabinose than those from the inner endosperm, which is due to a higher proportion of unsubstituted xylose residues and a lower proportion of disubstituted xylose residues (Delcour *et al.,* 1999).

In our study, the ash content was the same in both fractions, around 9%. Concerning proteins, in WEM and PWEM from spelt it was respectively 24.5% and 20.8%; (Table 5.3). As spelt bran contained galactose, it can be inferred that most of the remaining proteins in PWEM may be part of the AGP structure (Fincher *et al.,* 1974) and consequently for PWEM A/X ratio was corrected by the presence of AGP (Loosveld *et al.,* 1997).

On the HPSEC chromatogram (Fig. 5.2A), in WEM, a peak at 6.3 kDa corresponded to the population of WE-AX while the peak with the lowest MW corresponded to oligomers of glucose which disappeared on the PWEM graph. In PWEM, two populations of WE-AX were present with MWs of 6.7 and 28 kDa. WE-AX from wheat display close MWs, of 5 and 20 kDa in the study by Maes and Delcour (2002).



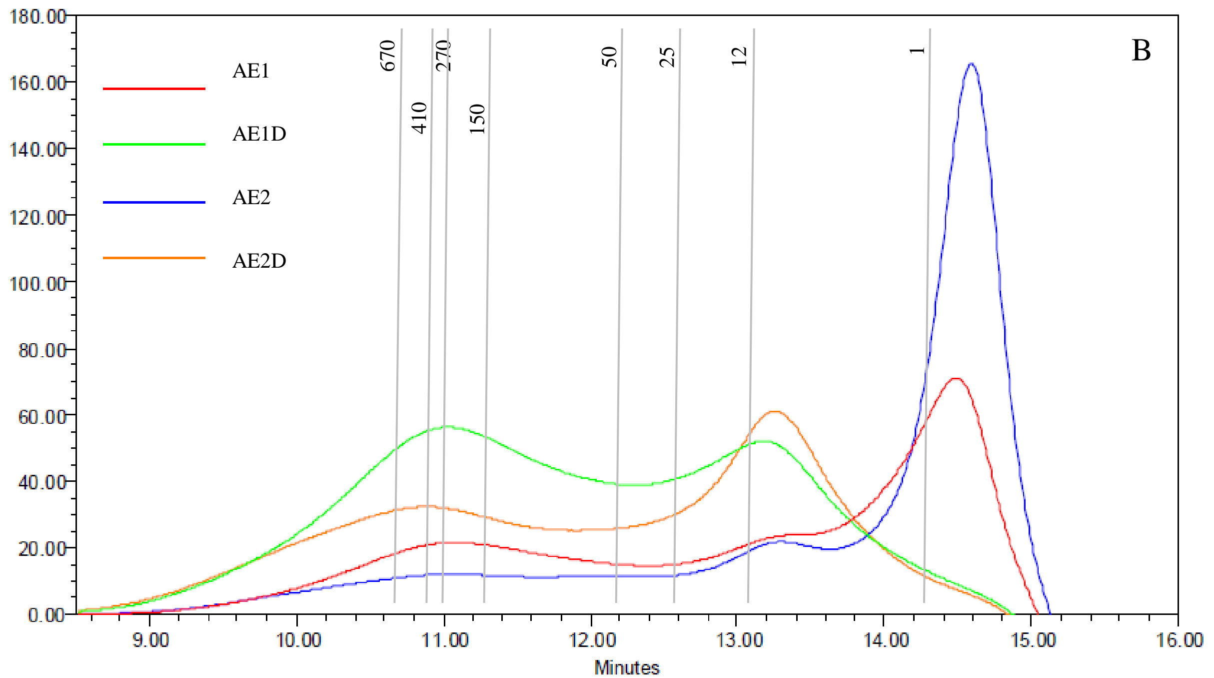


Figure 5.2. Chromatograms of WEM (red) and PWEM (green) (A) and of AE1 (red), AE1D (green), AE2 (blue) and AE2D (orange) (B). Standard dextrans MW values in kDa are indicated with grey vertical lines.

5.3.3.5 Chemical composition of WUM

Yield of WUM was 29.6% for spelt bran which was much lower than results from previous studies on wheat bran. The differences in the bran composition (starch and protein contents) and in the purification methods may cause a high variation in the reported yields of WUM (40-65%) (Selvendran *et al.,* 1980; Brillouet and Mercier, 1981; Carré and Brillouet, 1986; Bergmans *et al.,* 1996; Bataillon *et al.,* 1998, Maes and Delcour, 2002; Kabel *et al.,* 2002b). In the case of spelt, the low yield originated from the high loss of starch initially contained in the bran.

Spelt WUM contained 27.0% AX, 7.7% glucose, 10.9% proteins, 5.6% ashes and 0.8% starch. Literature data for the bulk of the dry matter of WUM from wheat bran consisted of 45-55% cellulosic sugar residues, 4-9% proteins, 0.2-5% ash, 6-14% lignin, <0.1-1.5% starch (Maes and Delcour, 2002). Considering the starch content in spelt WUM, it was obvious that glucose came from another unknown source. Bergmans et al. (1996) observed in the WU-cell wall material that 21% of the non-starch glucose was not accounted for by cellulose nor (1->3),(1->4)-β-D-glucans. It was not known whether this was attributable to shortcomings of the methods used or to the presence of other polysaccharides. This glucose might also come from degradation of cellulosic sugars during the acid hydrolysis of the monosaccharides analysis.

Fraction	WUM (%)	AE1 (%)	AE1D (%)	AE2 (%)	AE2D (%)	CRR (%)
Yield/bran	29,6	22,2	9,5	13,9	3,8	11,4
Yield/WUM	-	74,9	32,2	46,9	12,8	38,6
Ashes	5,6	39,1	6,2	64,7	10,3	18,0
Proteins	10,9 ± 0,1	10,5 ± 0,0	14,5 ± 0,1	5,3 ± 0,0	13,6 ± 0,0	2,3 ± 0,0
Total non cellulosic sugars	40,1	23,3	44,1	9,4	28,7	22,5
Arabinose	9,6 ± 1,2	8,3 ± 0,0	16,6 ± 0,1	3,4 ± 0,0	10,3 ± 0,1	3,9 ± 0,1
Xylose	21,1 ± 1,5	12,2 ± 0,1	25,4 ± 0,1	5,4 ± 0,1	17,0 ± 0,2	17,0 ± 0,8
Mannose	0,4 ± 0,0	0,2 ± 0,1	0,1 ± 0,2	0,0 ± 0,0	0,0 ± 0,0	0,2 ± 0,0
Glucose	7,7 ± 0,4	1,7 ± 0,1	0,9 ± 0,1	0,4 ± 0,0	0,7 ± 0,0	0,8 ± 0,0
Galactose	1,2 ± 0,1	0,7 ± 0,1	1,0 ± 0,1	0,2 ± 0,0	0,7 ± 0,0	0,5 ± 0,0
Total	56,7	72,9	64,9	79,5	52,6	42,8
A/X	0,46	0,68	0,65	0,63	0,60	0,23
AX	27,0	18,0	36,9	7,7	24,1	18,5

AX for WU-AX=(0,88(%ara+%xyl))

Table 5.4. Composition of the WUM, WU-AX fractions and CRR in percent (d.m.) from spelt.

5.3.3.6 Characterization of WU-AX

Two fractions of WU-AX were successively obtained and each one was purified through a dialysis especially to lower the ashes content because the alkaline extracts (AE1 and AE2) contained a lot of ashes resulting from salt formation during neutralisation of the WUM- alkaline hydrogen peroxyde suspension. AE1 represented 22.2% of the bran, AE2 13.9%. AE1D 9.5% and AE2D 3.8% (Table 5.4). The material loss throughout the dialysis was very variable. Between AE1D and AE1 it was 57% and between AE2D and AE2 it was 73%. The dialysis succeeded in increasing twofold the AX content

for AE1D and threefold for AE2D, and in reducing significantly the ash concentration by 84%. Consequently, AE1D contained 37% of AX and AE2D 24%, which correspond respectively to 32.2 and 12.8% of WUM. Spelt extraction yield of WU-AX (48.2%), was in the range of results reported for wheat bran by Schooneveld-Bergmans (1998), Maes and Delcour (2002) or Hollmann and Lindhauer (2005), i.e. 40 to 50% of the AXs. The spelt extraction yield of WU-AX was however lower (4.4%) than the results from Shiiba et al. (1993): 14% (weight of AX/weight of wheat bran). A/X ratios were 0.65 and 0.60 for spelt fractions while in literature they were comprised between 0.8 and 0.92 (Shiiba et al., 1993; Schononveld-Bergmans, 1999; Maes and Delcour, 2002; Hollmann and Lindhauer, 2005) or 0.35-0.71 (Mandalari et al., 2005) for wheat bran WU-AX. AE1 and AE2 presented three peaks on the HPSEC chromatograms (Fig. 5.2B). As for WE-AX the last peak on the right of the chromatogram corresponded to oligomers with MWs of 790 Da (AE2) and 960 Da (AE1). The two other peaks from AE1 and AE2 were the WU-AX with respectively MWs of 6.7 kDa and 280 kDa, and 7.1 kDa and 290 kDa. AE1D clearly showed two populations: one with 8.1 kDa and one with 310 kDa and AE2D 7.4 kDa and 415 kDa. This proved the absence of degradation of AX molecules which might be observed if conditions are too severe (Persson et al., 2009). Reported MWs from wheat AX are distributed in two populations, one between 100 and 120 kDa (Hollmann and Lindhauer, 2005; Maes and Delcour, 2002) and one between 300 and 622 kDa (Shiiba *et al.,* 1993; Bergmans, 1996; Schononveld-Bergmans, 1999). These authors applied rather aggressive methods such as the use of NaOH (Shiiba *et al.,* 1993), barium hydroxide plus autoclave, peroxyde or chlorite (Bergmans *et al.,* 1996), and finally again barium hydroxide to extract AX or GAX (Schooneveld-Bergmans, 1998 and 1999). On the contrary Maes and Delcour (2002) and Hollmann and Lindhauer (2005) used a more environment friendly method with H_2O_2. In the case of spelt, the H_2O_2 method enabled to extract WU-AXs of 310-415 kDa, i.e. much higher MWs than those from wheat bran in the same conditions (Maes and Delcour, 2002; Hollmann and Lindhauer, 2005).

The different published results concerning the contents in arabinose and xylose, A/X ratios and MWs obtained through chemical extractions are highly variable. Indeed they depend on the methods used for hydrolysis and probably for extraction. Due to environmental awareness, enzymatic methods were also developed, for instance Beaugrand *et al.* (2004a) that managed to extract 40-55% of the AX from wheat bran and Benamrouche *et al.* (2002) that solubilised 50% of the AX from wheat bran. Moreover, genotype, environment and the genotype/environment interaction also had influence on AX in wheat grain which may lead to significant differences in the results (Li *et al.,* 2009). The same effects can be expected on spelt growth and grain composition, and consequently on spelt AXs.

5.3.3.7 Balance sheet of material

Concerning yield, it is interesting to analyse the distribution of the AX in the different fractions purified: PWEM, AE1D and AE2D. The three fractions together enabled to extract 62.5% of the total arabinose, 47.5% of the xylose and so 55.3% of the arabinoxylans initially present in spelt bran, distributed among AE1D 38.3%, AE2D 9.9% and PWEM 7.1%. Among AX, the majority were WU-AX, 87%. The majority of the AX (69%) was concentrated in the AE1D fraction (38.2% of the initial AX from spelt bran). Finally, it was possible to get 4.4 g of WU-AX and 0.6 g of WE-AX from 100 g of spelt bran.

5.3.3.8 Characterization of CRR

The yield of CRR was 11.4% of the bran and 38.6% of WUM. CRR contained 26.4% of the AX present in WUM. It was mainly composed of ashes 18.0% and AX 18.5%. For Maes and Delcour (2002), in wheat bran arabinose and xylose amount to respectively 38 and 24% of each of the sugars originally present in WUM, while it was 16 and 31% here for spelt. These figures showed that more xylose and less arabinose were extracted from spelt bran than from wheat bran. In spelt CRR, the A/X ratio 0.23, was half that found in WUM (0.46). It could be argued that this was caused by previous selective extraction of the most substituted AX. Indeed, AXs of high A/X ratio are more easily extracted by alkali than less branched AX (Dupont and Selvendran, 1987; Bergmans *et al.,* 1996). Moreover, considering the A/X ratios of WUM, alkali fractions and CRR, an increase of 35% between WUM and alkali fractions and a decrease of 50% between WUM and CRR was observed. During the process, components rich in arabinose are more easily extracted and are more accessible. Kabel *et al.* (2007) showed that unsubstituted linear xylan domains favoured adsorption to cellulose and as in CRR A/X ratio is very low, it is difficult to extract more AX.

5.3.4 Conclusion

It is to the best of our knowledge the first study on spelt AX extraction and characterization. The most adapted conditions of total hydrolysis for the study of monosaccharides of the spelt extraction fractions were selected: all the samples were hydrolysed during 3 h at 100°C, with TFA 2 M for the solid group, H_2SO_4 1 M for WE-AX fractions and TFA 0,5 M for WU-AX fractions. The destarching and deproteinisation conditions adapted here were efficient, with initial starch and protein contents of 41% and 19%. The present method enabled to extract 55.3% of the AXs from spelt bran, composed of 87% of WU-AX and 13% of WE-AX. A/X ratios were 0.47 for WE-AX and 0.60-0.65 for WU-AX. Populations of WE-AX from spelt had MWs of 7 and 28 kDa and populations of WU-AX from spelt had MWs of 7-8 kDa and 310-415 kDa. According to literature data, wheat bran yields more WE-AXs than spelt bran. This can be explained by a difference in the solubility behaviour of the polymers which is influenced by the chain length, the presence and the distribution of side-chain groups and the linkages (Saulnier *et al.,* 2007). Besides, spelt WE-AXs were bigger molecules than wheat WE-AXs.

In wheat, it has been demonstrated that genotype had an impact on the average size of water-soluble polysaccharides (Udy, 1956 in Saulnier *et al.,* 2007) and that both genotype and environment had an effect on the WE-AX content (Li *et al.,* 2009). Concerning the A/X ratio, literature data on wheat bran is so variable that it can hardly be compared with the results of spelt. The extractability of spelt WU-AXs was in the range of the one of wheat. The A/X ratios were in most cases higher for wheat than for spelt bran WU-AXs, indicating less arabinose branching in spelt bran WU-AXs. However, in the light of the monosaccharides contents, it should be reminded that A/X ratio is influenced by the conditions of hydrolysis. WU-AXs extracted with alkaline H_2O_2 had MWs three times bigger in spelt bran than in wheat bran. The differences concerning A/X ratios and MWs of WU-AXs of wheat bran and spelt bran may lead to differing techno-functional properties. In that case, it could be interesting to look for specific potential applications as food additives for spelt bran WU-AXs that would require water-binding capacity and swelling properties. Besides, spelt AXs could be preferred for organic food as spelt is often grown under organic farming in comparison to wheat. The study of spelt bran AX fractions orientates now towards a more extensive characterization of the molecules structure and composition, and of their techno-functional properties (water-holding capacity, lipid-holding capacity, gelation, film formation, viscosity…).

5.3.5 Acknowledgments

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5.3.6 References

Bacic A., Harris P. J., Stone B.A., 1988. Structure and function of plant cell walls. *The Biochemistry of Plants,* 14, 297-370.

Bacic A., Stone B. A., 1981b. Isolation and ultrastructure of aleurone cell walls from wheat and barley. *Australian Journal of Plant Physiology*, 8, 453-475.

Bataillon M., Mathaly P., Nunes Cardinali A.-P, Duchiron F., 1998. Extraction and purification of arabinoxylan from destarched wheat bran in a pilot scale. *Industrial Crops and Products*, 8, 37-43.

Beaugrand J. (2004). Bases cytologiques et moléculaires de la dégradation enzymatique du son de blé tendre. Laboratoire INRA, UNIR-614 Fractionnement des AgroRessources et Emballages, Reims Champagne-Ardenne, 172p.

Beaugrand J., Crônier D., Debeire P., Chabbert B., 2004a. Arabinoxylan and hydroxycinnamate content of wheat bran in relation to endoxylanase susceptiblity. *Journal of Cereal Science*, 40, 223-230.

Benamrouche S., Crônier D., Debeire P., Chabbert B., 2002. A chemical and histological study on the effect of ((1->4)-β-endo-xylanase treatment on wheat bran. *Journal of Cereal Science*, 36, 253-260.

Bergmans M.E.F., Beldman G., Gruppen H., Voragen A.G.J., 1996. Optimisation of the selective extraction of (glucurono)arabinoxylans from wheat bran: use of barium and calcium hydroxide solution at elevated temperatures. *Journal of Cereal Science*, 23, 235-245.

Blakeney A.B., Harris P.J., Henry RJ., Stone B.A., 1983. A simple and rapide preparation of alditol acetates for monosaccharide analysis. *Carbohydrate Research* 113, 291-299.

Bonafaccia G., Galli V., Francisci R., Mair V., Skrabanja V., Kreft I., 2000. Characteristics of spelt wheat products and nutritional value of spelt wheat-based bread. *Food Chemistry*, 68, 437-441.

Brillouet J.-M., Mercier C., 1981. Fractionation of wheat bran carbohydrates. *Journal of the Science of Food and Agriculture*, 32, 243-251.

Brillouet J.M., Joseleau J.P., 1987. Investigation of the structure of a heteroxylan from the outer pericarp (beeswing bran) of the wheat kernel. *Carbohydrate Research*, 159, 106-126.

Campbell K.G., 1997. Spelt: agronomy, genetics and breeding. Plant breeding reviews, 15, 187-213.

Chen W.-J. L., and Anderson J.W., 1981.Soluble and insoluble plant fiber in selected cereals and vegetables. *The American Journal of Clinical Nutrition*, 34, 1077-1082.

Courtin C. M. and Delcour J., 2002. Arabinoxylans and endoxylanases in wheat flour bread-making. *Journal of Cereal Science*, 35, 225-243.

Delcour J. A., Van Win H., Grobet P., 1999. Distribution and structural variation of arabinoxylans in common wheat mill streams. *Journal of Agricultural and Food Chemistry*, 47, 271-275.

Dupont M. S., Selvendran R.R., 1987. Hemicellulosic polymers from the cell walls of beeswing wheat bran: part I, polymers solubilised by alkali at 2°. *Carbohydrate Research*, 163, 99-113.

Edwards S., Chaplin M.F., Blackwood A.D., Dettmar P.W., 2003. Primary structure of arabinoxylans of ispaghula husk and wheat bran. *Proceedings of the Nutrition Society*, 62, 217-222.

Englyst H.N., Cummings J.H., 1984. Simplified method for the measurement of total non-starch polysaccharides by gas-liquid chromatography of constituent sugars as alditols acetates. *Analyst*, 109, 937-942.

Fincher G.B., Sawyer W.H., Stone B.A., 1974. Chemical and physical properties of an arabinogalactan-peptide from wheat endosperm. *The Biochemical Journal*, 139, 535-545.

Hollmann J., Lindhauer M.G., 2005. Pilot-scale isolation of glucuronoarabinoxylans from wheat bran. *Carbohydrate Polymers*, 59, 225-230.

Iiyama K., Lam T.B.T., Stone B.A., 1994. Covalent cross-links in the cell wall. *Plant Physiology*, 104, 315-320.

Kabel M.A., Kortenoeven L., Schols H.A., Voragen A.G.J., 2002a. In vitro fermentability of differently substituted xylooligosaccharides. *Journal of Agricultural and Food Chemistry*, 50: 6205-6210.

Kabel M.A., Carvalheiro F., Garrote G., Avgerinos E., Koukios E., Parajo J.C., Girio F.M., Schols H.A., Voragen A.G.J., 2002b. Hydrothermally treated xylan rich by-products yield different classes of xylo-oligosaccharides. *Carbohydrate Polymers*, 50, 47-56.

Kabel M.A., Van den Borne H., Vincken J.-P., Voragen A.G.J., Schols H.A., 2007. Structural differences of xylans affect their interaction with cellulose. *Carbohydrate Polymers*, 69, 94-105.

Lempereur I., Rouau X., Abecassis J., 1997. Genetic and agronomic variation in arabinoxylan and ferulic acid contents of durum wheat (Triticum durum L.) grain and its milling fractions. *Journal of Cereal Science*, 25, 103-110.

Li S., Morris C.F., Bettge A.D., 2009. Genotype and environment variation for arabinoxylans in hard winter and spring wheats of the U.S. Pacific Northwest. *Cereal Chemistry*, 86, 88-95.

Loosveld A.-M. A., Grobet P.J., Delcour J.A., 1997. Contents and structure features of water extractable arabinogalactan in wheat flour fractions. *Journal of Agricultural and Food Chemistry*, 45, 1998-2002.

Maes C., Delcour J.A., 2001. Alkaline hydrogen peroxide extraction of wheat bran non-starch polysaccharides. *Journal of Cereal Science*, 34, 29-35.

Maes C., Delcour J.A., 2002. Structural characterisation of water-extractable and water-unextractable arabinoxylans in wheat bran. *Journal of Cereal Science*, 35, 315-326.

Mandalari G., Faulds C.B., Sancho A.I., Saija A., Bisignano G., LoCurto R., Waldron K.W., 2005. Fractionation and characterisation of arabinoxylans from brewers' spent grain and wheat bran. *Journal of Cereal Science*, 42, 205-212.

Mankarios A.T., Christopher F., Jones G., Jarvis M.C., Threlfall D.R., Friend J., 1979. Hydrolysis of plant polysaccharides and GLC analysis of their constituent neutral sugars. *Phytochemistry*, 18, 419-422.

Mares D.J., Stone B.A., 1973. Studies on wheat endosperm I. Chemical composition and ultrastructure of the cell walls. *Australian Journal of Biological Sciences*, 26, 793-812.

Persson T., Ren J.L., Joelsson E., Jönsson A.-S., 2009. Fractionation of wheat and barley straw to access high-molecular-mass prior to ethanol production. *Bioresource Technology*, 100, 3906-3913.

Pomeranz Y., 1988. Chemical composition of kernel structure. In: Pomeranz, Y. (Ed), Wheat and chemistry technology. AACC, St Paul, Minnesota, U.S.A., pp 97-158.

Ralet M.-C., Thibault J.-F., Della Valle G., 1990. Influence of extrusion cooking on the physico-chemical properties of wheat bran. *Journal of Cereal Science*, 11, 249-259.

Ranhotra G. S., Gelroth J. A., 1995. Baking and nutritional qualities of a spelt wheat sample. *Lebensmittel-Wissenschaft und Technologie*, 28, 118-122.

Ring S.G., Selvendran R.R., 1980. Isolation and analysis of cell wall material from beeswing wheat bran (Triticum aestivum). *Phytochemistry*, 19, 1723-1730.

Ruibal-Mendieta N. L., Rozenberg R., Delacroix D., Petitjean G., Dekeyser A., Baccelli C., Marques C., Delzenne N.M., Meurens M., Habib-Jiwan J.-L., Quetin-Leclercq J., 2004a. Spelt (Triticum spelta L.) and winter wheat (Triticum aestivum L.) wholemeals have similar sterols profiles, as determined by quantitative liquid chromatography and mass spectrometry analysis. *Journal of Agricultural and Food Chemistry*, 52, 4802-4807.

Ruibal-Mendieta N. L., Delacroix D., Mignolet E., Pycke J.-M., Marques C., Rozenberg R., Petitjean G., Habib-Jiwan J.-L., Meurens M., Quetin-Leclercq J., Delzenne N.M., Larondelle Y., 2005. Spelt (Triticum aestivum ssp. Spelta) as a source of breadmaking flours and bran naturally enriched in oleic acid and minerals but not phytic acid. *Journal of Agricultural and Food Chemistry*, 53, 2751-2759.

Saulnier L., Guillon F., Sado P.-E., Rouau X., 2007. Plant cell wall polysaccharides in storage organs : xylans (food applications). In: Kamerling, J.-P., Boons, G.-J., Lee, Y.C., Suzuki, A., Taniguchi, N., Voragen, A.G.J. (Eds), Comprehensive glycoscience. From chemistry to systems biology. Analysis of glycans. Polysaccharide functional properties, Vol 2., Elsevier, Oxford, pp. 653-687.

Schober T. J., Bean S. R., Kuhn M., 2006. Gluten proteins from spelt (Triticum aestivum ssp. spelta) cultivars: A rheological and size-exclusion highperformance liquid chromatography study. *Journal of Cereal Science*, 44, 161–173.

Schooneveld-Bergmans M. E. F., Hopman A.M.C.P., Beldman G., Voragen A.C.J., 1998. Extraction and partial characterization of feruloylated glucuronoarabinoxylans from wheat bran. *Carbohydrate Polymers*, 35, 39-47.

Schooneveld-Bergmans M. E. F., Beldman G., Voragen A.G.J., 1999. Structural features of (glucurono)arabinoxylans extracted from wheat bran by barium hydroxide. *Journal of Cereal Science*, 29, 63-75.

Selvendran R.R., Ring S.G., O'Neill M.A., DuPont M.S., 1980. Composition of cell wall material from wheat bran used in clinical feeding trials. *Chemistry and Industry*, 11, 885-888.

Shiiba K., Yamada H., Hara H., Okada K., Nagao S., 1993. Purification and characterization of two arabinoxylans from wheat bran. *Cereal Chemistry*, 70, 209-214.

Southgate D.A.T., 1995. Analytical Strategies. In : Belton, P.S. (Eds.), Dietary fibre Analysis, Royal Society of Chemistry, Cambridge, pp. 34-35.

6 CHAPTER 6

Characterization of hemicellulosic fractions from spelt hull extracted by different methods

Excerpt from Article 3:

Escarnot, E., Aguedo, M., Paquot, M., 2011b. Characterization of hemicellulosic fractions from spelt hull extracted by different methods. *Carbohydrate Polymers*, 85, 419-428.

6.1 The context of the study

After the work on one by-product of spelt milling, bran, a second by-product of spelt milling that is hull will be investigated. Bran is a part of the grain whereas hull is a vegetative part of the spikelet, as straw is a vegetative part of the plant. They make part of the biomass from the annual crop. Spelt hull composition is unknown however it is supposed to be close to that of straw which is a rich source of hemicelluloses. Consequently, the focus will be done on hemicelluloses from spelt hull.

Hemicelluloses from the vegetative part of the plant may be slightly different from those of the grain. Xylans are mainly present in the lignified secondary walls where they account for 20-30 % of the mass tissues (Saulnier *et al.*, 2007). In some tissues of monocotyl plants xylans occur up to 50% (Stephen, 1983 in Ebringerova *et al.*, 2005). Xylans can be arabinoxylans, arabinoglucuronoxylans, glucuronoarabinoxylans (GAXs) and heteroxylans. The distinction between AX and GAX depends on the amount of glucuronic acid. In endosperm AX has very little glucuronic acid whereas in vegetative part AX contain more glucuronic acid and GAX should be a more appropriate name (Scheller and Ulvskov, 2010). As in the bran and endosperm, phenolic acids such as ferulic acids are attached to the arabinofuranosyl residues and esters of p-coumaric acids have also been found. Xylans from the vegetative part have interactions with other constituents from the cell wall such as lignin, other cell wall polymer and protein. As no information on AXs from spelt hull is available, the closest material, wheat straw, will serve of reference at a first stage. The detailed structure of the AX/GAX from wheat straw will be described in the following chapter.

The extractability of the AX/GAX in the vegetative part is difficult. Hemicelluloses have been extracted from several annual crops such as wheat straw (Sun *et al.*, 1998; Sun *et al.*, 2000; Xu *et al.*, 2006a), barley straw and maize stems (Jin *et al.*, 2009), barley husk (Hoije *et al.*, 2005), sugar beet pulp (Sun and Hughes, 1998), buckwheat hull (Hromadkova *et al.*, 2003), grape stalks (Spigno *et al.*, 2008), sugarcane bagasse (Xu *et al.*, 2006b), rye and rice straw (Xiao *et al.*, 2001). Several chemical methods were experimented in this purpose: one-step procedure with H_2O_2 (Sun *et al.*, 2000) or organic acids (Xu *et al.* 2006a), two-step procedure with $NaOH/H_2O_2$ (Sun *et al.*, 2002) or three-step procedure with $NaOH/NaClO_2/KOH$ (Sun *et al.*, 1998). Physical methods such as ultrasounds were shown to be very effective during the alkaline extraction (Sun *et al.*, 2002). The presence of protein and non-separated starch can complicate the isolation of hemicelluloses (Hoije *et al.*, 2005). Generally low pH treatments tend to produce hemicellluloses under an oligomeric form (Xu *et al.*, 2006a) while alkaline treatments hydrolyse the ester linkages between plant polysaccharides and lignin which increases the solubility of hemicelluloses without reducing their molecular mass (Persson *et al.*, 2009).

Firstly, the method applied to bran will be tested on hull and as hull is hypothesized to be close to straw, methods that have been employed on this material will be tested on the hull. Different

chemicals, concentration and duration will be experimented in the following chapter and the result from each one will be discussed regarding the efficiency and the profile of the obtained products.

6.2 Characterization of hemicellulosic fractions from spelt hull extracted by different methods

Abstract

Arabinoxylans (AXs) from spelt hull were extracted by three different alkaline methods and by two methods with several modalities and two organic acids. The first method enabled to extract 1.4% of the water-extractable AXs (WE-AXs) of the hull with molecular weight (MW) of 5400 g/mol and arabinose to xylose (A/X) ratio of 0.55. The two successive extractions with 2% alkaline peroxide hydrogen at 60°C during 4h enabled to extract 20.4% of the water-unextractable AXs (WU-AXs) of the hull. Those fractions had MWs of 6800-7800 g/mol and 227 700-274 600 g/mol and A/X ratios of 0.32 and 0.45. The second method with 2% alkaline peroxide hydrogen at 50°C during 24 h enabled to extract 25.4% of the hemicelluloses of the hull. The two AX populations had MW of 9200-11400 g/mol and 256 800-273 200 g/mol with an A/X ratio of 0.36. The third method with NaOH gave the highest yield, 41.9% after a 16 h extraction. A/X ratios (0.18) and MWs (two populations of 9300-9500 g/mol) were lower than those of the previous methods. Organic acids method offered insufficient yields and compounds of very low MW (1300-11300 g/mol). Methods with alkaline peroxide hydrogen enabled to obtain high MWs and high A/X ratios with moderate yields while the NaOH method gave the highest yield efficiency with lower MWs and A/X ratios. Organic acids method did not bring any advantage neither on the yield neither on the MWs of the compounds.

6.2.1 Introduction

Spelt is a traditional cereal mainly grown in middle and Eastern Europe countries. It is an environmental-friendly crop well-known for its rusticity and its adaptation to low input cultivation. As a hulled cereal, spelt is mainly used for feeding however it is developing in “niche” markets for human food thanks to its image of “more natural, less over-bred” cereal (Schober *et al.,* 2006) and thanks to health peculiar properties. Spelt is recommended in the treatment of colitis ulcerosa, neurodermitis and other allergies and high blood cholesterol (Hertzka and Strechlow, 1988; Strechlow *et al.,* 1991; Portman, 1991; all in Zielinski *et al.,* 2008). In this case, the hull is separated from the grain and returns generally for feeding with very low added value. Hull represents 21 to 32% of the harvest (Percival, 1921) and nowadays in Belgium in average 15 000 to 22 000 tons of hulls are produced per year (according to Direction générale de la Statistique et de l’Information économique, 2010).

To the best of our knowledge, composition of spelt hull is not reported in literature, however it should not be far from the composition of the straw. Wheat straw contains 30-50% cellulose, 20-40% hemicelluloses and 10-30% lignin. The main sugars from wheat straw are glucose (39.6%), xylose (24.3%), arabinose (2.1%) and galactose (0.2%) (Lequart *et al.,* 1999).

Hemicelluloses are branched polymers of low molecular weight (MW), with general formulas $(C_5H_8O_4)_n$ and $(C_6H_{10}O_5)_n$, i.e. respectively pentosans and hexosans (Cai and Paszner, 1988). Wheat

straw hemicelluloses are mainly composed of xylose, and to a lesser extent of arabinose, while glucose, galactose, mannose and rhamnose are minor sugars. The β-(1-4)-D-xylopyranose backbone is substituted at C-3 by α-L-arabinofuranose and at C-2 by α-D-glucuronic acid (mainly 4-O-methyl-D-glucuronic acid). Galactose and xylose residues are potentially linked to arabinofuranosyl branches (Sun *et al.,* 1996). As well as glycol-proteins, pectins, waxes and ashes, the cell walls of wheat straw also contain small amounts of p-hydroxycinnamic acids (mainly ferulic and p-coumaric acids), esterified on arabinofuranosyl residues (Sun *et al.,* 1995; Sun *et al.,* 2001). All the cell wall constituents are closely bound, either by weak bonds such as hydrogen or Van der Waals bonds, either by covalent bonds (Sun *et al.,* 2000). Xylans form hydrogen bonds with cellulose, covalent bonds with lignins, and ester linkages with acetyl units and p-hydroxycinnamic acids (Sun *et al.*, 2000). The liberation of xylan from the cell wall of cereal straws is restricted by the presence of the lignin network and their ester or ether linkages (Xu *et al.*, 2006a). The covalent bonds between cellulose and xylan are extremely resistant and cannot be broken even by treatment with acids (Taiz, 1984 in Heredia *et al.,* 1995).

Hemicelluloses can be used to produce health food supplement. For instance, xylans from cereals contribute as dietary fibers upon some biochemical and physiological processes in human and animal organisms by lowering blood cholesterol and decreasing post-prandial glucose and insulin responses (Asp *et al,* 1993; Chesson, 1995; Baghurst *et al.,* 1996). Besides, non-digestible arabino-xylo-oligosaccharides originating from arabinoxylans were described as prebiotics (Courtin *et al.,* 2009). Polymeric hemicelluloses can be used to produce barrier films, hydrogels, paper additives (Persson *et al.,* 2009) and also adhesives, thickeners, stabilisers and emulsifiers (Doner and Hicks, 1997).

It is difficult to separate lignin from hemicelluloses and cellulose without modifying the hemicelluloses since the components of lignocellulose are tightly associated (Puls and Schuseil, 1993 in Lundqvist *et al.,* 2002). The different ways to fractionate the hemicelluloses from lignocelluloses include physical methods such as steam treatment (Han *et al.,* 2010) and chemical methods such as the organosolv process (Paszner *et al.,* 1993). A traditional hypochlorite bleaching process as used in chemical pulp bleaching causes a serious environmental problem (Fang *et al.,* 1999). Gould (1984) demonstrated that approximately half of the lignin and most of the hemicelluloses present in wheat straw are solubilized when the material is treated at 25°C in an alkaline solution of hydrogen peroxide. According to Sun *et al.* (2000), the macromolecular hemicelluloses are only solubilized and partially degraded with no monocarboxylic acid formed. Indeed, alkalis hydrolyse the ester linkages between plant polysaccharides and lignin, which increases the solubility of the hemicelluloses without reducing their MWs, provided the conditions are not too severe (Persson *et al.,* 2009). Besides, hydrogen peroxide presents mild oxidation during the delignification process and environmental compatibility.

In the present work, spelt hull AXs were extracted by four different methods described in the literature: the first comes from Maes and Delcour (2002) on wheat bran, the second from Sun *et al.* (2000) on wheat straw, the third from Sun *et al.* (1998) on wheat straw and the fourth from Xu *et al.* (2006a) also on wheat straw. The two first methods use H_2O_2 at different temperatures and times of extraction and the first includes successive extractions. They were chosen because H_2O_2 is an environmental-friendly chemical and because they were described as providing a compromise between MW and yield of extraction. The third method was performed with NaOH and KOH and included a delignification with sodium chlorite. The fourth and last method relied on organic acids to obtain high yields and to evaluate the effects of such chemicals on hull. The characteristics of AXs and their extraction yields with the different methods were compared.

6.2.2 Material and methods

6.2.2.1 Material

Hulls came from the spelt variety Ressac harvested in August 2007. The spikelets were dehulled at "Le Moulin de Hollange" in November 2007 and hulls were stored at 4°C until they were micronized with jet milling (Alpine 100 AFG, Augsburg, Germany) with 6-7 bars of pressure and turbine at 3000 rpm. All reagents were of analytical grade. All enzymes were a kind gift from Novozymes (Bagsvaerd, Denmark).

6.2.2.2 Methods

Hull deproteinisation and destarching

Micronized hulls in 0.05 M phosphate buffer pH 6.5 (ratio 1:7, w/v) were heated under continuous stirring until 75°C and α-amylase Termamyl 120L (Novozymes, Bagsvaerd, Denmark) was added (5 µL/g hull). The suspension was then heated to 90°C and maintained 1 h. It was then cooled down to 50°C and Fungamyl 800L (Novozymes) was added (0.4 µL/g hull). The suspension was kept 30 min at 50°C. Three proteases were successively added: Neutrase® 50 µL/g hull, Alcalase® 50 µL/g hull and Flavourzyme 1000L® 2µL/g hull (all three from Novozymes). The suspension was heated during 4h at 55°C under continuous stirring. In order to desactivate the enzymes, the suspension was then heated at 100°C during 15 min. The mixture was vacuum-filtered through a 20 µm nylon filter. The residue, *i.e.* destarched and deproteinised spelt hull or water-unextractable material (WUM), was washed several times with distilled water and dried at 50°C in a drying oven for 24 h. The filtrate containing WE-AX was kept for the first method of extraction.

First method of AX extraction: two successive extractions with H_2O_2

This method was adapted from Maes and Delcour (2002) and was previously described elsewhere (Escarnot *et al.,* 2011a).

Second method of AX extraction: one extraction with H_2O_2

This method was adapted from Sun *et al.* (2000), however the durations of the experiment and the volumes of precipitation with ethanol were different. A solution of 2% H_2O_2 was freshly prepared and the pH was adjusted to 11.5 with NaOH 4 M. The destarched deproteinised hulls were dissolved at a ratio 1:10 (w/v). The solutions were maintained at 50°C during 4 h, 8 h, 16 h or 24 h under slow continuous stirring. The residue was recovered by vacuum filtration through a 20 µm nylon filter, washed with distilled water until the pH of the filtrate was stable. Consequently four filtrates (F) were obtained, F4, F8, F16 and F24 which pH was neutralized. For precipitation, each filtrate was then set to pH 5.5 with 10% HCl and 3 or 4 volumes of ethanol were added and left overnight at 4°C. On the next day, they were centrifuged (10,000 g, 30 min, 4°C), the ethanol was removed and the precipitates were recovered, dried under air flux and then milled (Ika A11 basic). Eight different precipitates (P) were obtained according to the duration of the experiment and the volume of precipitation with ethanol (V): P4 3V, P4 4V, P8 3V, etc. Residues (R) (R4, R8, R16 and R24) were washed with distilled water, dried at 60°C in a drying oven for 24h and milled (Ika A11 basic).

Third method of AX extraction: successive NaOH and $NaClO_2$-KOH extractions

It was from Sun *et al.* (1998) however times of extraction were different. Destarched deproteinised hulls were dissolved in a fresh 3% NaOH solution at a ratio 1:27 (w/v). The solution was maintained at 45°C for 2 h, 5 h or 16 h under continuous stirring. The suspension was vacuum filtered through a 20 µm nylon filter and the pH of the supernatant was neutralized. Three filtrates were obtained F2, F5 and F16, and for precipitation their pH were set to 5.5 with 20% HCl, 3 or 4 volumes of ethanol were added and left overnight at 4°C. On the next day, they were centrifuged (10,000 g, 30 min, 4°C), the ethanol was removed and the precipitates were recovered, dried under air flux and milled (Ika A11 basic). Residues were washed with distilled water, with 70% ethanol, dried at 60°C in a drying oven for 16 h and milled (Ika A11 basic).

A solution of 1.3% $NaClO_2$ was freshly prepared and the pH was adjusted to 4 with 10% acetic acid. The previous residues were dissolved in this solution at a ratio 1:50 (w/v) and maintained at 75°C during 2 h. Again, residues were vacuum filtered through a 20 µm nylon filter, washed with distilled water, dried at 60°C in a drying oven for 16h and milled (Ika A11 basic).

Then this residue was dissolved in a 10% KOH solution at a ratio 1:25 (w/v). It was left 16 h at 25°C under continuous stirring. The solution was vacuum filtered through a 20 µm nylon filter. Filtrates F2K, F5K and F16K were neutralized. For precipitation, the filtrate was set at pH 5.5 with 20% HCl and 3 or 4 volumes ethanol were added. The precipitates (P2K 3V, P2K 4V, P5K 3V, etc.) were left overnight at 4°C and were centrifuged the day after (10,000g, 30 min, 4°C), then the ethanol was removed and the precipitates were recovered, dried under air flux and milled (Ika A11 basic). Residues

(R2, R5, R16) were washed with distilled water, dried at 60°C in a drying oven for 24 h and milled (Ika A11 basic).

Fourth method of AX extraction: acidic extractions

This method comprises several modalities of extraction with organic acids and is based on the work of Xu *et al.* (2006a). Micronized destarched deproteinised hull was dissolved in 5 fresh solutions at a ratio 1:20 (w/v) of acetic acid and distilled H_2O, with proportions of 65/35, 80/20 and 90/10 and in solutions of acetic acid, formic acid and distilled H_2O with proportions of 60/20/20 and 60/30/10. 0.1% HCl was added as a catalyst and the suspension was stirred during 4 h at 85°C. It was then filtered on a glass filter of porosity 4 (10-16 µm). The filtrate was collected and 3 volumes of ethanol were added and left 12 h at room temperature. The residue was washed with distilled water and ethanol, dried at 60°C in a drying oven for 24 h and milled (Ika A11 basic). The precipitates were recovered by centrifugation (10,000 g, 30 min, 4°C), washed with 70% ethanol, dried under air flux and then milled (Ika A11 basic).

6.2.2.3 Analyses

Granulometry was measured in triplicate by a Mastersizer 2000 (Malvern Instruments Ltd, Worcestershire, UK).

Protein content was determined (in duplicate) by the Kjeldahl method (Kjeltec 2300, Foss) and by multiplying the N content by 5.7.

For the ashes content, samples were put at 600°C in a muffle furnace during 5 h and were then cooled in a dessicator.

The monosaccharides analysis was based on the method of Englyst and Cummings (1984), previously described in Escarnot *et al.* (2011a). The hydrolysis were performed during 3h at 100°C with TFA 2M for solids, with TFA 0.5M for liquids from chemical extraction and with H_2SO_4 1M for liquids from water extraction.

Molecular weights were determined in triplicate by High Performance Size Exclusion Chromatography (HPSEC) on a Waters 2690-HPLC system (Waters INC., Milford MA, USA), equipped with a TSKgel GMPWxl column (300 x 7.8 mm) (Tosoh Co. Ltd., Tokyo, Japan) and coupled on-line with a single detector system: a Waters 2410 differential refraction index (RI) detector. Arabinoxylan solutions were filtered through a 0.45 µm membrane filter (Millipore Co., Milford MA, USA) and 100 µL were injected in HPSEC. Elution was carried out at room temperature, at a flow rate of 0.7mL/min with 50 mM sodium nitrate (NaNO3) solution containing 0.05% sodium azide (NaN3) as preservative. Dextrans of 1000, 5000, 12 000, 25 000, 50 000, 150 000, 270 000, 410

000, 610000 g/mol (Fluka, Sigma-Aldrich, Buchs, Switzerland) were used to establish a calibration curve to calculate the MWs.

6.2.3 Results

6.2.3.1 Hull characterization

Hull contained 12.6% starch coming from the dehulling, 5.7% of lignin, 30.2% of cellulose and 35.5% of hemicelluloses (Table 6.1, Appendix Fig.A.7 and A.8). The total composition of hull was close to what is reported in literature for wheat straw (Lequart *et al.*, 1999), however hull's contents are lower for lignin and cellulose and are higher for hemicelluloses. Besides, the spelt hull is richer in non-starch polysaccharides than spelt bran. Indeed spelt bran contains 19% hemicelluloses, 2% lignin and 6% cellulose (Escarnot *et al.,* 2010).

Component	% of micronised hull*
Dry Matter	92,7
Proteins	7,0 ± 0,0
Cellulose	30,2 ± 0,1
Hemicellulose	35,5 ± 0,5
Lignin	5,7 ± 0,1
Arabinose	3,4 ± 0,0
Xylose	18,1 ± 0,3
Mannose	0,6 ± 0,0
Glucose	18,9 ± 1,5
Galactose	1,6 ± 0,0
Ashes	6,1
Starch	12,6 ± 0,6
Total proteins + fibers + ashes + starch	97,1 ± 3.1

*Granulometry

Distribution	d(0,1)	d(0,5)	d(0,9)
Particle size µm	12	109	686

Table 6.1. Composition of the micronized hull (%, d.m.) with standard deviation (SD %).

6.2.3.2 Extraction by the first method: two successive extractions with H_2O_2

The hull WE-AX fraction was poorly concentrated in AX: 12.3% and the A/X ratio was 0.55. The extraction yield (AX of the fraction/original AX of the hull) was low: 1.4%. WE-AX was composed of one population of 5.4 kDa (Table 6.2 and Fig. 6.1A).

Concerning WU-AX, EA1D (the first extraction fraction after dialysis) contained 28.6% AX with an A/X ratio of 0.45. Two AX populations were detected with MWs of 6800 and 230 000 g/mol. EA2D (the second extraction fraction after dialysis) contained 28.7% AX with an A/X ratio of 0.32. The decrease of A/X ratio between the first and the second alkali extraction was marked. Populations from

this fraction had MWs of 7800 and 275 000 g/mol. WU-AX extraction yields were respectively 10.6 and 9.8 % for each fraction.

Fraction	Hull	PWEM	WUM	AE1D	AE2D	CRR
Yield/hull	100.0	1.9	75.8	7.0	6.4	46.2
Yield/WUM	-	-	-	9.3	8.5	60.9
Ashes	6.1	6.8	6.4	7.6	6.3	9.9
Proteins	7.0 ± 0.0	18.5 ± 0.3	2.7 ± 0.1	7.6 ± 0.1	4.3 ± 0.1	0.5 ± 0.0
Total non-cellulosic sugars	42.6 ± 1.8	29.8 ± 3.4	25.1 ± 1.8	34.4 ± 0.6	33.8 ± 2.9	15.2 ± 0.8
Arabinose	3.4 ± 0.0	7.4 ± 0.6	3.6 ± 0.2	10.1 ± 0.1	7.9 ± 0.7	2.0 ± 0.1
Xylose	18.1 ± 0.3	9.0 ± 0.4	20.7 ± 1.3	22.4 ± 0.2	24.7 ± 2.1	13.1 ± 0.6
Mannose	0.6 ± 0.0	3.3 ± 0.3	0.0 ± 0.0	0.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0
Glucose	18.9 ± 1.5	6.4 ± 1.3	0.2 ± 0.3	0.3 ± 0.1	0.2 ± 0.0	0.0 ± 0.0
Galactose	1.6 ± 0.0	3.5 ± 0.8	0.6 ± 0.0	1.5 ± 0.1	0.9 ± 0.1	0.1 ± 0.1
Starch	10.9 ± 0.6	0.1	0.1	-	-	-
Total	55.6 ± 2.4	55.1 ± 3.7	34.2 ± 1.9	49.6 ± 0.7	44.4 ± 3.0	25.6 ± 0.8
AX = (0,88(%ara+%xyl-0,7%gal))	17.9	12.3	-	-	-	-
AX = (0,88(%ara+%xyl))	-	-	21.3	28.6	28.7	13.3
A/X (%ara-0,7%gal)/%xyl	0.1	0.5	-	-	-	-
A/X	-	-	0.2	0.4	0.3	0.1
Yield* of extraction	-	1.4	-	10.6	9.8	32.6

* AX fraction/AX hull

Table 6.2. Composition (%, d.m.) of the different fractions extracted with the first method, A/X ratios and yields of extraction.

6.2.3.3 Extraction by the second method: one extraction with H_2O_2

The composition of the liquid fractions obtained after 4h and 8h of extraction was similar (Table 6.3, Appendix Fig.A.9 and A.10). AX content of the four filtrates varied between 7.3 and 10.1%. A/X ratios were 0.33 for the filtrates of 4h and 8h of reaction and 0.41 for the filtrates of 16 and 24h of reaction. Short extractions resulted in a slightly higher AX content and less substituted AX. Ashes contents were very high for all filtrates: from 45 to 50% of the total material. Concerning the extraction efficiency (filtrate AX/hull AX), percentages were close for most of the filtrates, from 20.5 to 22.1% except for the filtrate from 16h of reaction: 15.6%. MWs of the two main populations of AXs were similar for the four filtrates, i.e. 918-976 g/mol and 5100-6200 g/mol (Fig. 6.1B).

Concerning the precipitates, AX contents varied from 26.1 to 33.5% and A/X ratios from 0.25 to 0.42. The AX content did not increase with the volume of ethanol neither the A/X ratio. The richest fractions in AX were obtained with an extraction time of 8 h and they also had the highest A/X ratios. AX populations were very broad; one low MW population could be distinguished and two other populations with higher MWs of 8800-14000 g/mol and 257000-367000 g/mol (depending on the extraction time and the volume of ethanol) (Fig. 6.1C). Three volumes of ethanol gave higher MWs than 4 volumes. Whatever the volume of ethanol and the population, the highest MWs were obtained after 8 h of extraction. Ash content was again high however lower than in filtrates, from 17.5% to 41.3%.

Concerning the extraction efficiency, the yields varied from 17.9 to 20.6% for the majority of the fractions, the highest being obtained for the 24h-extraction precipitate, with 3 volumes of ethanol (23.9%) and for the 24h-precipitate with 4 volumes of ethanol (25.4%). This last contained 31.7% AX with an A/X ratio of 0.36 and with two main populations of 9200 and 273000 g/mol. The precipitates from the 8h-extraction had low ash content, high AX content, high A/X ratio and high MW, turning them the most interesting fractions.

Concerning the residues, their AX content varied from 20.7 to 24.9% and the AX chains were very lowly substituted, since A/X ratios varied from 0.18 to 0.21. Residues contained between 55.9 and 63.8% of the original total AX of the hull, indicating that only part of the AX was extracted.

Fraction	Duration of extraction	Arabinose	Xylose	Mannose	Glucose	Galactose	Proteins	Ashes	AX*	A/X	Yield** of extraction
Hull	-	3.4 ± 0.0	18.1 ± 0.3	0.6 ± 0.0	18.9 ± 1.5	1.6 ± 0.0	7.0 ± 0.0	6.1	18.9	0.19	-
Filtrate	4h	2.7 ± 0.0	8.7 ± 0.3	0.1 ± 0.0	0.6 ± 0.0	0.7 ± 0.0	4.8 ± 0.0	50.0	10.1	0.32	21.3
	8h	2.8 ± 0.0	8.3 ± 0.5	0.1 ± 0.0	0.4 ± 0.0	0.5 ± 0.0	4.4 ± 0.2	45.6	9.8	0.34	22.1
	16h	2.4 ± 0.1	5.8 ± 0.2	0.1 ± 0.0	0.4 ± 0.0	0.4 ± 0.0	5.1 ± 0.2	45.9	7.3	0.42	15.6
	24h	2.9 ± 0.0	6.9 ± 0.1	0.0 ± 0.0	0.4 ± 0.0	0.5 ± 0.0	4.6 ± 0.3	49.4	8.6	0.41	20.5
Precipitate	4h-3v	9.9 ± 0.3	25.1 ± 0.4	0.4 ± 0.0	1.6 ± 0.1	2.9 ± 0.2	5.8 ± 0.0	31.1	30.7	0.39	19.0
	4h-4v	9.2 ± 0.5	23.1 ± 1.6	0.4 ± 0.1	1.5 ± 0.2	2.8 ± 0.4	6.3 ± 0.0	33.2	28.4	0.40	19.3
	8h-3v	11.3 ± 0.9	26.8 ± 1.9	0.5 ± 0.1	1.5 ± 0.1	3.3 ± 0.2	6.5 ± 0.1	17.5	33.5	0.42	18.1
	8h-4v	11.2 ± 1.0	26.1 ± 1.9	0.4 ± 0.0	1.3 ± 0.2	2.8 ± 0.3	6.6 ± 0.0	21.6	32.8	0.43	20.6
	16h-3v	8.4 ± 0.1	22.7 ± 0.6	0.1 ± 0.1	0.5 ± 0.0	1.3 ± 0.1	6.6 ± 0.1	41.4	27.3	0.37	17.9
	16h-4v	7.9 ± 0.4	21.7 ± 0.8	0.0 ± 0.0	0.5 ± 0.1	1.2 ± 0.1	6.8 ± 0.0	38.3	26.1	0.36	18.7
	24h-3v	10.2 ± 0.4	28.0 ± 1.1	0.0 ± 0.0	0.5 ± 0.0	1.5 ± 0.0	5.5 ± 0.0	33.8	33.7	0.37	23.9
	24h-4v	9.5 ± 0.1	26.5 ± 0.2	0.0 ± 0.0	0.6 ± 0.1	1.5 ± 0.1	5.9 ± 0.0	35.6	31.7	0.36	25.4
Residue	4h	3.6 ± 0.1	19.8 ± 1.0	0.1 ± 0.1	0.9 ± 0.1	0.8 ± 0.0	0.7 ± 0.0	2.3	20.7	0.18	55.9
	8h	4.9 ± 0.2	23.4 ± 2.2	0.0 ± 0.0	0.9 ± 0.2	1.0 ± 0.1	0.6 ± 0.0	2.3	24.9	0.21	63.8
	16h	4.4 ± 0.3	21.0 ± 1.3	0.0 ± 0.0	0.9 ± 0.1	0.9 ± 0.1	0.7 ± 0.0	2.0	22.3	0.21	62.2
	24h	4.3 ± 0.3	21.0 ± 2.5	0.0 ± 0.0	0.9 ± 0.1	0.8 ± 0.0	0.6 ± 0.0	2.1	22.3	0.21	59.1

*AX = (0,88(%ara+%xyl))

**AX fraction/AX hull

v: volume of ethanol for precipitation

Table 6.3. Composition (%, d.m.) of the different fractions extracted with the second method, A/X ratios and yields of extraction.

6.2.3.4 Extraction by the third method: successive NaOH and $NaClO_2$-KOH extractions

NaOH-extraction filtrates contained between 7.2 and 8.2 % of AX with A/X ratios between 0.14 and 0.20 (Table 6.4, Appendix Fig.A.11 and A.12). The longest extraction time, 16 h, yielded the richest filtrate in AX with the highest A/X ratio. NaOH-extraction filtrates had much lower A/X ratios than H_2O_2-extraction filtrates even with the shortest extraction time (2 h) respectively 0.17 and 0.37. The KOH-extraction filtrates contained between 3.5 and 5 % of AX with a very low A/X ratio around 0.11, whatever the extraction time. Concerning AX extraction yield, it varied between 45.6 and 49.6% for NaOH filtrates, and then between 25 and 37.5% for KOH filtrates; the highest corresponding to 5h and 2h of extraction.

For NaOH precipitates, AX content ranged from 26.7 to 33.3% and A/X ratios from 0.17 to 0.19. In the case of the 2 h-extraction, the use of 4 instead of 3 volumes of ethanol enabled to increase the extraction yield by 47% which was not observed for other durations. Here the precipitation of NaOH filtrates enabled to increase the A/X ratio of 0.04 which means that some xylose units were not recovered through precipitation.

Residues contained between 3.3 and 4.3 % of AX, which were more substituted (A/X of 0.25) than the extracted AX. In other words, between 5.8 and 7.6% of the original AX remained in the hull, indicating a very efficient extraction.

All NaOH and KOH filtrates gave one peak on the HPSEC chromatogram corresponding to a small compound with MW around 880-900 g/mol (Fig. 6.1D). All chromatograms of NaOH precipitates had the same shape with three populations whatever the extraction duration: one of 1000 g/mol in average, the second of 8700-10500 g/mol and the third of 99000-188000 g/mol (depending on the extraction time and the volume of ethanol). The highest MWs (10400 and 188000 g/mol) were obtained with an extraction of 5h and precipitation with 3 volumes of ethanol. The KOH precipitates had only one population with an average of 933 g/mol for all extraction times.

Fraction	Duration of extraction	Arabinose	Xylose	Mannose	Glucose	Galactose	Proteins	Ashes	AX*	A/X	Yield** of extraction
Hull	-	3.4 ± 0.0	18.1 ± 0.3	0.6 ± 0.0	18.9 ± 1.5	1.6 ± 0.0	7.0 ± 0.0	6.1	18.9	0.19	-
Filtrate NaOH	2h	1.1 ± 0.1	7.1 ± 0.3	0.1 ± 0.1	0.5 ± 0.1	0.4 ± 0.1	1.1 ± 0.0	79.9	7.2	0.15	45.6
	5h	1.2 ± 0.0	8.1 ± 0.4	0.0 ± 0.0	0.5 ± 0.0	0.4 ± 0.0	1.3 ± 0.0	77.3	8.1	0.14	49.6
	16h	1.6 ± 0.1	7.8 ± 1.4	0.1 ± 0.1	0.5 ± 0.0	0.5 ± 0.1	1.5 ± 0.0	73.5	8.2	0.20	48.1
Filtrate KOH	2h	0.6 ± 0.0	5.8 ± 0.3	0.0 ± 0.0	0.3 ± 0.0	0.1 ± 0.1	0.1 ± 0.0	90.6	5.6	0.11	37.5
	5h	0.6 ± 0.1	5.1 ± 0.3	0.0 ± 0.0	0.3 ± 0.0	0.1 ± 0.1	0.1 ± 0.0	91.7	5.0	0.11	33.2
	16h	0.4 ± 0.0	3.6 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	93.2	3.5	0.12	25.0
Precipitate NaOH	2h-3v	4.9 ± 0.2	25.5 ± 1.1	0.4 ± 0.1	1.9 ± 0.1	2.0 ± 0.1	3.0 ± 0.1	27.9	26.7	0.19	24.7
	2h-4v	6.0 ± 0.6	31.9 ± 1.0	0.4 ± 0.0	2.1 ± 0.1	2.3 ± 0.1	3.4 ± 0.0	30.0	33.3	0.19	36.3
	5h-3v	5.5 ± 0.1	32.4 ± 0.1	0.4 ± 0.1	2.0 ± 0.1	2.3 ± 0.1	2.9 ± 0.0	29.0	33.4	0.17	33.7
	5h-4v	6.0 ± 0.5	31.9 ± 2.4	0.4 ± 0.0	2.2 ± 0.2	2.5 ± 0.2	3.3 ± 0.0	27.4	33.3	0.19	33.1
	16h-3v	5.4 ± 0.4	29.7 ± 1.3	0.7 ± 0.4	2.0 ± 0.2	2.3 ± 0.2	3.2 ± 0.1	35.7	30.8	0.18	41.9
	16h-4v	5.5 ± 0.2	31.5 ± 0.1	0.8 ± 0.7	2.2 ± 0.2	2.4 ± 0.2	0.1 ± 0.0	30.7	32.6	0.17	39.5
Residue	2h	0.9 ± 0.0	3.4 ± 0.2	0.0 ± 0.0	2.5 ± 0.2	0.5 ± 0.0	0.3 ± 0.0	13.6	3.8	0.25	6.6
	5h	1.0 ± 0.1	3.9 ± 0.2	0.1 ± 0.2	2.9 ± 0.1	0.6 ± 0.1	0.3 ± 0.0	16.3	4.3	0.26	7.6
	16h	0.8 ± 0.0	3.0 ± 0.1	0.1 ± 0.1	2.6 ± 0.3	0.5 ± 0.0	0.3 ± 0.1	13.2	3.3	0.25	5.8

*AX = (0,88(%ara+%xyl))

**AX fraction/AX hull

v: volume of ethanol for precipitation

Table 6.4. Composition (%, d.m.) of the different fractions extracted with the third method, A/X ratios and yields of extraction.

6.2.3.5 Extraction by the fourth method: acidic extractions

Concerning the filtrates, AX extraction yields increased with the concentration of acetic acid, respectively, 10.9, 24.7 and 39.1%. Formic acid increased the extraction yields to 48.5 and 64.2%. Concerning the precipitates, the maximum AX extraction yield was obtained with 90% acetic acid and 10% H_2O and it rose with the concentration of acetic acid, from 0.9 to 5.1% (Table 6.5, Appendix Fig.A.13 and A.14). Extraction yields with acetic and formic acids were lower, 4.0 and 0.3%. A higher solubilization of hemicelluloses was observed with acetic and formic acids than with acetic acid alone however it was associated with the inability to recover the hemicelluloses through the precipitation with 3 volumes of ethanol.

In filtrates, xylose content increased with the concentration in acetic acid and formic acid. In precipitates, xylose content increased with the concentration in acetic acid and decreased with the one of formic acid. Glucose content varied between 4.0 and 9.2% in filtrates and decreased with the concentration of acetic and formic acids. In precipitates, glucose content decreased with the concentration of acetic acid but it increased drastically with the concentration of formic acid, 13.5%. The precipitation of filtrates extracted with the blend acetic acid/formic acid/H_2O, decreased the concentration of AX.

In precipitates, A/X ratios varied from 0.05 to 0.19 and in filtrates from 0.18 to 0.95. Precipitation of extracts from acetic acid provoked a loss of arabinose, while it was much less important from the blend formic and acetic acids. Indeed, A/X ratios of filtrates with acetic acid alone were high (0.28 and 0.95), they decreased with acetic acid concentration and were of 0.18-0.24 from the blend formic and acetic acids.

Extracted hemicelluloses had low MW and could be splitted into 3 populations, the first population had a MW of 9400-11300 g/mol with acetic acid alone (depending on the amount of acetic acid) and 5300-7200 g/mol for formic and acetic acids (depending on the used proportions). The second and third populations had MWs of 1300-1500 g/mol and 280 g/mol respectively (Fig. 6.1E).

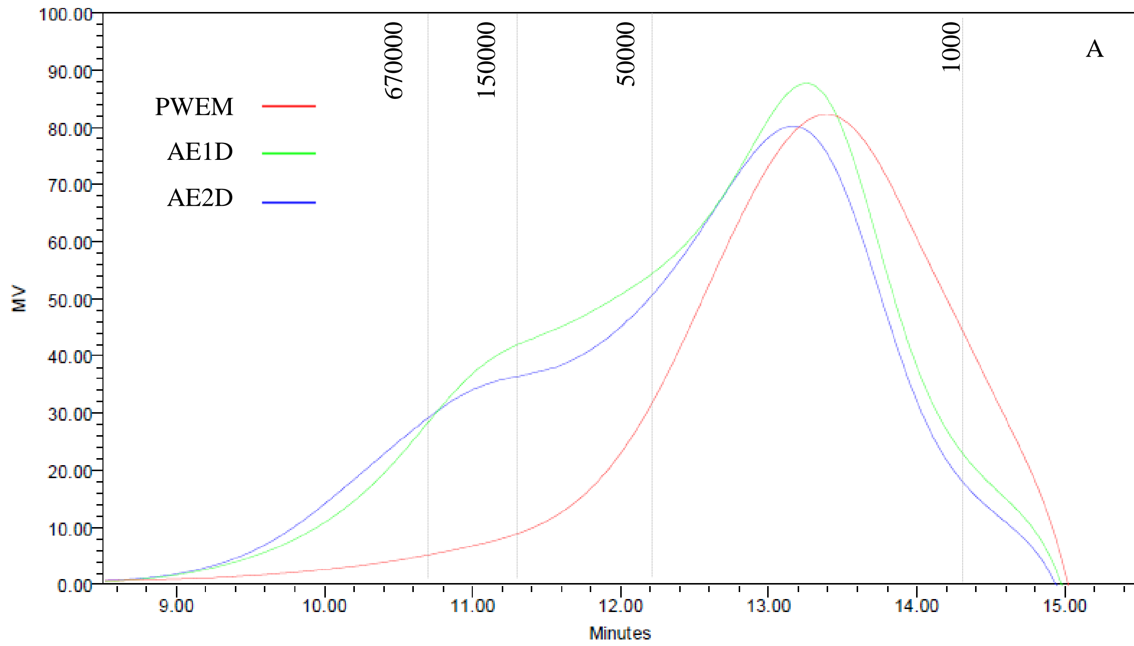
Fraction	Experiment number	Rhamnose	Arabinose	Xylose	Mannose	Glucose	Galactose	AX*	A/X	Yield** of extraction
Hull		0.1 ± 0.1	3.4 ± 0.0	18.1 ± 0.3	0.6 ± 0.0	18.9 ± 1.5	1.6 ± 0.0	18.9	0.19	-
Precipitate	1	0.3 ± 0.0	2.8 ± 0.0	24.6 ± 0.6	1.6 ± 0.0	4.7 ± 0.1	4.1 ± 0.0	24.1	0.11	0.9
	2	0.0 ± 0.0	2.5 ± 0.1	28.5 ± 1.0	0.8 ± 0.0	3.0 ± 0.1	2.3 ± 0.1	27.3	0.09	1.5
	3	0.0 ± 0.0	2.3 ± 0.1	42.2 ± 1.7	0.4 ± 0.0	1.5 ± 0.1	1.2 ± 0.1	39.2	0.05	5.1
	4	0.0 ± 0.0	3.1 ± 0.1	35.8 ± 1.6	0.5 ± 0.0	3.3 ± 0.1	2.4 ± 0.0	34.2	0.09	4.0
	5	0.0 ± 0.0	1.8 ± 0.0	9.4 ± 0.3	0.7 ± 0.0	13.5 ± 0.4	2.6 ± 0.1	9.9	0.19	0.3
Residue	1	0.0 ± 0.0	2.4 ± 0.1	22.1 ± 0.5	0.0 ± 0.0	1.1 ± 0.1	1.1 ± 0.0	21.6	0.11	73.6
	2	0.0 ± 0.0	1.8 ± 0.1	19.3 ± 0.5	0.0 ± 0.0	1.0 ± 0.1	0.9 ± 0.0	18.6	0.09	61.8
	3	0.0 ± 0.0	1.4 ± 0.0	17.5 ± 0.5	0.1 ± 0.1	1.2 ± 0.0	0.8 ± 0.0	16.6	0.08	51.6
	4	0.0 ± 0.0	1.1 ± 0.0	13.8 ± 0.5	0.1 ± 0.1	1.4 ± 0.1	0.6 ± 0.0	13.1	0.08	34.6
	5	0.0 ± 0.0	0.5 ± 0.0	7.2 ± 0.2	0.0 ± 0.0	1.3 ± 0.0	0.3 ± 0.0	6.8	0.07	15.1
Filtrate	1	0.0 ± 0.0	11.5 ± 0.0	12.1 ± 0.2	0.6 ± 0.0	9.2 ± 0.4	3.2 ± 0.0	20.7	0.95	10.9
	2	0.0 ± 0.0	11.6 ± 0.0	26.2 ± 0.2	0.3 ± 0.2	6.1 ± 0.1	3.6 ± 0.1	33.2	0.44	24.7
	3	0.0 ± 0.0	8.2 ± 0.2	29.2 ± 0.7	0.6 ± 0.1	4.0 ± 0.2	3.2 ± 0.1	32.9	0.28	39.1
	4	1.2 ± 0.2	9.2 ± 0.2	38.3 ± 0.9	0.5 ± 0.3	7.5 ± 0.4	3.3 ± 0.2	41.8	0.24	48.5
	5	1.7 ± 0.1	7.4 ± 0.2	41.2 ± 0.8	0.5 ± 0.2	4.0 ± 0.2	2.8 ± 0.1	42.8	0.18	64.2
Residue	1	0.0 ± 0.0	2.3 ± 0.1	21.8 ± 0.8	0.2 ± 0.0	1.3 ± 0.1	1.2 ± 0.1	21.2	0.10	71.5
	2	0.0 ± 0.0	1.5 ± 0.1	18.9 ± 0.7	0.1 ± 0.1	1.3 ± 0.0	0.9 ± 0.0	18.0	0.08	55.4
	3	0.0 ± 0.0	0.7 ± 0.0	10.2 ± 0.1	0.0 ± 0.0	1.4 ± 0.0	0.4 ± 0.0	9.5	0.07	24.4
	4	0.0 ± 0.0	1.2 ± 0.1	13.8 ± 0.2	0.1 ± 0.1	1.4 ± 0.0	0.7 ± 0.0	13.2	0.09	34.7
	5	0.0 ± 0.0	0.5 ± 0.0	7.3 ± 0.0	0.0 ± 0.0	1.4 ± 0.0	0.3 ± 0.0	6.9	0.07	15.1

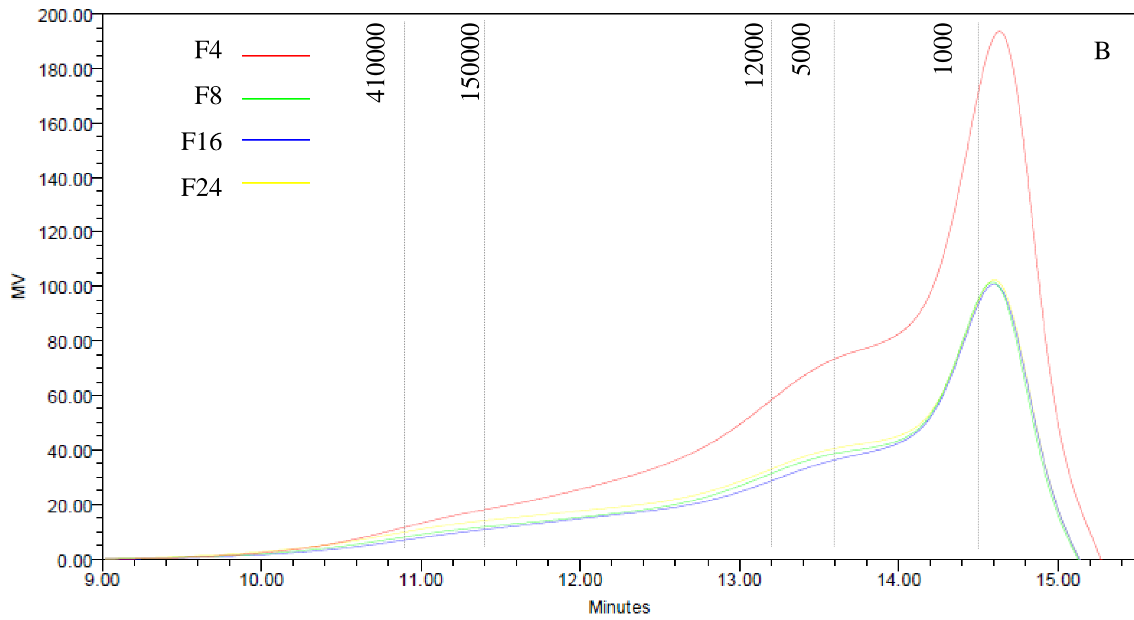
*AX = (0,88(%ara+%xyl))

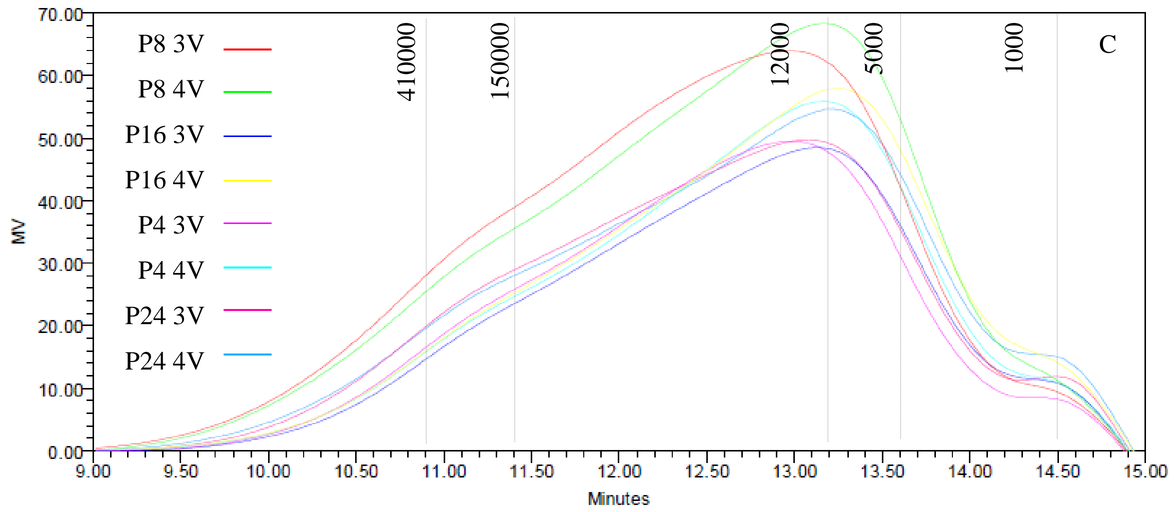
**AX fraction/AX hull

1: 65% acetic acid and 35% H_2O; 2: 80 % acetic acid and 20% H_2O; 3: 90 % acetic acid and 10% H_2O; 4: 60 % acetic acid, 20% formic acid and 20% H_2O; 5: 60 % acetic acid, 30% formic acid and 10% H_2O

Table 6.5. Composition (%, d.m.) of the different fractions extracted with the fourth method, A/X ratios and yields of extraction.


A
PWEM
AE1D
AE2D
670000
150000
50000
1000
MV
Minutes


B
F4
F8
F16
F24
410000
150000
12000
5000
1000
MV
Minutes

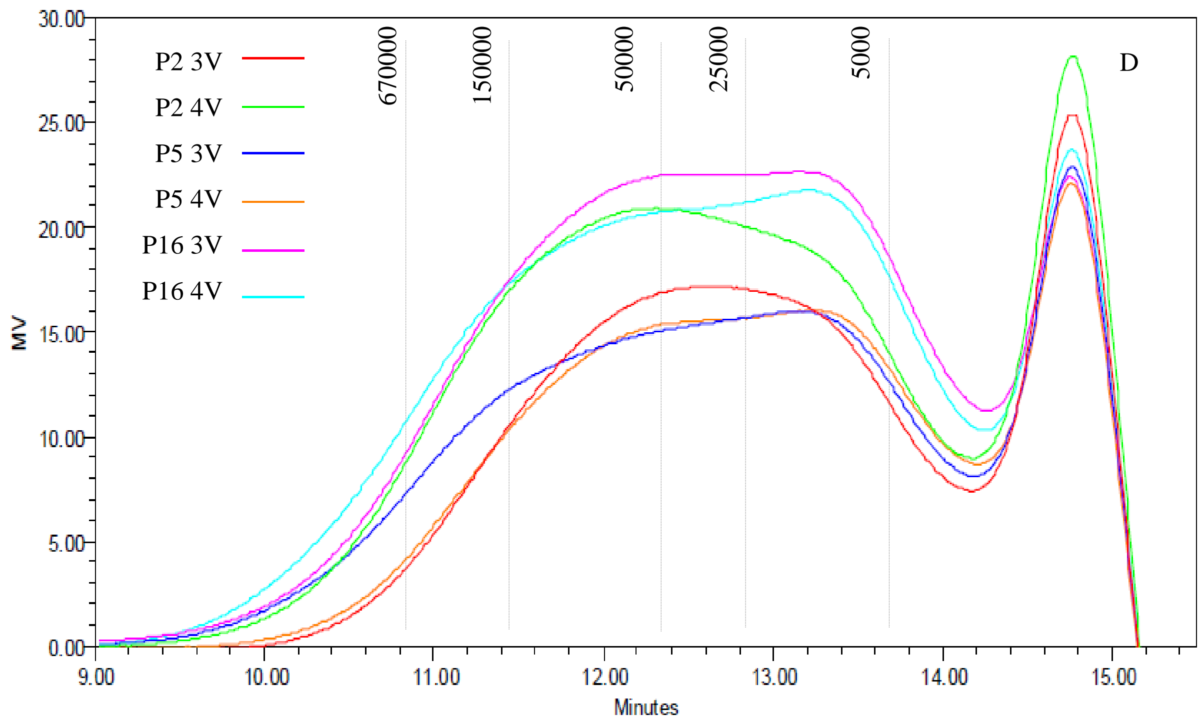


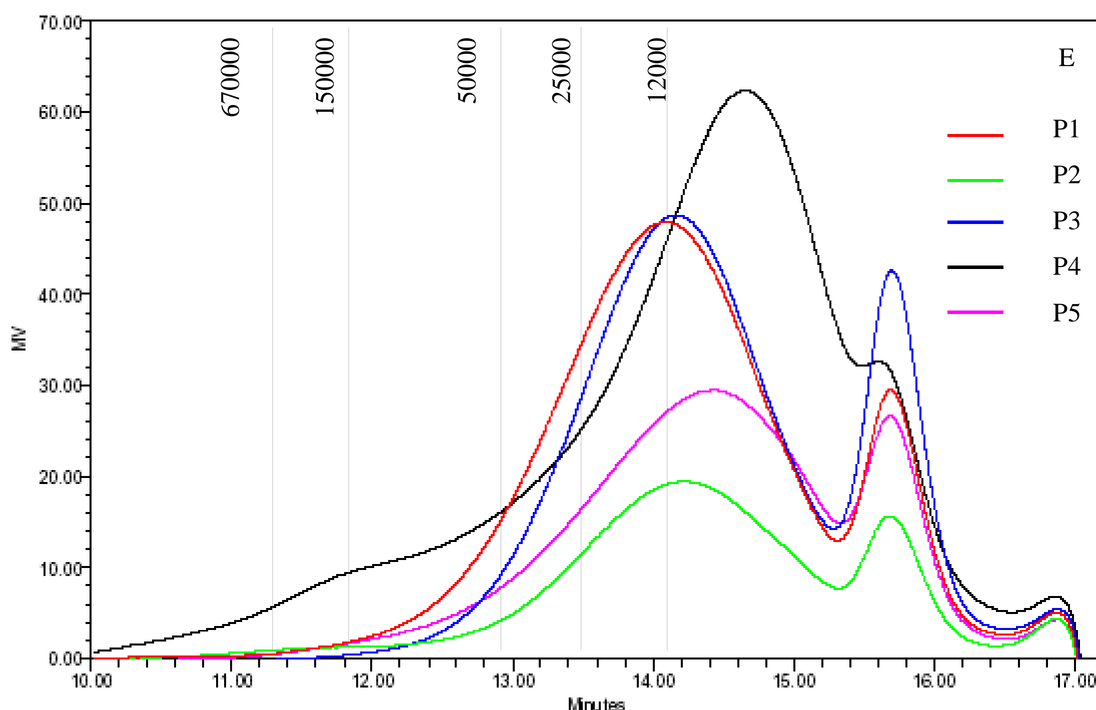
F4/8/16/24: filtrate 4, 8, 16 and 24h of reaction

P2/4/5/8/16/24: precipitate 2, 4, 5, 8, 16 and 24h of reaction

3V: precipitation with 3 volumes of ethanol

4V: precipitation with 4 volumes of ethanol





P1: precipitate from 65% acetic acid and 35% H_2O

P2: precipitate from 80% acetic acid and 20% H_2O

P3: precipitate from 90% acetic acid and 10% H_2O

P4: precipitate from 60% acetic acid, 20% formic acid and 20% H_2O

P5: precipitate from 60% acetic acid, 30% formic acid and 10% H_2O

Figure 6.1. HPSEC chromatographic profiles of (A) PWEM, AE1D and AE2D from the first method; (B) the four filtrates from the second method; (C) the eight precipitates from the second method; (D) the two precipitates from the third method; (E) the five precipitates from the fourth method. Standard dextrans MW values in g/mol are indicated in grey on the corresponding vertical lines.

6.2.4 Discussion

With both methods of extraction using H_2O_2, the AX contents of the different extraction fractions were close (around 30%) and the extraction yields were similar. Concerning the liquid fractions of each experiment, the successive extractions of the first method enabled to get AX with higher MWs than those of the second method. In the second method, precipitation enabled to get higher MWs than those of the first method. A/X ratios were similar, depending on the time of extraction and the fraction considered. In the case of the filtrates, the longer the extraction time the higher the A/X ratio which means that more and more arabinose was released from the substrate. In the case of the precipitates, the contrary was observed: the longer the extraction time the lower the A/X ratio, indicating that part of the arabinose could not be precipitated as it might have been cut from the chain of xylan and might be under a monomeric or oligomeric form. The high yield and high MW of the AX can be explained

by the use of alkaline peroxide processes which are efficient for delignification and removal of hemicelluloses (Fang *et al.,* 1999; Sun *et al.,* 2004b).

The method with NaOH enabled to obtain higher extraction yields but less substituted AX and polysaccharides with lower MWs. The longest extraction with H_2O_2 (24 h) did not result in AX degradation, while a short extraction with NaOH was sufficient to provoke a disruption of the polymers and to a greater extent with $NaClO_2$-KOH, which yielded only compounds with low MW (1000 g/mol in average). The original amount of arabinose was not found in the filtrate, neither in the precipitate and residue, consequently it seemed rapidly degraded by the treatment with $NaClO_2$-KOH, and precipitation managed to recover only a weak part of the arabinose in the hemicellulosic fraction. Alkali metal hydroxide can induce the intracrystalline swelling of cellulose and a widening of the lattice dimensions or even a crystal lattice conversion but no dissolution, and KOH causes less swelling than NaOH (Nevell and Zeronian, 1985 in Wertz *et al.,* 2010). Furthermore, $NaClO_2$ causes oxidation of cellulose, randomly in the accessible regions of the fibers, and the degradation may occur simultaneously (Lewin and Epstein, 1962 in Wertz *et al.,* 2010). It can be hypothesized that the changes in the configuration of the cellulose such as it could have happened in this experiment facilitated the liberation of hemicelluloses. This could also explain the higher yields than with the H_2O_2 methods, however degradation of hemicelluloses occurred. It should be mentioned that in spite of the environmental concern, chlorite was experimented to resolve the problem of coloration due to lignin (5-10%) and to phenolic compounds (Shin *et al.,* 2001).

The organic acids used in the fourth method, associated with a temperature much higher than that of the other methods, lead to a considerable degradation of the polymers. Concerning filtrates, higher yields than with all other methods were obtained when formic acid was used. For similar yields, A/X ratios were higher with acetic acid showing that arabinose would be less degraded than with other chemicals (NaOH, $NaClO_2$, KOH and formic acid). Concerning the precipitates, as explained before, precipitation was impeded by the small size of the molecules. As reported by Wertz *et al.* (2010), formic acid in aqueous solution of suitable concentration can cause intercrystalline and intracrystalline swelling and dissolution of cellulose. Moreover, in wheat straw, the linkages between lignin and hemicelluloses and cellulose are relatively strong and can only be cleaved in acidic media (Sun *et al.,* 2004a). Such a cleavage could have happened in this experiment, liberating the hemicelluloses with a high yield. The degradation of hemicelluloses led to oligomers with very low MW, this has also been observed on commercial straw pulping where inorganic agents achieve high cellulose extraction at the expense of the hemicellulosic fraction which undergoes hydrolysis and degradation (Sun *et al.,* 2004a).

The advantage of the first method was the splitting into WE- and WU-AX, the short extraction time (maximum 8 h) and the preservation of high MWs while the other H_2O_2 methods required 24 h.

Concerning yields of AX recovery, the methods with NaOH and $NaClO_2$-KOH were the most efficient. Organic and inorganic acids caused too much degradation to enable a good recovery of the hemicelluloses. It should be highlighted that fractions coming from the last two methods without dialysis had high ash contents, thus a demineralisation would be necessary in order to get a higher AX purity.

The quantity and quality of AXs obtained here from spelt hull can be compared to literature data on fractions obtained from other cereal sources such as wheat straw, wheat bran, spelt bran and different hull and husk (Table 6.6). Sun *et al.* (2000) managed to extract from 77 to 91% of the hemicelluloses from wheat straw while with the same method here, AX extraction yields from hull varied from 17.9 to 25.4%; such a discrepancy may be due to different ligno-cellulosic structures, and different amounts of minor sugars (glucose, rhamnose, mannose, galactose). However MWs ranged from 24000 g/mol to 31000 g/mol for straw while MWs of hull AX were higher, from 8700 to 370000 g/mol. In Sun *et al.*'s study (1998), the fraction extracted with NaOH represented from 32.7 to 41.5% of the dewaxed wheat straw after 2 to 15h of reaction, while it was here between 24.0 and 29.2% for the destarched hull. Also, the final straw residue (after NaOH and $NaClO_2$-KOH extractions) represented 38.0-39.9% of the dewaxed straw while it made up here 40.6 to 43.3% of the destarched hull (not dewaxed). MWs of the compounds extracted with NaOH from straw ranged from 26000 to 32000 g/mol and those obtained from $NaClO_2$-KOH extraction from 60000 to 62000 g/mol. This was very different from the profile of the populations of hull which respectively ranged from 8700 to 188000 g/mol with NaOH extraction and 930 g/mol with $NaClO_2$-KOH extraction. In the case of straw, the extraction with $NaClO_2$-KOH resulted in higher MWs than with NaOH, i.e. the contrary of what happened with hull. Xu *et al.* (2006a) dissolved 42.4-76.5% of the hemicelluloses from wheat straw with organic acids, with MWs between 10000 and 16100 g/mol. These figures are far from those obtained for spelt hull in the same conditions, with the highest yield of 5.1% and MW of 10300 g/mol. Whatever the conditions, organic acids led to very divergent results between wheat straw and spelt hull (Table 6.6).

Reference	Material	Extraction yield (AX%)	MW of AX (g/mol)	Method
Results from present work	spelt hull	41.9%	9300 and 177000	NaOH; 16h; 45°C
		20.4%	6800-7800 and 230000-275000	H_2O_2 2%; pH 11.5; 4h twice; 60°C
		25.4%	9200 and 273000	H_2O_2 2%; pH 11.5 ; 24h; 50°C
		5.1%	283; 1300 and 10300	90% acetic acid - 10% H_2O; 4h; 85°C
		4.0%	288; 1500 and 5300	60% formic/20%acetic acid/20% H_2O; 4h; 85°C
Hromadkova *et al.,* 2003	buckwheat hull	16.5%	61 and 3600	NaOH 5%; 60°C; 10 min
Hoije *et al.* , 2005	barley husk	57%	39100	HCl 0.05M; 16h then acetic acid with NaCLO2; 2h; 75°C then NaOH 1M + 0.5% $NaBH_4$; 16h
Roos *et al.* , 2009	barley husk	9%	52000	microwave irradiation 5 min; 200°C; NaOH 0.5%; pH 12.3 + steam
	barley husk	50%	13000	microwave irradiation 5 min; 210°C; pH 6.5 + steam
Jin *et al.* , 2009	barley straw	94.6%	14600-28840	neutral dioxane 90%; 4h; 80°C then acidic dioxane 80%; 4h; 70°C then DMSO; 4h; 80°C then KOH 8%; 4h; 50°C
Sun *et al* ., 1998	wheat straw	32.7-41.5%	27740 to 31810	NaOH 3%; 45°C; 2-15h
		4.5-9.8%	60200-62260	KOH 10%; 25°C; 16h
Sun *et al.,* 2000	wheat straw	91%	28800	H_2O_2 2%; 50°C; 16h; pH 11.5
		90.0%	30700	H_2O_2 2%; 50°C; 12h; pH 11.5
Persson *et al.* , 2009	wheat straw	47%	10000	steam pretreatment in alkaline environment NaOH 5%; 30 min; 190°C; pH 11.1
		40%	20000	steam pretreatment in alkaline environment NaOH 2%; 30 min; 190°C; pH 8.8
Thomsen *et al.* , 2008	wheat straw	83%	-	hydrothermic treatment: 80°C pre-soaking then 170-180°C for hemicelluloses and 195°C for cellulose
Sun *et al.* , 2002	wheat straw	53.4%	32520	methanol 60% and 0.5M NaOH; 60°C; 2.5h; without sonication + H_2O_2 2% and tetraacetylethylenediamine 0.2%; pH 11.8; 12h; 48°C
		44.5-50.8%	28400-31160	methanol 60% and 0.5M NaOH; 60°C; 2.5h; with 5-35 min sonication + H_2O_2 2% and tetraacetylethylenediamine 0.2%; pH 11.8; 12h; 48°C
Sun *et al.* , 2005a	wheat straw	32.3-37.5%	42710 and 44080	aqueous alcohol pre-treated wheat straw; 4h; 85°C then H_2O_2 1,8%; cyanamide 0.18%; 4h; 50°C; pH 10
		19.6-45%	12980 to 15950	organic acid pre-treated straw; 4h; 85°C then H_2O_2 1,8%; cyanamide 0.18%; 4h; 50°C; pH 10
		45%	15950	organic acid pre-treated straw; 4h; 85°C then H_2O_2 1,8%; cyanamide 0.18%; 4h; 50°C; pH 10
Xu *et al.* , 2006a	wheat straw	42.4-70%	8500-16100	acetic acid-H_2O; 4h; 85°C
		65.1-76.5%	10000-12100	formic acid-acetic acid-H_2O; 4h; 85°C

Table 6.6. Synthesis of the results from the present work and literature data on hemicelluloses and AXs extraction from hull, straw and husk.

Hoije *et al.* (2005) extracted hemicelluloses from barley husks and obtained fractions with 50-83% of AX. This high purity was facilitated by the chlorite which removed proteins and starch. Extraction yields ranged from 25.1 to 57% and MWs from 35000 to 45000 g/mol. Yields were lower for the spelt hull extraction even with NaOH (41.9% for 16 h) but MWs were higher, from 9400 to 178000 g/mol. Another example comes from Hromadkova *et al.* (2003) who extracted hemicelluloses from buckwheat hulls with maximum yield of 16.5% and MWs of 61 and 3600 g/mol respectively. It should be highlighted that Roos *et al.* (2009) also worked on barley husks and managed to extract hemicelluloses of 40000 g/mol with a yield of 8.6%, interestingly without chemicals (water, heat treatment, microwave irradiation).

Different applications for the obtained hemicellulosic fractions could be considered, depending on their MWs. High MW xylans may be used as adhesives and emulsifiers (Doner and Hicks, 1997). Xylans from cereal straw can also be used as gel-forming or thermoplastic materials, as fillers for polypropylene, as a component for paint formulation and as a coating for cellulosic fibers (Xu *et al.,* 2006b). Endoxylanases can yield arabino-xylo-oligosaccharides from AX, which present interesting properties as prebiotics (Courtin *et al.,* 2009). Monomeric xylose can be further fermented into ethanol (Hahn-Hägerdal *et al.,* 2007) or transformed into xylitol (Granström *et al.,* 2007) which has many industrial applications for instance in medical fields (Xu *et al.,* 2006b).

6.2.5 Conclusion

The MW of the AXs extracted from spelt hull varied a lot depending on the conditions of extraction. The most efficient extraction of AXs from spelt hull was obtained with the method using NaOH and $NaClO_2$-KOH during 16 h and precipitation with 3 volumes of ethanol, which yielded 41.9% of the total AX. The AX fraction obtained through these conditions had an A/X ratio of 0.17 and two main populations with MWs of 9300 and 177000 g/mol. However, this method also led to the highest degradation of the polysaccharides. In order to obtain higher MWs, the method with 2% H_2O_2 at 50°C during 8h and 3 volumes of ethanol was the most adapted: it produced a fraction with two main AX populations of 14000 and 367000 g/mol with an extraction yield of 18.1%.

The tested methods offered diverse results with regard to the extraction yields, the MWs, the A/X ratios; therefore it may be possible to select the most adapted method for a chosen result. It may also be possible to further adapt and/or combine the methods. At this point, physical methods should also be explored and eventually combine with the chemical ones. Some perspectives of this work consist in focusing on the techno-functional properties of the hemicelluloses extracts in order to define potential applications.

6.2.6 Acknowledgments

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6.2.7 References

Asp H.G., Bjorck I ., Nyman M., 1993. Physiological effect of cereal dietary fibre. *Carbohydrate Polymers*, 21, 183-187.

Baghurst P.A., Baghurst K.I., Record S.J., 1996. Dietary fibre, non-starch polysaccharides and resistant starch- a review. *Food Australia*, 48, S3-S35.

Cai Z.-S., and Paszner, L., 1988. Salt catalysed wood bonding with hemicellulose. *Holzforschung*, 42, 11-20.

Chesson A., 1995. Dietary fiber. In A. Stephen, Food polysaccharides and their applications, pp 547-551. New-York, Marcel Dekker.

Courtin C.M., Swennen K., Verjans P., Delcour J.A., 2009. Heat and pH stability of prebiotic arabinoxylooligosaccharides and fructooligosaccharides. *Food Chemistry*, 112, 831-837.

Direction générale de la Statistique et de l'Information économique, SPF Economie, P.M.E., Classes moyennes et Energie, Belgium, 2010.
http://statbel.fgov.be/fr/modules/publications/statistiques/economie/estimation_de_la_production_des_cultures_agricoles_annee_de_recolte_2009.jsp

Doner L.W., and Hicks K.B., 1997. Isolation of hemicellulose from corn fiber by alkaline hydrogen peroxide extraction. *Cereal Chemistry*, 74, 2, 176-181.

Englyst H.N. and Cummings J.H., 1984. Simplified method for the measurement of total non-starch polysaccharides by gas-liquid chromatography of constituent sugars as alditols acetates. *Analyst*, 109, 937-942.

Escarnot E., Agneessens R., Wathelet B., Paquot M., 2010. Quantitative and qualitative study of spelt and wheat fibres in varying milling fractions. *Food Chemistry*, 122, 857-863.

Escarnot E., Aguedo M., Agneessens R., Wathelet B., Paquot M., 2011a. Extraction and characterization of water-extractable and water-unextractable arabinoxylans from spelt bran: Study of the hydrolysis conditions for monosaccharides analysis. *Journal of Cereal Science*, 53, 45-52.

Fang J.M., Sun R.C., Salisbury D., Fowler P., Tomkinson J.T., 1999. Comparative study of hemicelluloses from wheat straw by alkali and hydrogen peroxide extractions. *Polymer Degradation and Stability*, 66, 423-432.

Gould J.M., 1984. Alkaline peroxide delignification of agricultural residues to enhance enzymatic saccharification. *Biotechnology and Bioengineering*, 26, 1, 46-52.

Granström T.B., Izumori K., Leisola M., 2007. A rare sugar xylitol. Part II: biotechnological production and future applications of xylitol. *Applied Microbiology and Biotechnology*, 74, 273-276.

Hahn-Hägerdal B., Kahrumaa B., Fonseca C., Spencer-Martins I., Gorwa-Grauslund M.F., 2007. Towards industrial pentose-fermenting yeast strains. *Applied Microbiology and Biotechnology*, 74, 937-953.

Han G., Deng J., Zhang S., Bicho P., Wu Q., 2010. Effect of steam explosion treatment on characteristics of wheat straw. *Industrial Crops and Products*, 31, 28-33.

Heredia A., Jimenez A., Guillen R.Z., 1995. Composition of plant cell walls. *Lebensmittel Unters Forsch*, 200, 24-31.

Hoije A., Grondahl M., Tommeraas K., Gatenholm P., 2005. Isolation and characterization of physicochemical and material properties of arabinoxylans from barley husks. *Carbohydrate Polymers*, 61, 266-275.

Hromadkova Z. and Ebringerova A., 2003. Ultrasonic extraction of plant materials-investigation of hemicellulose release from buckwheat hulls. *Ultrasonics Sonochemistry*, 10, 127-133.

Jin .X., Ren J.L., Peng F., Xu F., Zhou G.Y., Sun R.C., Kennedy J.F., 2009. Comparative characterization of degraded and non-degradative hemicelluloses from barley straw and maize stems: composition, structure, and thermal properties. *Carbohydrate Polymers*, 78, 609-619.

Lequart C., Nuzillard J. M., Kurek B., Debeire P., 1999. Hydrolysis of wheat bran and straw by an endoxylanase: production and structural characterization of cinnamoyl-oligosaccharides. *Carbohydrate Research*, 319, 102-111.

Lundqvist J., Teleman A., Junel L., Zacchi G., Dalman O., Tjernel F., 2002. Isolation and characterization of galactoglucomannan from spruce (Picea abies). *Carbohydrate Polymers*, 48, 1, 29-39.

Maes C., and Delcour J.A., 2002. Structural characterisation of water-extractable and water-unextractable arabinoxylans in wheat bran. *Journal of Cereal Science*, 35, 315-326.

Paszner L. Jeong C., Quinde A., Awrdel-Karim S., 1993. Two-stage, continuous hydrolysis of wood by the acid catalysed organosolv saccharification (ACOS) process. *Energy Biomass Wastes*, 16, 629-64.

Percival J., 1921. The wheat plant. A monograph. London: Duckworth and Co., 325–334.

Persson T., Ren J. L., Joelsson E., Jönsson A.-S, 2009. Fractionation of wheat and barley straw to access high-molecular-mass hemicelluloses prior to ethanol production. *Bioresource Technology,* 100, 3906-3913.

Roos A.A., Persson T., Krawczyk H., Zacchi G., Stalbrand H., 2009. Extraction of water-soluble hemicelluloses from barley husks. *Bioresource Technology*, 100, 763-769.

Schober T. J., Bean S. R., Kuhn M., 2006. Gluten proteins from spelt (Triticum aestivum ssp. spelta) cultivars: A rheological and size-exclusion high-performance liquid chromatography study. *Journal of Cereal Science*, 44, 161–173.

Shin H., Guebitz G., Cavaco-Paulo A., 2001. "In situ" enzymatically prepared polymers for wool coloration. *Macromolecular Materials and Engineering*, 286, 691-694.

Sun R.C., Lawther J.M., Banks W.B., 1995. Influence of alkaline pre-treatments on the cell wall components of wheat straw. *Industrial Crops and Products*, 4, 127-145.

Sun R.C., Lawther J.M., Banks W.B., 1996. Fractional and structural characterization of wheat straw hemicelluloses. *Carbohydrate Polymers*, 29, 325-331.

Sun R.C., Fang J.M., Rowlands P., Bolton J., 1998. Physicochemical and thermal characterization of wheat straw hemicelluloses and cellulose. *Journal of Agricultural Chemistry*, 46, 2804-2809.

Sun R.C., Tomkinson J., Wang Y.X., Xioa B., 2000. Physico-chemical and structural characterization of hemicelluloses from wheat straw by alkaline peroxide extraction. *Polymer*, 41, 2647-2656.

Sun R.C., Sun X.F., Zhang S.H., 2001. Quantitative determination of hydroxycinnamic acids in wheat, rice, rye, and barley straws, maize stems, oil plam frond fiber, and fast-growing poplar wood. *Journal of Agricultural and Food Chemistry*, 49, 5122-5129.

Sun R.C., Wang X.Y., Sun X.F., Sun J.X., 2002. Physicochemical and thermal characterisation of residual hemicelluloses isolated by TAED activated peroxide from ultrasonic irradiated and alkali organosolv pre-treated wheat straw. *Polymer Degradation and Stability*, 78, 295-303.

Sun X.F., Sun R.C., Fowler P., Baird M.S., 2004a. Isolation and characterisation of cellulose obtained by a two-stage treatment with organosolv and cyanamide activated hydrogen peroxide from wheat straw. *Carbohydrate Polymers*, 55, 379-391.

Sun J.X., Sun X.F., Sun R.C., Su Y.Q., 2004b. Fractional extraction and structural characterization of sugarcane bagasse hemicelluloses. *Carbohydrate Polymers*, 56, 195-204.

Sun X.F., Xu F., Zhao H., Sun R.C., Fowler P., Baird M.S., 2005a. Physicochemical characterisation of residual hemicelluloses isolated with cyanamide-activated hydrogen peroxide from organosolv pre-treated wheat straw. *Bioresource Technology*, 96, 1342-1349.

Thomsen M.H., Thygesen A., Thomsen A.B., 2008. Hydrothermal treatment of wheat straw at pilot plant scale using a three-step reactor system aiming at high hemicelllulose recovery, high cellulose digestibility and low lignin hydrolysis. *Bioresource Technology*, 99, 4221-4228.

Wertz J.-L., Bédué O., Mercier J.P., 2010. In: Cellulose science and technology (364 p). Lausanne, Switzerland: EPFL Press.

Xu F., Liu C.F., Geng Z.C., Sun J.X., Sun R.C., Hei B.H., Lin L., Wu S.B., Je, J., 2006a. Characterisation of degraded organosolv hemicelluloses from wheat straw. *Polymer Degradation and Stability*, 91, 1880-1886.

Xu F., Sun J.-X., Liu C.F., Sun R.C., 2006b. Comparative study of alkali- and acidic organic solvent-soluble hemicellulosic polysaccharides from sugarcane bagasse. *Carbohydrate Polymers*, 341, 253-261.

Zielinski H., Ceglinska A., Michalska A., 2008. Bioactive compounds in spelt bread. *European Food Research Technology*, 226, 537-544.

7 CHAPTER 7

Enzymatic hydrolysis of arabinoxylans from spelt bran and hull

Excerpt from Article 4:

Escarnot, E., Aguedo, M., Paquot, M., 2012. Enzymatic hydrolysis of arabinoxylans from spelt bran and hull. *Journal of Cereal Science*, 55, 243-253.

7.2 The context of the study

In the two previous chapters, it has been shown that it was possible to extract efficiently AXs from bran and hemicelluloses from hull through chemical methods. Nowadays, the societal concern regarding the way of production orientates this research towards more environmentally-friendly methods. In the case of solubilization of AXs and hemicelluloses, enzymatic procedures are elected. Usually, the use of enzymes is oriented towards AXOS production. The objective of the following chapter is not the creation of AXOS *per se* but a prior step to evaluate the effects of the different enzymatic preparations on solubilization of AXs from bran and hull. Attention is paid to the parameters of the AXOS as this preliminary work is intended to be followed by their development.

Choice of enzymes is consequently a key point in the process. Microorganisms can be divided into three groups according to their strategies to hydrolyse hemicelluloses (Shallom and Shoham, 2003): complete hydrolysis to monosaccharides and disaccharides by several and synergic extracellular hemicellulases (Fusarium, Trichoderma and Aspergillus species); partial extracellular hydrolysis to oligosaccharides further hydrolysed by cell-associated or intracellular hemicellulases (Bacilli) which has advantages regarding sugar competition against non-hemicellulolytic microorganisms; hydrolysis by cellulosomes, extracellular cell-associated multi-enzymes complexes, harbouring cellulases and hemicellulases .

Xylanases are a group of enzymes responsible for the hydrolysis of xylan, the main being endo-1,4-β-xylanase (Girio *et al.*, 2010) which generates unsubstituted or substituted oligosaccharides depending on the initial configuration of the xylan. They have distinct ways of working: they can hydrolyse the WU-AX without solubilising them, they can reduce the level of WU-AX by solubilising them, which leads to an increase of the level of S-AX (solubilized-AX). Moreover, endoxylanases can reduce the MW of the S-AX and finally can reduce the MW of WE-AX (Courtin *et al.*, 1999). Xylan is not attacked randomly, but the bonds selected for the hydrolysis depend on the nature of the substrate molecule *i.e.* on the chain length, the degree of branching and the presence of substituents (Li *et al.*, 2000). Then other enzymes can complete the work of endoxylanases but they are more adapted to the production of monomers: β-xylosidase which hydrolyses small XOS and xylobiose (Girio *et al.*, 2010); α-L-arabinofuranosidase which liberates the α-L-arabinofuranosyl substituents (Saha, 2000); esterases which release ferulic and p-coumaric acids (Maes, 2004); α-D-glucuronidase which hydrolyses backbone units found in glucuronoxylan (Girio *et al.*, 2010; Puchart and Biely, 2008). The same type of enzymes are naturally present in the grain (xylosidase, arabinofuranosidase, endoxylanase) and at the same time inhibitors of enzymes are present, which are Triticum aestivum xylanase inhibitor (TAXI) and Xylanase Inhibiting Protein (XIP) (Maes, 2004).

The extent, type and distribution of the substituents along the backbone of AX greatly influence the susceptibility to enzymic degradation. The ratio A/X is a particular indication of the ability for the

enzymes to attack the AX. Solubilizations with enzymes have been successful on wheat bran (Maes *et al.*, 2004) and wheat straw (Lequart *et al.*, 1999) and the production of AXOS has been fulfilled from wheat bran (Swennen *et al.*, 2006b).

The following chapter will set up an experiment with different enzymes on spelt bran and hull in order to evaluate their effect. A preliminary step will characterize the way of working of enzymes on a model, oat spelt xylan, and will enable to make a choice among the different commercial enzymatic preparations tested.The hydrolysates will be evaluated through their sugar composition, the content in monomers and the molecular weight. The content in monomers is not necessary to evaluate the efficiency of the enzymes regarding the solubilization of AX however as it is known that this kind of solutions will be developped as AXOS, this parameter is a key feature of the hydrolysates.

7.3 Enzymatic hydrolysis of arabinoxylans from spelt bran and hull

Abstract

Spelt bran and hull were submitted to enzymatic treatment during 1h, 4h and 24h to release arabinoxylans (AXs). Several commercial enzymatic preparations with mainly endoxylanase activity from *Trichoderma reesei* (Rohalase WL), *Thermomyces lanuginosus* (Pentopan Mono conc. BG), *Bacillus subtilis* (Belfeed B MP and Grindamyl Powerbake 900), *Humicola insolens* (Ultraflo L), *Aspergillus aculeatus* (Shearzyme 2X), *Aspergillus aculeatus* plus *Trichoderma reesei* (Shearzyme Plus) were tested and combined with a commercial cellulolytic preparation from *Trichoderma reesei* (Celluclast 1.5L). The xylanolytic activity of the commercial enzyme preparations was heterogeneous. The production of monomers by the different enzymes was evaluated on oat spelt xylan. Spelt bran and hull hydrolysates were analysed regarding their content in total and free sugars. Monomers of glucose were mainly released with Celluclast, Ultraflo L and Shearzyme 2X. The percentage of released AXs ranged from 18.1 to 69.7% for bran and 0.5 to 6.4% for hull (% of the AXs originally present in the substrate). The solution of AXs hydrolysed by endoxylanases from spelt bran contained polysaccharides of degree of polymerisation (DP) of 2-71 while those hydrolysed with endoxylanases plus Celluclast had DPs of 1-1164. The hydrolysates from spelt hull had much lower DPs (between 2 and 17).

7.3.1 Introduction

Spelt (*Triticum aestivum* ssp. *spelta*) is an ancient subspecies of common wheat (*Triticum aestivum* ssp. *aestivum*) whose main characteristic is that the hull is attached to the grain. In agronomical terms, spelt might be more resistant to diseases and perform better than wheat under less advantageous growing conditions, such as wet, cold soils and high altitudes (Campbell, 1997). Spelt is an environmental-friendly cereal : with the hull covering the seed, chemical treatment before sowing might not be necessary and due to its long straw, it does not withstand a high level of nitrogen fertilization (Bonafaccia *et al.*, 2000). Moreover, it contributes to agrobiodiversity. In Belgium, it covers 10,000 ha and represents an average 70 000 T of grain per year (2005-2009) (according to Direction générale de la Statistique et de l'Information économique, 2010). From the point of view of composition, spelt has higher protein content than wheat, higher lipid content, especially Δ7-avenasterol (Bonafaccia *et al.*, 2000; Ruibal-Mendieta *et al.*, 2004a). Over the past few decades, spelt has attracted renewed and increasing interest as human food due to its image of "healthier, more natural, less over-bred" cereal than modern wheat (Schober *et al.,* 2006). It falls into a niche product, being important in specialty breads, organic food and food products with characteristics that differ from regular wheat products (Ranhotra *et al.*, 1995). Technological characteristics of spelt showed that it can be used for milling, bread making and pasta production (Ranhotra *et al.*, 1995; Bonafaccia *et al.*, 2000).

Bran and hull from spelt are by-products of the milling industry which are generally used for feeding. Bran accounts for 14 to 19% of the wheat grain. It comprises the outer layers, the aleurone layer and remnants of the starchy endosperm (Pomeranz, 1988). Spelt bran is composed of 5.7% cellulose; 18.6% hemicelluloses, 2.3% lignin (d.m.; w/w) (Escarnot *et al.*, 2010) and 41.2% of starch (Escarnot *et al.*, 2011). When considering destarched bran, the arabinose plus xylose content reaches 30.7% for spelt (Escarnot *et al.*, 2011a). Hull represents 24-28% of the grain (Hucl *et al.*, 1995). The hull of the present study contains 12.6% starch, 30.2% cellulose, 35.5% hemicelluloses and 5.7% lignin (Escarnot *et al.*, 2011b).

Arabinoxylans (AXs) from spelt bran and hull have not been extensively characterized. In wheat bran, AXs are constituted by a main chain of β-linked (1->4)-3-D-xylose substituted mainly by side chains of α-(O-2) and/or α-(O-3)-L-arabinose (Bacic *et al.*, 1988). Some AXs in wheat bran are physically, chemically inter-linked and also linked to lignin and cellulose through diferulate bridges and hydrogen bonds (Iiyama *et al.*, 1994). Alkaline extraction of AXs was previously done on wheat and spelt bran (with alkaline peroxide hydrogen) (Maes and Delcour, 2002; Escarnot *et al.*, 2011a). Moreover, several treatments using alkaline peroxide hydrogen, NaOH and then $NaClO_2$-KOH, acetic and formic acids were applied on spelt hull and different hemicelluloses extractions were achieved concerning yields and profiles (Escarnot *et al.,* 2011b). Besides, some enzymatic hydrolyses of AXs from wheat bran (Lequart *et al.*, 1999; Beaugrand *et al.,* 2004b; Van Craeyveld *et al.,* 2010) and from wheat straw (Lequart *et al.*, 1999) were described. The enzymatic methods using endoxylanases on bran provided fractions different from those obtained by chemical methods and were as efficient as the chemical ones. It should be mentioned that the enzymatic procedures are more acceptable from an environmental point of view than the chemical procedures. Polymerised AXs can form gels and consequently can be used in food industry for texturization and stabilization (Saulnier *et al.,* 2007) or as adhesives and emulsifiers while monomeric xylose can be transformed into ethanol (Hahn-Hägerdal *et al.,* 2007) or xylitol (Granström *et al.,* 2007). Arabino-xylo-oligosaccharides (AXOS) present interesting properties as prebiotics (Courtin *et al.,* 2009). Wheat bran AXOS produced by enzymic or chemical cleavage of AX have been shown to selectively stimulate *Bifidobacterium* species *in vitro* pure cultures, and in *in vivo* trials with rats, mice, chickens and humans (Van Craeyveld et al., 2009a).

In the present work, xylanases, cellulases and combinations of both were tested on spelt bran and hull. The hydrolysates were characterized through the content in total mono- and polysaccharides. The molecular weights (MWs) were determined to characterize the fractions. Yields of extraction were also calculated to compare the efficiency of the different enzymes.

7.3.2 Material and methods

7.3.2.1 Material

Bran and hull came from the spelt cv. Ressac, harvested in August 2007. The spikelets were dehulled and the grains milled at "Le Moulin de Hollange" (Hollange, Fauvillers, Belgium) in November 2007. Bran and hulls were stored at 4°C, their moisture contents were respectively 5.3 and 0.9%. Bran was screened at 1mm to remove parts of the hulls. Screened bran and hulls were micronized by jet milling (Alpine 100 AFG, Augsburg, Germany) with 6-7 bars of pressure and turbine at respectively 2000 and 3000 rpm. The granulometry was measured in triplicate with a Mastersizer 2000 (Malvern Instruments Ltd, Worcestershire, UK). All reagents were of analytical grade.

7.3.2.2 Methods

The enzymes

Height multi-enzymes commercial preparations were chosen for their different activities and origin. Two preparations have only endo-1-4-β-xylanase activity: Belfeed B 1100 MP from *Bacillus subtilis* (Puratos, Groot-Bijgarden, Belgium) with 105 IU/g (according to product data sheet) and Grindamyl Powerbake 900 from *Bacillus subtilis* (Danisco, Copenhagen, Denmark). Shearzyme 2X from *Aspergillus oryzae* (both Novozymes, Bagsvaerd, Denmark) displays as main activity endo-1-4-β-xylanase with 1000 FXU-S/g (according to product data sheet) and it may also have β-xylosidase and β-glucanase activities, and in a lesser extent β-glucosidase and α-arabinofuranosidase activities (Rantanen *et al.,* 2007). The endo-1-4-β-xylanase activity of Pentopan Mono conc. BG is 20 times higher than in Pentopan Mono BG. Pentopan Mono BG from *Thermomyces lanuginosus* has mainly endo-1-4-β-xylanase activity with 2500 FXU-W/g (according to product data sheet) and an endoglucanase activity of 52 nkat/mL was detected (Flander et al., 2008). Celluclast 1.5L from *Trichoderma reesei* (Novozymes, Bagsvaerd, Denmark) has cellulase activity with 700 EGU/g (according to product data sheet), xylanase (680 U/mL) and endoglucanase (26 U/mL) activities (Pala *et al.,* 2007). Shearzyme Plus from *Aspergillus oryzae* and *Trichoderma reesei* (Novozymes, Bagsvaerd, Denmark) has cellulase (350 EGU/g) and endo-1,4-β-xylanase (250 FXU-S/g) activities (according to product data sheet) and a side activity of β-glucanase (Novozymes, personal communication). Ultraflo L from *Humicola insolens* (Novozymes, Bagsvaerd, Denmark) has according to the product data sheet endo-1,3-4-glucanase as main activity (45 FBG/g) and xylanase and cellulase as side activities; it also contains arabinanase (5.2 U/mL), endoglucanase (13.5 U/mL), beta-glucanase (16.7 U/mL) and feruloyl esterase (Moore *et al.,* 2006; Vafiadi *et al.,* 2008). Xylanase activity was quantified to 700.7 U/mL (Vafiadi *et al.,* 2008). Rohalase WL from *Trichoderma reesei* (AB Enzymes, Darmstadt, Germany) has three activities: mainly endo-1,4-β-xylanase (400 kBXU/g) with cellulase and β-glucanase as side activities (according to product data sheet) and a weak β-glucosidase activity (AB Enzymes, personal communication).

Activities of the enzymes

The xylanolytic activity of the commercial enzymes was evaluated in the optimal working conditions of each enzyme with the Xylazyme AX tablets during 10 min (Megazyme, Bray, Wicklow, Ireland). Each enzyme corresponded with a buffer, a pH and a temperature which were respectively: Belfeed, NaAc, pH 5, 55°C; Rohalase, NaP, pH 5.5, 55°C; Ultraflo L, NaAc, 4.7, 40°C; Celluclast 1.5L, NaAc, pH 5, 60°C; Pentopan mono conc BG, NaAc, pH 5, 55°C; Shearzyme 2X, NaAc, pH 4.7, 40°C; Shearzyme Plus, NaAc, pH 4.7, 40°C; Grindamyl Powerbake 900, NaP, pH 6.3, 40°C. The absorbance was read at 590 nm and the amount of enzyme (µg) required to yield an absorbance of 1.0 was deduced (Van Crayeveld *et al.*, 2009b).

Enzymatic reactions on oat spelt xylan

Oat spelt xylan (85%, Sigma-Aldrich, Saint-Louis, USA) was suspended in adequate buffer at a ratio 1:100 (w/v). The aim was to evaluate how the different commercial enzymatic preparations work. Oat spelt xylan was used for this characterization. Enzyme was added so as to be equivalent to an absorbance of 2250. Each suspension was set at the adequate temperature (Table 7.1) and aliquotes were taken immediately after enzyme addition (0 h), after 1h of reaction, after 4h of reaction, after 7h and after 24h. After sampling, enzymes were inactivated at 100°C for 10 min.

Destarching and deproteination

The production of destarched and deproteinised spelt bran was previously extensively described in Escarnot *et al.* (2011a). The content of starch after reaction was verified with a total starch assay kit and with a resistant starch assay kit (Megazyme, Bray, Wicklow, Ireland).

Enzymatic hydrolysis

Destarched and deproteinised spelt bran and hull were suspended in adequate buffer (25mM) at a ratio 1:20 (w/v). The content of enzyme was defined so that each sample benefited from an equivalent xylanolytic activity whatever the enzyme. The quantity of enzyme (g) was calculated so that the theoretical absorbance was 1000 for 1 g of bran or hull. The ratio between enzymatic preparation and the substrate was decided on the basis of information found in literature (Sorensen *et al.,* 2003, 2005; Pastell *et al.,* 2008) and from preliminary works (results not shown). A synthesis of the different ratios lead to choose a theoretical absorbance of 1000 for 1 g of substrate. The needed quantity for each enzyme was deduced from the absorbance. When comparing the effects of several enzymatic preparations it is essential that the xylanolytic activity is equivalent among the preparations otherwise the comparison is not right. Each suspension was set at the adequate temperature (Table 7.1) and aliquotes were taken immediately after enzyme addition (0h), after 1 h of reaction, after 4 h and after 24 h. After sampling, enzymes were inactivated at 100°C for 10 min. Aliquotes were centrifuged (3

min, 2000 rpm for Belfeed hydrolysates and 1 min, 2000 rpm for all others, depending on consistency) and were filtered through a 0.45 µm membrane filter (Millipore Co., Milford MA, USA).

Enzyme	Buffer	pH	Temperature °C	Quantity	Enzyme concentration	Experiment number		
						Oat spelt xylan	Bran	Hull
Belfeed	NaAc	5	55	22 mg	solid	1	9	20
Celluclast 1,5L	NaAc	5	60	550 µl	100 mg/ml	2	10	21
Grindamyl Powerbake 900	NaP	6,3	40	1,3 g	solid	3	-	-
Pentopan mono conc BG	NaAc	5	55	400 µl	0,1 mg/ml	4	11	22
Rohalase WL	NaP	5,5	55	100 µl	10 mg/ml	5	12	23
Shearzyme Plus	NaAc	4,7	40	800 µl	100 mg/ml	6	-	-
Shearzyme 2X	NaAc	4,7	40	470 µl	100 mg/ml	7	13	24
Ultraflo L	NaAc	4,7	40	410 µL	100 mg/ml	8	14	25
Belfeed + Celluclast 1,5L	NaAc	5	55	22 mg	solid	-	15	-
Pentopan mono conc BG + Celluclast 1,5L	NaAc	5	55	400 µl	0,1 mg/ml	-	16	-
Rohalase WL + Celluclast 1,5L	NaP	5,5	55	100 µl	10 mg/ml	-	17	-
Shearzyme 2X + Celluclast 1,5L	NaAc	5	45	470 µl	100 mg/ml	-	18	-
Ultraflo L + Celluclast 1,5L	NaAc	5	45	410 µL	100 mg/ml	-	19	-
Celluclast 1,5L in combination	-	-	-	550 µl	100 mg/ml	-	-	-

Table 7.1. Conditions of reaction for each enzyme and combination of enzymes: buffer, pH, temperature °C, contents and concentrations of enzyme and experiment number for oat spelt xylan (0.2 g), spelt bran (1 g) and spelt hull (1 g).

Analytical procedures

Monosaccharide analysis was based on the method of Englyst and Cummings (1984). A sample (in duplicate) was accurately weighed and the acid was added. For chemical total hydrolysis of polysaccharides, enzymatic hydrolysates were set 3 h at 100°C with 1 M H_2SO_4 and spelt bran and hull were set 3 h at 100°C with 2 M TFA (Escarnot *et al.*, 2011a). The solution was then neutralized with concentrated NaOH and the alkalinity of the solution was checked. Sugars (0.4 mL of supernatant) were reduced to their corresponding alditols with 2 mL of DMSO containing 2 mL NaBH4. Reduction was performed for 90 min at 40°C. The excess of NaBH4 was eliminated by adding 6 mL glacial acetic acid. Acetylation was then performed with acetic anhydride (4 mL, 15 min at room temperature) in the presence of 1-methyl-imidazole (0.4 mL) as a catalyst. Acetylation was stopped with 10 mL deionized water and the acetylated alditols were partitioned between dichloromethane (4 mL) and water. After the phase separation, 1 mL of the lower one was transferred in a septum-cap vial. 2-Deoxy-D-glucose was used as internal standard and standards of D-arabinose, D(+)-xylose, D (+)-mannose, D(+)-glucose, D(+)-galactose (purity >99.5%, Sigma Chemical Co., St-Louis MO, USA) were used. The analyses were performed with a HewlettePackard Agilent 6890 series gas chromatograph equipped with a high-performance capillary column, HP1-methylsiloxane (30 m x 0.32 mm, 0.25 mm film thickness) (Scientific Glass Engineering, Melbourne, Australia). 0.2 mL of derivatized sugars in dichloromethane was injected. Helium was the carrier gas with a flow of 1.6 mL/min. The injection temperature was 290°C, and the temperature program was 1 min at 120°C,

followed by a linear increase in 4 min to 220°C and then in 35 min to 290°C which was maintained for 4 min. Compounds were detected using a flame ionization detector at 320°C.

For monomers analysis, samples followed the same process except that no acid hydrolysis was performed and that acetylation was not performed at room temperature but at 93°C during 2h. The previous solution of standards also contained 1,4-β-D-xylobiose (purity >95%, Megazyme, Bray, Wicklow, Ireland).

Yield of extraction (%) was calculated by a ratio between the quantity of AXs in the hydrolysate and in the substrate. The quantity of AX was obtained by multiplying the content in sugar (%; d.m.; w/w)) and the quantity of matter (g; d.m.).

Molecular weights (MWs) were determined in triplicate by High Performance Size Exclusion Chromatography (HPSEC) on a Waters 2690-HPLC system (Waters INC., Milford MA, USA), equipped with a Progel-TSK G3000 PWXL column (300 x 7.8 mm) (Supelco, Bellefonte, Pennsylvania, USA) and coupled on-line with a single detector system: a Waters 2410 differential refraction index (RI) detector. Enzymatic hydrolysates were filtered through a 0.45 µm membrane filter (Millipore Co., Milford MA, USA) and 100 µL were injected in HPSEC. Elution was carried out at a flow rate of 0.7 mL/min with 50 mM sodium nitrate (NaNO3) solution containing 0.05% sodium azide (NaN3) as preservative. Dextrans of 1, 5, 12, 25, 50 kDa (Fluka, Sigma-Aldrich, Buchs, Switzerland) were used to establish a calibration curve to calculate the MWs. The polymerisation degree (DP) was the ratio of MW and MW of C5 sugar residues (132 Da).

7.3.3 Results and discussion

7.3.3.1 Enzymes' activity

The choices of buffer, pH and temperature were made according to information provided by the enzyme suppliers and according to literature (Sorensen *et al.*, 2003). The xylanolytic activity of the commercial preparations of enzymes was highly variable; the ratio of the highest and the lowest activity was 883. The amount of enzyme required to yield an absorbance of 1.0 at 590 nm was 14.85 µg of Belfeed, 0.87 µg of Rohalase, 27.06 µg of Ultraflo L, 36.92 µg of Celluclast 1.5L, 0.03 µg of Pentopan conc mono BG, 31.27 µg of Shearzyme 2X, 51.85 µg of Shearzyme Plus and 883.23 µg of Grindamyl Powerbake 900. The two enzymes with the highest xylanolytic activities were Rohalase WL and Pentopan Mono conc. BG. Belfeed, Ultraflo L, Shearzyme 2X and Celluclast 1.5L displayed intermediate activities. Finally Shearzyme Plus had a low xylanolytic activity. Grindamyl Powerbake 900 displayed the lowest xylanolytic activity. Grindamyl Powerbake 900 was not tested on bran and hull since theoretically 1.3 g of the enzyme was necessary for 1 g of bran or hull.

7.3.3.2 Evaluation of the release of monomers by enzymes on oat spelt xylan

AXs obtained by enzymatic way may have different potential applications according to the profile of the hydrolysates. Within the framework of the production of prebiotic AXOS it is important to restrict the content of monomers of the hydrolysates because they are not considered as dietary fibers or prebiotics. Consequently, the enzymes were first experimented on commercial oat spelt xylan to evaluate the production of monomers (Table 7.2). No monomers of rhamnose, mannose and galactose were detected in the hydrolysates. Shearzyme Plus produced high amounts of free xylose and glucose, respectively 48.6% and 17.7%; the same was observed for Celluclast with respectively 28.8% and 14.3% of the dry weight (DW) after 24 h reaction. Such contents being much too high within an AX fraction, Shearzyme Plus was consequently discarded. Celluclast had mainly cellulase activity and it was maintained in order to test if further attack of cellulose would make the release of AXs easier. Shearzyme 2X also produced free xylose and glucose but in a much lesser extent, respectively 9.1% and 4.5% of the fraction. Ultraflo L produced free xylose and glucose but also arabinose, respectively 10.1%, 7.3% and 5.3%. Even if the monomers obtained with Shearzyme 2X and Ultraflo L were not suitable in the case of the production of AXOS, as they are digestible sugars, these enzymes were tested on spelt bran and hull to evaluate their potential of hydrolysis of AXs. The release of monomers by Grindamyl, Belfeed, Pentopan and Rohalase was almost null. Hydrolysate from Shearzyme 2X displayed the highest content of xylobiose (73.3%) followed by Pentopan and Ultraflo (22.5%; 21.3%), Rohalase and Belfeed in a lesser extent. The release of xylobiose by Grindamyl was negligible. Shearzyme Plus and Celluclast released xylobiose which was hydrolysed into xylose during the reaction. The production of monomers of glucose was higher in the hydrolysates 2 and 6 for which enzymatic preparations had cellulase as main activity and, in a lesser extent, hydrolysates 7 and 8 for which enzymatic preparations had β-glucanase activities (Table 7.2).

Experiment number	Time (h)	Free monomers and xylobiose			
		Ara	Xyl	Glu	Xylob
1	0	n.d.	n.d.	n.d.	n.d.
	1	n.d.	n.d.	n.d.	n.d.
	4	n.d.	n.d.	n.d.	n.d.
	7	n.d.	n.d.	n.d.	0.7 ± 0.7
	24	n.d.	n.d.	n.d.	3.1 ± 0
2	0	n.d.	n.d.	11.1 ± 0	3.1 ± 0.3
	1	n.d.	16.1 ± 0.2	11.7 ± 0	2.6 ± 0.1
	4	n.d.	22.2 ± 0.1	11.3 ± 0.1	n.d.
	7	n.d.	28.3 ± 0.1	12.8 ± 0	n.d.
	24	n.d.	28.8 ± 0.3	14.3 ± 0.1	n.d.
3	0	n.d.	n.d.	n.d.	n.d.
	1	n.d.	n.d.	n.d.	n.d.
	4	n.d.	n.d.	n.d.	n.d.
	7	n.d.	n.d.	n.d.	n.d.
	24	n.d.	n.d.	0.2 ± 0	0.1 ± 0.1
4	0	n.d.	n.d.	n.d.	n.d.
	1	n.d.	n.d.	n.d.	3.3 ± 0.2
	4	n.d.	n.d.	n.d.	9.6 ± 0.2
	7	n.d.	n.d.	n.d.	13.6 ± 0.1
	24	n.d.	0.3 ± 0.3	n.d.	22.5 ± 0.8
5	0	n.d.	n.d.	n.d.	n.d.
	1	n.d.	n.d.	n.d.	3.7 ± 0.1
	4	n.d.	n.d.	n.d.	5.5 ± 0.2
	7	n.d.	n.d.	n.d.	6.2 ± 0.2
	24	n.d.	1.6 ± 0	n.d.	7.5 ± 0.2
6	0	n.d.	0.7 ± 0	16.2 ± 0.3	3.2 ± 0.1
	1	0.3 ± 0	8 ± 0.1	16.1 ± 0.2	11.8 ± 0.3
	4	0.8 ± 0	23.9 ± 0.1	16.6 ± 0.1	11.4 ± 0.2
	7	1.1 ± 0	32.4 ± 0.2	15.9 ± 0	9.3 ± 0.1
	24	2.1 ± 0	48.6 ± 0	17.7 ± 0.1	4 ± 0
7	0	n.d.	n.d.	2.7 ± 0	1.9 ± 0.1
	1	n.d.	1.2 ± 0	2.8 ± 0	19.6 ± 1.2
	4	n.d.	3.9 ± 0.02	3 ± 0	52 ± 0.1
	7	n.d.	5.4 ± 0.1	3.2 ± 0	61.1 ± 0.5
	24	n.d.	9.1 ± 0	4.5 ± 0	73.3 ± 0.9
8	0	n.d.	n.d.	6 ± 0.1	n.d.
	1	1.8 ± 0	n.d.	6 ± 0	1.8 ± 0.2
	4	4 ± 0	1.8 ± 0	6.2 ± 0	8.5 ± 0.9
	7	4.5 ± 0.1	3.1 ± 0	6.3 ± 0	13.2 ± 0.5
	24	5.3 ± 0.1	10.1 ± 0.1	7.3 ± 0.1	21.3 ± 0.4

Table 7.2. Concentration of monomers and xylobiose (%, d.m.) (± SD) in hydrolysates from oat spelt xylan at 0 h, 1 h, 4 h, 7 h and 24 h. See Table 7.1 for experiment numbers.

7.3.3.3 Composition of the substrates

Granulometry of bran and hull was similar with the following respective repartition in the populations: d(0.1) 14 µm; d(0.5) 111 µm and d(0.9) 688 µm and d(0.1) 12 µm; d(0.5) 109 µm and d(0.9) 686 µm. The repartition of the populations must be interpreted this way: d(0.5) is the volume median diameter, 50% of the population is below the corresponding value; d(0.1) means that 10% of the volume distribution is below the corresponding value; d(0.9) means that 90% of the volume distribution is below the corresponding value. Destarched and deproteinised bran and hull contained respectively 0.8% and 0.1% total starch according to the total starch assay kit. According to the resistant starch assay kit which enabled also to calculate the total starch, bran contained 0.3% resistant starch and 2.9% solubilised starch, resulting in 3.2% and hull it was respectively 0.1 % resistant starch and 0% solubilised starch, resulting in 0.1%. The difference between both tests comes from the fact that in the total starch assay kit, the material is washed with ethanol to remove glucose and maltodextrins. Consequently, the solubilised starch from the resistant starch assay kit accounts the glucose and maltodextrins, explaining the higher value of total starch in the destarched and deproteinated bran, which is not the case in the destarched and deproteinated hull. Finally, destarched and deproteinated bran and hull contained respectively 0.3% and 0.1% resistant starch, 0.5% and 0% starch, 2.4% and 0% glucose and maltodextrins. Xylose content of destarched and deproteinated bran and hull was similar, 15.1% and 16.4%, while arabinose content was 6.0% and 2.6%, giving respectively A/X ratios of 0.39 and 0.16. The AXs from hull were less substituted by arabinose than those of the bran. Glucose content was higher in bran (10.2%) than in hull (1.3%) (Table 7.3A and B).

7.3.3.4 Bran hydrolysates

Table 7.3A displays the content in total sugars and in free monosaccharides of spelt bran hydrolysates. No rhamnose (neither as monomer neither as a polysaccharide) and no free galactose were detected. For experiments (exp.) 9 to 14, after 24 h of reaction, total glucose content of the hydrolysates ranged from 23.9% to 32.3%; total xylose content from 26.9% to 37.9% and total arabinose content from 8.1% to 11.7% (Table 7.3A). The profiles of the total sugar content of the different enzymatic hydrolysates were similar, but those obtained after 24 h with Rohalase and Celluclast displayed lower xylose and arabinose contents. The association of Celluclast with the other enzymes increased the content of total glucose but did not increase the total content of xylose and arabinose (Table 7.3A).

Experiment number	Time (h)	Total sugars						Free monomers and xylobiose				
		Ara	Xyl	Man	Glu	Gal	A/X ratio	Ara	Xyl	Man	Glu	Xylob
DD BRAN		6 ± 0.1	15.1 ± 0.3	0.4 ± 0	10.2 ± 0	1 ± 0	0.39					
9	0	7 ± 0	23.6 ± 0.2	0.8 ± 0.1	34.5 ± 0.4	0.8 ± 0	0.29	n.d.	n.d.	0.5 ± 0	4 ± 0.1	n.d.
	1	8 ± 0.1	32.4 ± 0.2	0.7 ± 0	27.4 ± 0.2	0.7 ± 0	0.25	n.d.	n.d.	0.5 ± 0	4 ± 0	1.6 ± 0.1
	4	8.1 ± 0.2	33.5 ± 0.1	0.6 ± 0	27.8 ± 0.3	0.7 ± 0	0.24	n.d.	n.d.	0.5 ± 0	4 ± 0	2.2 ± 0
	24	8.5 ± 0.1	35.5 ± 0.3	0.6 ± 0	29.6 ± 0.1	0.8 ± 0	0.24	n.d.	n.d.	0.5 ± 0	4 ± 0.1	2.9 ± 0.1
10	0	7.4 ± 0	17 ± 0.3	1.1 ± 0.1	36.3 ± 1	0.9 ± 0	0.44	n.d.	n.d.	0.6 ± 0	11.5 ± 0.1	0.9 ± 0
	1	8.3 ± 0.2	26.2 ± 1.1	1.1 ± 0	30.8 ± 1.6	0.7 ± 0	0.32	n.d.	6.9 ± 0.1	0.5 ± 0	10.3 ± 0.1	1.4 ± 0
	4	8.6 ± 0.1	28.9 ± 0.8	1.2 ± 0	32.4 ± 1	0.7 ± 0	0.3	n.d.	11.7 ± 0	0.5 ± 0	11.8 ± 0	0.7 ± 0
	24	9 ± 0.2	28.4 ± 0.3	1.3 ± 0	32.3 ± 0.6	0.8 ± 0	0.32	n.d.	13.7 ± 0	0.6 ± 0	14.7 ± 0.1	n.d.
11	0	7.1 ± 0.2	17.1 ± 0.7	0.6 ± 0	33 ± 1.4	0.9 ± 0	0.42	n.d.	n.d.	0.7 ± 0	5.2 ± 0	n.d.
	1	10 ± 0.6	34 ± 1.5	0.5 ± 0	28.3 ± 0.2	0.9 ± 0.1	0.29	n.d.	n.d.	0.5 ± 0	4.1 ± 0	3 ± 0
	4	9.4 ± 0.2	32.4 ± 0.3	0.5 ± 0.1	25.2 ± 0.6	0.8 ± 0	0.29	n.d.	n.d.	0.5 ± 0	4 ± 0	5.7 ± 0.2
	24	10.4 ± 0.5	37.9 ± 0.2	0.5 ± 0	28.1 ± 1.1	0.9 ± 0	0.27	n.d.	0.6 ± 0	0.5 ± 0	4 ± 0	9.3 ± 0.1
12	0	5.7 ± 0	13 ± 0.1	0.8 ± 0	30.8 ± 1	0.8 ± 0.1	0.43	n.d.	n.d.	0.6 ± 0	4.6 ± 0	n.d.
	1	8.2 ± 0.2	26.2 ± 0	0.6 ± 0	24.6 ± 0.3	0.7 ± 0	0.31	n.d.	n.d.	0.5 ± 0	3.5 ± 0	1.8 ± 0
	4	8.4 ± 0.1	28 ± 0	0.7 ± 0	24.8 ± 0.1	0.7 ± 0	0.3	n.d.	n.d.	0.5 ± 0	3.8 ± 0	2.5 ± 0.1
	24	8.1 ± 0	26.9 ± 0.4	0.8 ± 0	24.3 ± 0.5	0.7 ± 0	0.3	n.d.	0.2 ± 0.2	0.5 ± 0	3.8 ± 0	2.8 ± 0
13	0	6.6 ± 1	14.6 ± 2	0.7 ± 0.1	35.4 ± 3.6	0.8 ± 0.1	0.45	n.d.	n.d.	0.8 ± 0	7.9 ± 0.1	3.3 ± 0
	1	10.2 ± 0.1	28.1 ± 0.2	0.6 ± 0	30.9 ± 0.2	0.8 ± 0	0.36	n.d.	0.8 ± 0	0.6 ± 0	6.2 ± 0.1	9.5 ± 0.1
	4	10.8 ± 0.4	35.5 ± 1.5	0.6 ± 0	28.6 ± 1	0.9 ± 0.1	0.3	n.d.	1.7 ± 0	0.5 ± 0	5.8 ± 0	14.5 ± 0
	24	9.9 ± 1.3	35.9 ± 3.9	0.5 ± 0	23.9 ± 2.4	0.8 ± 0.1	0.28	n.d.	3.5 ± 0	0.5 ± 0	6.4 ± 0.1	19.9 ± 0
14	0	8.8 ± 0.1	25.8 ± 0.3	1 ± 0.1	33.3 ± 0.5	0.9 ± 0	0.34	n.d.	n.d.	0.6 ± 0	8.6 ± 0	n.d.
	1	11 ± 0	35 ± 0	0.8 ± 0	28 ± 0.1	0.9 ± 0	0.31	1.7 ± 0	0.8 ± 0	0.5 ± 0	7.5 ± 0	7.9 ± 0
	4	11.4 ± 0.2	33.8 ± 0.3	0.8 ± 0	26.1 ± 0.7	1.1 ± 0	0.34	3.8 ± 0	2.5 ± 0	0.5 ± 0	7.6 ± 0.1	15.9 ± 0.3
	24	11.7 ± 0.2	35.1 ± 1.6	1 ± 0	27.7 ± 1.3	1.1 ± 0	0.33	6.6 ± 0	6.9 ± 0	0.6 ± 0	10.7 ± 0	24.2 ± 0.2
15	0	7.1 ± 0.1	21.1 ± 0	0.9 ± 0	39.2 ± 0.1	0.9 ± 0.1	0.34	n.d.	n.d.	0.6 ± 0	11.8 ± 0	1.6 ± 0
	1	9 ± 0.1	32.8 ± 0.1	1.1 ± 0	34.5 ± 0.3	0.9 ± 0	0.27	n.d.	8.7 ± 0	0.5 ± 0	11.1 ± 0	4.2 ± 0.1
	4	9.5 ± 0.2	35.5 ± 1.1	1.3 ± 0	37.3 ± 1.2	1 ± 0	0.27	0.5 ± 0	16.3 ± 0	0.6 ± 0	13.4 ± 0.1	1 ± 0
	24	9.5 ± 0.6	35.9 ± 1.3	1.4 ± 0	40.8 ± 1.7	1 ± 0.2	0.26	0.5 ± 0	18.8 ± 0.2	0.6 ± 0	18.7 ± 0.1	0 ± 0
16	0	6.8 ± 0	17 ± 0.1	0.9 ± 0	38.6 ± 0.5	1 ± 0	0.4	n.d.	n.d.	0.6 ± 0	12.3 ± 0	1.3 ± 0
	1	8.8 ± 0.3	31.1 ± 1.1	1.2 ± 0	35.6 ± 0.9	1 ± 0.1	0.28	n.d.	8.2 ± 0	0.5 ± 0	10.6 ± 0.1	3.8 ± 0.1
	4	8.6 ± 0.1	30.5 ± 0.4	1.2 ± 0	34.4 ± 0.3	1 ± 0	0.28	0.4 ± 0	14.7 ± 0	0.5 ± 0	12.2 ± 0.1	1.1 ± 0
	24	9.4 ± 0.2	33.3 ± 0.6	1.4 ± 0	38 ± 0.1	1.1 ± 0	0.28	n.d.	18.2 ± 0	0.6 ± 0	17.1 ± 0.2	n.d.
17	0	5.2 ± 0.2	12.6 ± 0	0.9 ± 0	36.9 ± 0.6	1 ± 0	0.42	n.d.	0 ± 0	0.6 ± 0	12.1 ± 0	n.d.
	1	7.2 ± 0	25.3 ± 0.7	1 ± 0	29.7 ± 1	0.8 ± 0	0.29	n.d.	5.9 ± 0	0.5 ± 0	10.1 ± 0.1	4.7 ± 0.1
	4	7.1 ± 0.3	25 ± 1.4	1.1 ± 0	29 ± 1.3	0.8 ± 0	0.28	n.d.	11.1 ± 0.1	0.5 ± 0	11.1 ± 0	2.1 ± 0
	24	7.6 ± 0.1	27.2 ± 0.4	1.2 ± 0	31.3 ± 0.4	0.9 ± 0	0.28	n.d.	14.1 ± 0.1	0.5 ± 0	14.5 ± 0	n.d.
18	0	7.4 ± 0.1	18.1 ± 0.2	1.2 ± 0	45.5 ± 0.6	1.3 ± 0	0.41	n.d.	0.9 ± 0	0.8 ± 0	16 ± 0.1	3.7 ± 0
	1	9.9 ± 0.3	34.1 ± 1.1	1.4 ± 0	36.9 ± 0.8	1.2 ± 0	0.29	n.d.	7.5 ± 0	0.5 ± 0	11.5 ± 0.1	13.3 ± 0.1
	4	10.5 ± 0.3	35 ± 0	1.4 ± 0	37 ± 0.3	1.3 ± 0	0.3	0.7 ± 0	15.9 ± 0.2	0.5 ± 0	12.5 ± 0.1	6.2 ± 0
	24	10.4 ± 0.1	34.6 ± 0.4	1.3 ± 0	36 ± 0.2	1.4 ± 0.1	0.3	1.7 ± 0	22.2 ± 0	0.5 ± 0	16 ± 0	1.2 ± 0
19	0	8.9 ± 0.4	26.5 ± 1.2	1 ± 0.1	36.1 ± 1.8	1.1 ± 0.1	0.34	0.1 ± 0.1	0.6 ± 0	0.7 ± 0	16.2 ± 0.1	2.7 ± 0.3
	1	9.5 ± 0	30 ± 0.5	1.2 ± 0	33.1 ± 0.3	1.2 ± 0	0.31	2.1 ± 0	6.4 ± 0	0.5 ± 0	13.8 ± 0.1	10.1 ± 0.2
	4	10 ± 0.4	31.6 ± 1.6	1.2 ± 0.1	34.9 ± 1.9	1.3 ± 0	0.32	3.8 ± 0	15.3 ± 0.1	0.6 ± 0	14.8 ± 0.1	7.4 ± 0.1
	24	11.4 ± 0.1	35.1 ± 0.4	1.3 ± 0	37.9 ± 0.6	1.5 ± 0	0.32	5.5 ± 0	26.8 ± 0.1	0.2 ± 0.2	21.2 ± 0.1	1.4 ± 0

Table 7.3.A Content of total saccharides, monomers and xylobiose (%, d.m.) (± SD) in hydrolysates from spelt bran at 0 h, 1 h, 4 h and 24 h. See Table 7.1 for experiment numbers.

Whatever the enzyme, the yield of extraction for arabinose plus xylose increased strongly after 1 h and then increased slightly between 1 h and 24 h (Table 7.4, Appendix Fig.A.15 and A.16). A high solubilization of AXs was already observed initially (0 h). Indeed, after 1 h for experiments 9 to 14, the extraction yield increased by 9.7-25.3% and for experiments 15 to 19 by 21.6-43.7%. For experiments 9 to 14, between 1 h and 4 h, the increase was 0-5.1% (except for hydrolysates from Shearzyme 2X with 13.9%) and 0-7.7% between 4 h and 24 h. The increase of matter in the hydrolysates is visible on Fig. 7.1. Combination with Celluclast also led to a limited improvement of the hydrolysis: 0.0-5.4% between 1 h and 4 h and 0.5-5.2% between 4 h and 24 h. Whatever the reaction time, the addition of Celluclast increased AXs' extraction yields. The yield range obtained without combination with Celluclast was 38.9-68.6% after 24 h and the yield range obtained with combination with Celluclast was 62.6-74.8% after 24 h. After 1 h, the addition of Celluclast to the

enzymes alone increased the extraction yield of AXs from 3.1% to 29.8%. This improvement displayed the xylanolytic action of Celluclast. Lequart *et al.* (1999) hydrolysed wheat bran AXs by a thermostable purified endoxylanase which released 35% of the cell-wall xylan content whereas Benamrouche *et al.* (2002) with the same type of endoxylanase achieved an enzymatic solubilization of 50%, with a composition of 69.2% xylose, 14.5% arabinose and 16.5% glucose. Van Craeyveld *et al.* (2010) tested 3 different xylanases on 15 wheat brans and extracted 32-55% of the total wheat bran AXs. The highest yield in the present work reached 68.8% of the AXs, with Ultraflo L. The composition of the hydrolysate after 24 h of reaction was 35.1% xylose, 11.7% arabinose, 27.7% glucose and 1.1% galactose (Table 7.3A). Andersson *et al.* (2003) solubilised 70% of the xylose and 58% of the arabinose of autoclaved rye bran after treatment with Bio-Feed Plus, a commercial enzyme preparation with endo-xylanase activity. In the present study Ultraflo L, after 24 h of reaction, released 71.7% of the xylose and 60.7% of the arabinose originally in the bran. In the study of Sorensen *et al.* (2003) on enzymatic hydrolysis of water-soluble wheat arabinoxylan, Ultraflo L alone released 53% of the theoretical maximum of arabinose after 48h of reaction while Celluclast alone enabled the release of 26% of the theoretical maximum of xylose after 48h of reaction. A synergistic interaction was observed between Celluclast 1.5L and Ultraflo L (Sorensen *et al.*, 2005). In the present study, the combination of Celluclast and Ultraflo L provided higher yields of extraction of AXs than did the Ulftraflo L alone. Indeed, the combination with Celluclast enabled a yield increase of 0.4-8.2% depending on the reaction time. Sorensen *et al.* (2007) explained that a synergism had been observed on soluble and insoluble arabinoxylan, which was the effect of positive interaction in arabinose release and xylan depolymerisation between the α-L-arabinofuranosidase and endo-1,4-β-xylanase activities present in Ultraflo L and between the β-xylosidase activity present in the Celluclast 1.5L. On rye bran, a combination of β-glucanase with one endoxylanase improved the AX solubilization (Figueroa-Espinoza *et al.*, 2004). Such an increase was also observed in the present work as commercial preparations with mainly endoxylanase activity (Belfeed, Rohalase, Pentopan and Shearzyme 2X) led to lower yields than the tested commercial preparation with endoxylanase plus glucanase activities (Ultraflo L) whatever the reaction time.

For spelt bran hydrolysates, in experiments 9, 11-14, A/X ratios were 0.24-0.33 and 0.26-0.32 in experiments 10, 15-19 (for 24 h reaction). In Van Craeyveld *et al.* (2010), the A/X ratios of the xylanase solubilised AXs were 0.27-0.34 (for 24 h reaction) and a significant negative correlation was observed between extraction yield and A/X ratio (r=-0.7). In the present study, yield of extraction and A/X ratio were also negatively correlated with r=-0.44 (P=0.05) (exp 9, 11-14) and r=-0.82 (P<0.0001) (exp. 10, 15-19). It was also observed that the A/X ratio of spelt bran of the present study was lower (0.39) than for wheat brans from other studies (0.53-0.71) (Beaugrand *et al.*, 2004a; Gebruers *et al.*, 2008). This characteristic could explain the higher extraction yield of AXs obtained for spelt bran. Indeed, Brillouet and Joseleau (1987), Schooneveld-Bergmans *et al.* (1999), Benamrouche *et al.*

(2002) and Beaugrand *et al.* (2004c) reported a resistance to enzymatic degradation of highly substituted wheat bran AX. Beaugrand *et al.* (2004a) pointed out the accessibility of the enzymes to the cross-linked polymer network of the bran as an important factor determining the hydrolysis of AXs. The differences in the extraction yields of AXs from brans could also be explained by the difference of susceptibility to xylanase attack of each tissue and by the proportion of the different tissues composing the bran. It was observed that the inner bran, aleurone and nucellar layers were attacked by endoxylanase while the outer bran, pericarp and testa remained unaltered (Benamrouche *et al.*, 2002; Beaugrand *et al.*, 2004c). AXs in nucellar epidermis and aleurone have a lower A/X ratio than those in pericarp (Antoine *et al.*, 2003; Barron *et al.*, 2007). According to Van Craeyveld *et al.* (2010), the difference in average A/X ratio of bran from different cultivars is due to varietal variation in AX content and/or to the proportion of the different tissues in the bran rather than to varietal differences in the A/X ratio of a specific tissue.

As explained above, the content of monomers is a key feature of the fractions. All hydrolysates contained monomers of glucose, in higher concentrations with Celluclast, Shearzyme 2X and Ultraflo L (Table 7.3A). Monomers of arabinose were only found in hydrolysates from Ultraflo L. Hydrolysates from Belfeed, Pentopan and Rohalase contained no monomers of xylose. Xylobiose content was the highest in hydrolysates from Ultraflo L and Shearzyme 2X. The combination of Celluclast with the other enzymes increased dramatically the content of monomers of glucose and xylose. In Van Craeyveld *et al.* (2010), after xylanase treatment monomeric arabinose and xylose contents were respectively 0.1-1.6% and 1.5-5.5% (for 24 h reaction). In the present work monomeric xylose ranged from 0 to 3.5%, arabinose was null and glucose was 3.8-6.4% for the hydrolysates from preparation with xylanase as main activity (exp. 9, 11-13) and were respectively 6.9%, 6.6% and 10.7% for the hydrolysate from Ultraflo L (exp.14) (for 24 h reaction). The hydrolysates obtained from Celluclast alone or combined were also richer in monomers of xylose 13.7-26.8%, arabinose 0.0-5.5% and glucose 14.5-21.2% (for 24 h reaction). Van Craeyveld *et al.* (2010) suggested that some wheat bran-associated enzymes (arabinofuranosidases and xylosidases) could produce monomers, however in the present case treatment at 100°C for 15 min after destarching et deproteination might decrease the enzymatic activity.

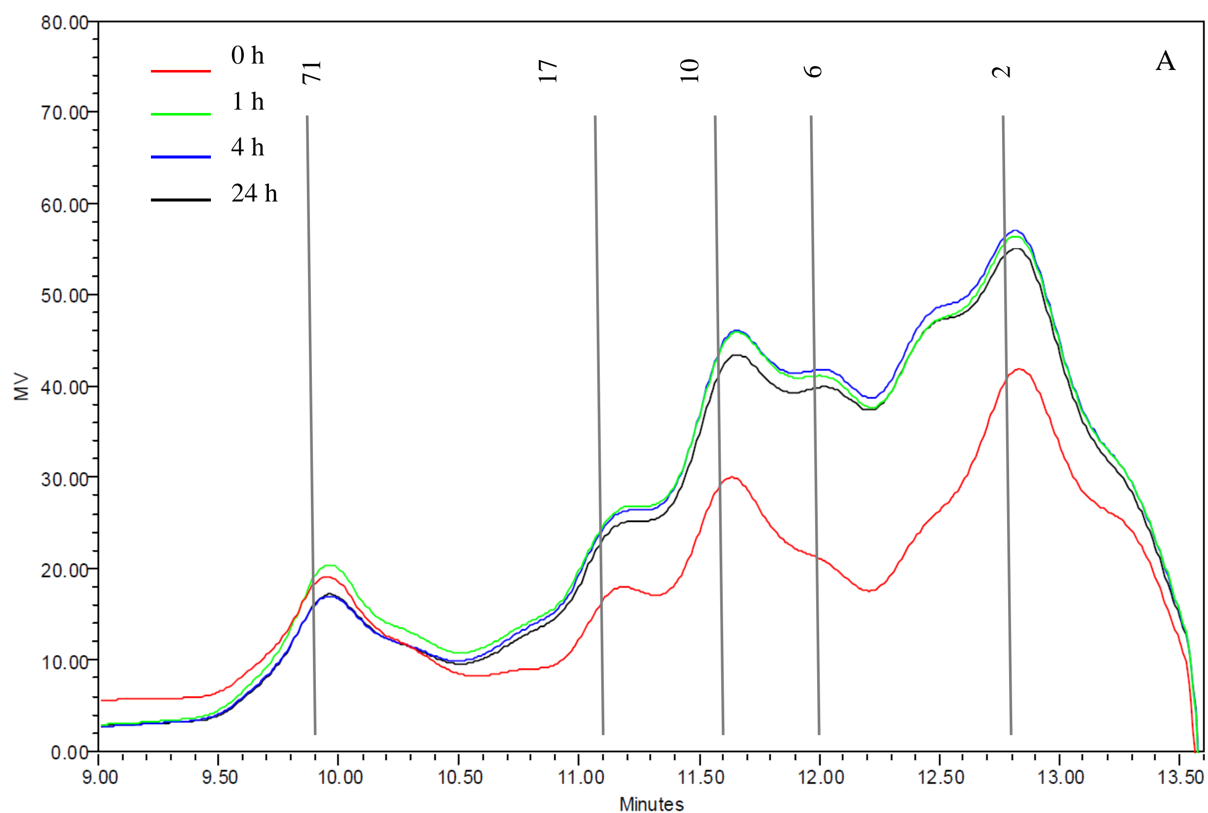
Concerning the proportion of the different DPs in the hydrolysates, for experiments 9, 11-14, DP 70-71 represented 18.7-22.8% of the initial fraction and decreased during the reaction until 10.9-13.4% of the fraction after 24 h (Table 7.5A). Shearzyme 2X (exp. 13) was an exception in this change since DP 71 remained stable around 10% during the reaction. At the beginning of the reaction, DP 2 was already present with 45.6-58.3% of the soluble fraction (exp. 9, 11-14). The DP 2 ratio remained stable during the reaction where it represented 47.1-53.1% of the fraction after 24 h (exp. 9, 11, 12), except for Shearzyme 2X and Ultraflo (exp. 13 and 14) where it represented 66.2-66.6% of the fraction after 24 h (Fig. 7.1E and 7.1F). Hydrolysates from Pentopan and Belfeed displayed similar

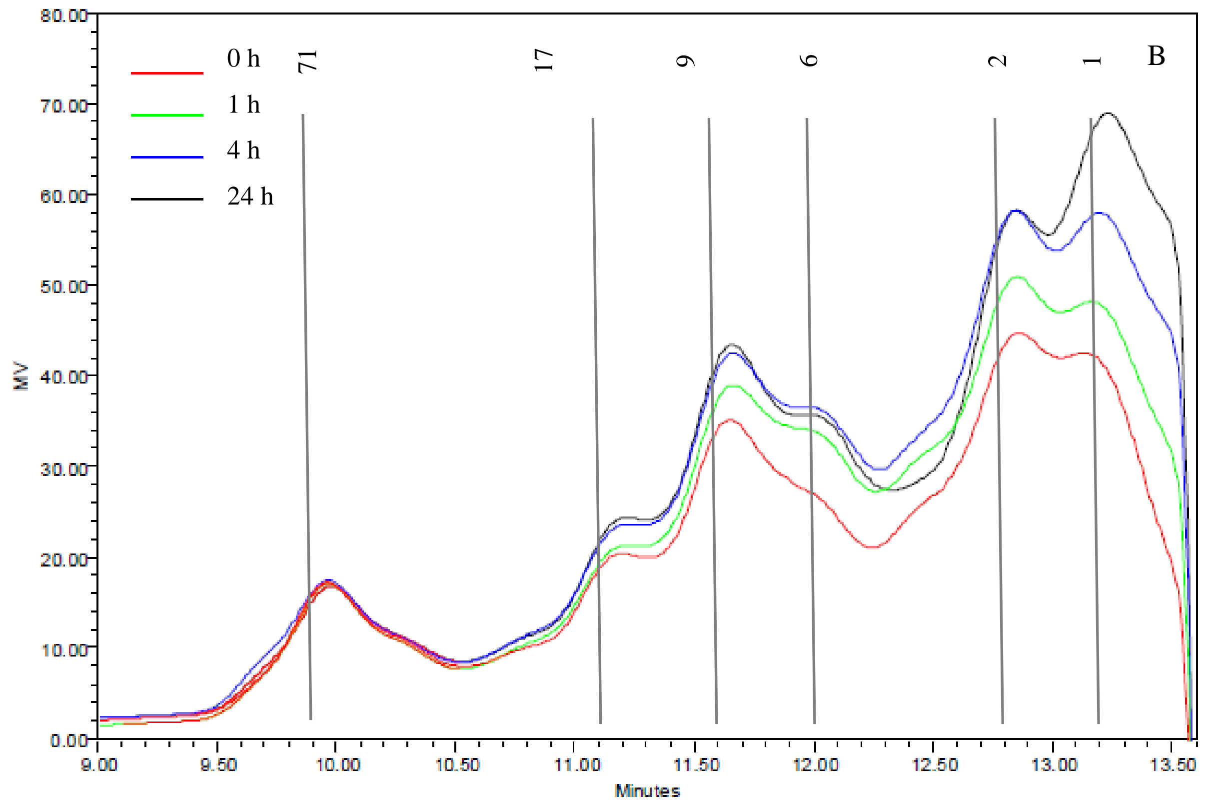
profiles while the one from Rohalase was different (Fig. 7.1A, 7.1C, 7.1D). The hydrolysate from Celluclast was the only one where DP1 had a peak (Fig. 7.1B). The proportion of others DPs (6-7 to 16-17) was changing during the reaction. Hydrolysates from combination with Celluclast contained a new fraction of DP 1138-1164 which represented 0.9-2.9% of the initial fraction and 0.5-3.3% after 24h of reaction. The change of this fraction during the reaction differed from enzyme to enzyme but it underwent generally an increase after 24 h, except for Ultraflo associated with Celluclast where a decrease was observed, together with an increasing fraction of monomers, in accordance with the measured content of monomers (Table 7.3A). Hydrolysates from combination with Celluclast had 54.5-70.9% of DP 1 after 24 h and the content in total free monomers was 29.1-55.1%. The DP 69-72 generally decreased during the reaction while the other DPs had different dynamics. Maes *et al.* (2004) observed on wheat bran that *Bacillus subtilis* endoxylanase released enzymatic-solubilised arabinoxylans with relative high MW (2000-30900 Da) while those released by *Aspergillus aculeatus* endoxylanase had relative low MW (1000-1200 Da). This was also the case in the present study since hydrolysates of spelt bran from *Bacillus subtilis* (Belfeed) contained 73.1% of DP 2-10 and 26.9% of DP 17-71 while those from *Aspergillus aculeatus* (Shearzyme 2X) contained 81.8% of DP 2-10 and 18.2% of DP 16-71. In Benamrouche *et al.* (2002) the main components released were short xylooligosaccharides (DP 2-4) with minor amounts of longer xylose-containing oligosaccharides (DP ≥5) as in the hydrolysate from Ultraflo L (after 24 h of reaction) in which the main oligosaccharides had a DP 2 (66.6% of the total) and DP 6 (5.4% of the total).

Destarched, deproteinated bran and hull contained respectively 10.2% and 1.3% of glucose and it was found in hydrolysates up to 50.4% in some cases. The presence of glucose in such fractions is quite common, however to our knowledge its origin is seldom if ever discussed. Glucose might have several origins: 1) from resistant starch whose content was respectively 0.3% and 0.1% in destarched deproteinated bran and hull, representing 0.33% and 0.11% glucose (results not shown); 2) from the starch which was not degraded, whose content was 0.5% in destarched deproteinated bran (0% in hull), which corresponded to 0.55% glucose; 3) from the remains of destarching (such as dextrins) in spite of intensive washing of the residue, which were evaluated to be 2.4% in the case of bran (0% for hull), representing 2.66% glucose (results not shown); 4) from the β-glucans, however they accounted for 0.54 % in destarched deproteinated bran (0% in hull), representing 0.6% glucose (results not shown); 5) from xylo-glucans nevertheless they are usually minor components of cell walls of monocotyledons (Bacic *et al.,* 1988); 6) from the commercial enzymatic preparations, though monosaccharides analysis of the commercial enzymatic preparations showed that none contained free glucose (results not shown); 7) from the degraded cellulose by cellulolytic enzymes however hydrolysates from endoxylanases alone also had high contents of glucose as observed in the hydrolysates from Belfeed and Pentopan for instance; 8) from amorphous cellulose (Willför *et al.,* 2009) whose proportion is unknown.

Consequently, the proportion of amorphous cellulose in spelt bran should be measured and the content of glucose liberated by the commercial enzymatic preparation should be quantified.

The introduction of an ultrafiltration step could reduce the content in free glucose of the fractions and thus improve their purity.





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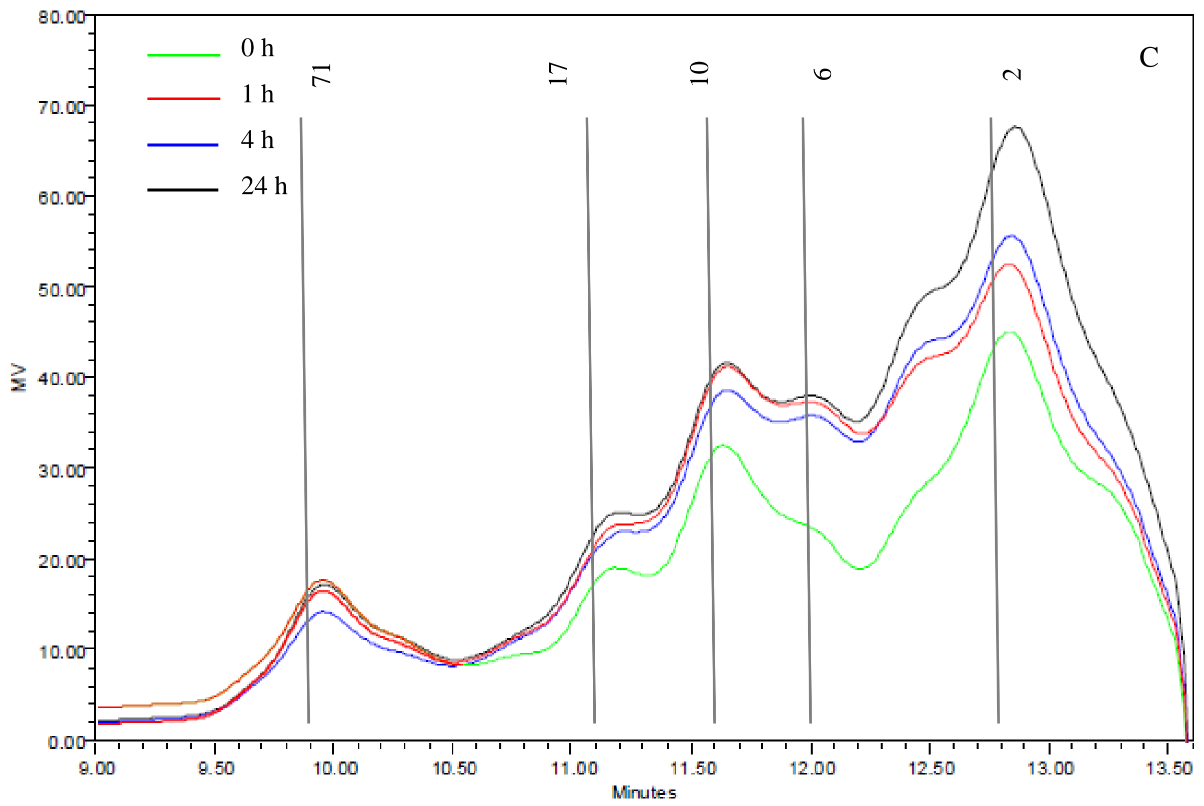

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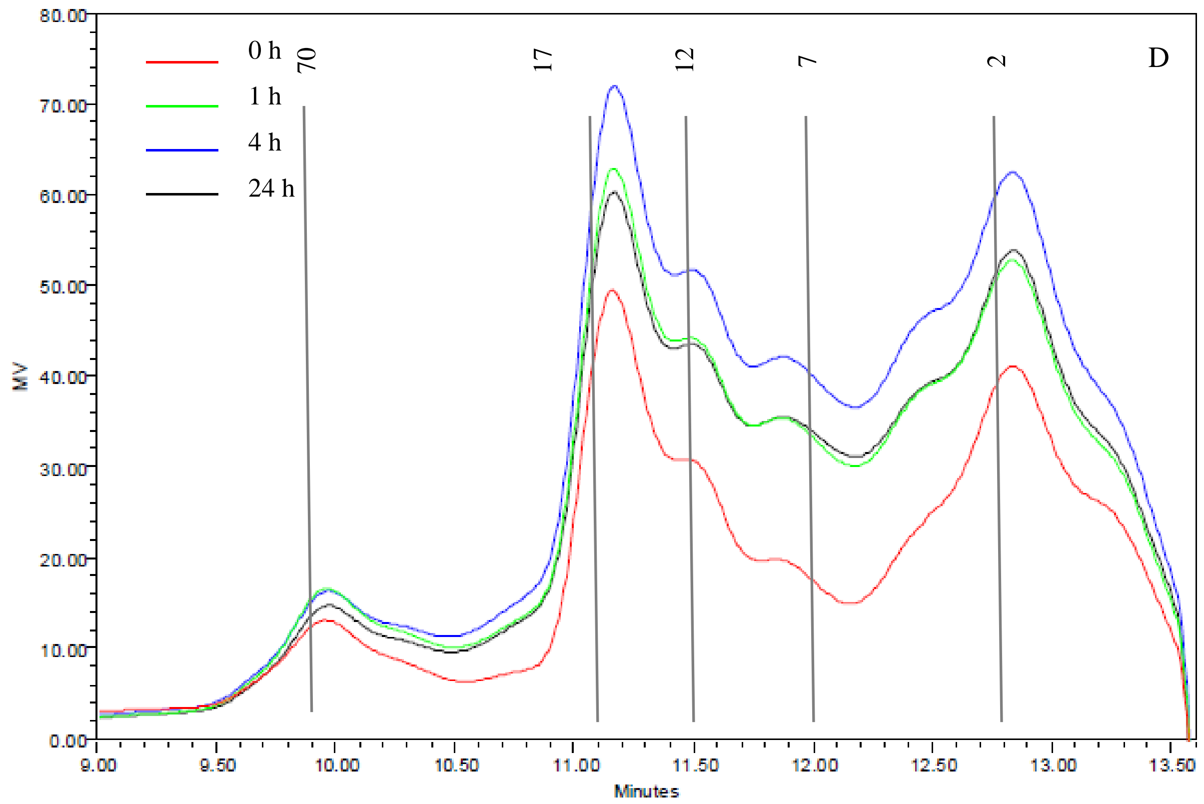
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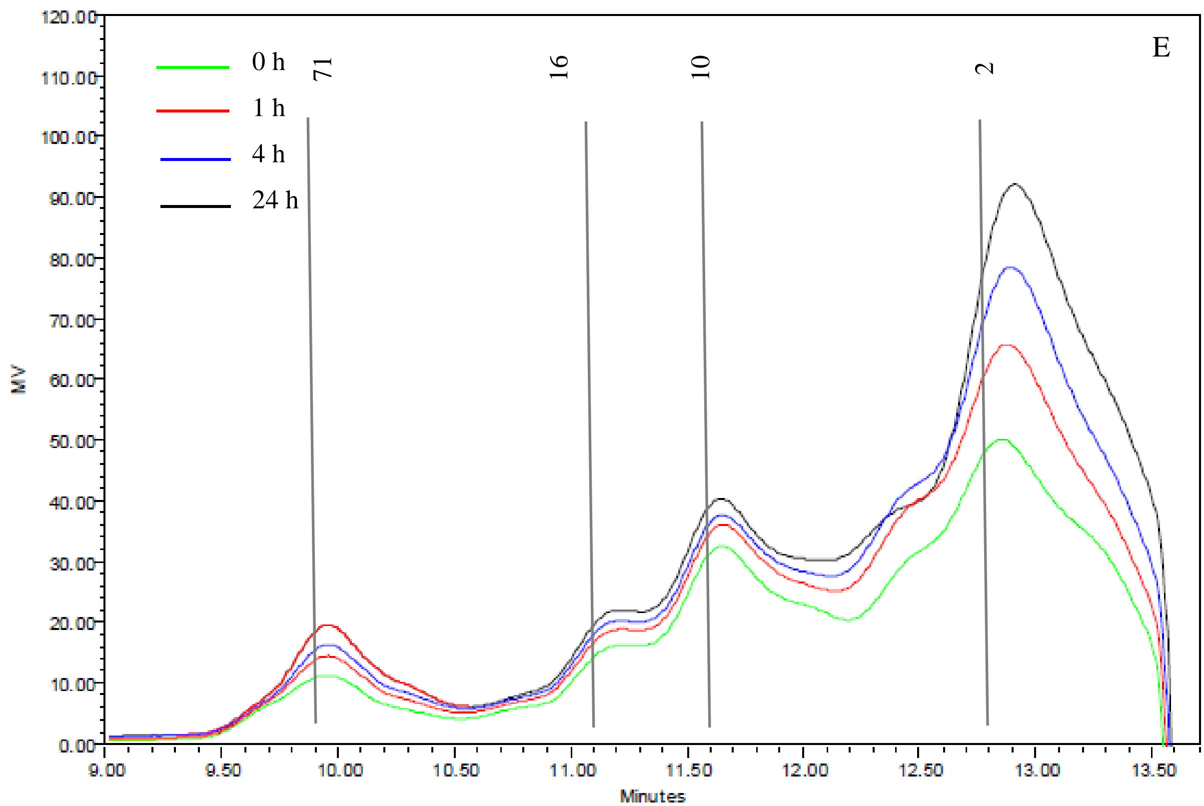
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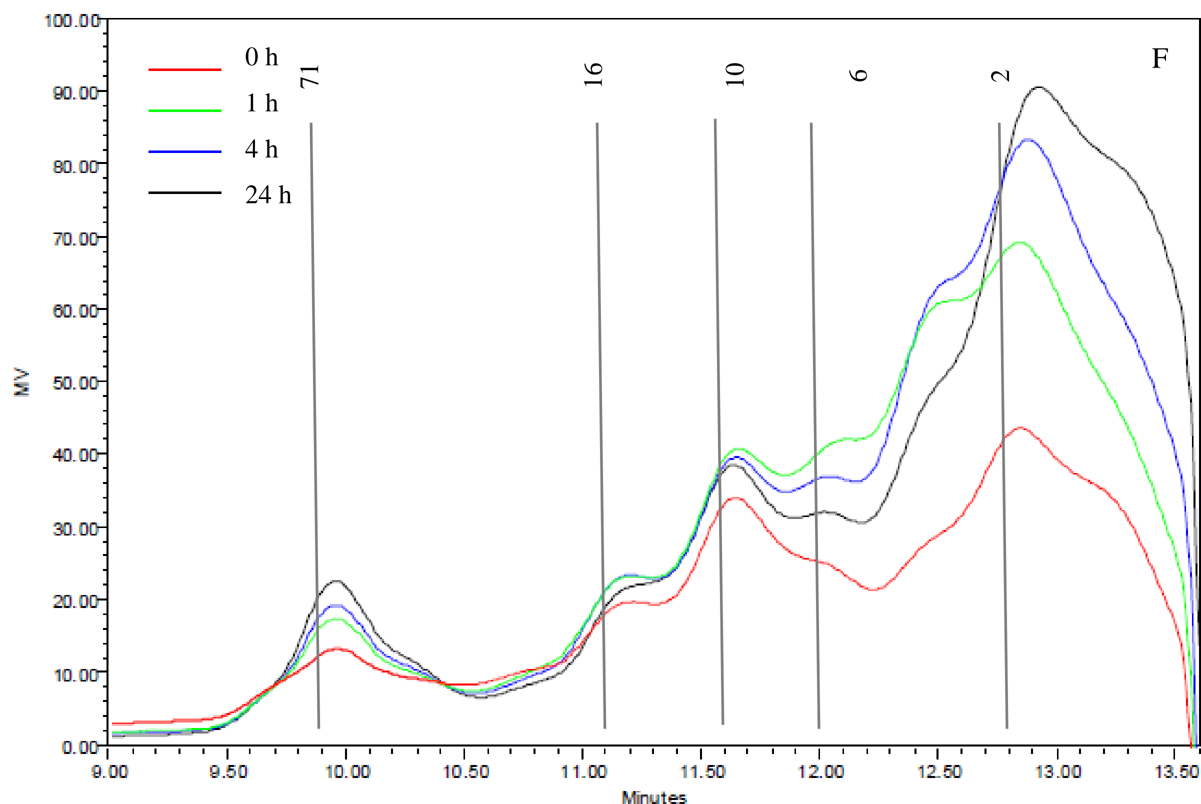

Figure 7.1. HPSEC Profiles of the spelt bran hydrolysates at 0 h, 1 h, 4 h and 24 h reaction from Belfeed (A), Celluclast (B), Pentopan (C), Rohalase (D), Shearzyme 2X (E), Ultraflo L (F). Estimated degrees of polymerisation (DP) are indicated in grey on the corresponding vertical lines.

Experiment number/ Time (h)	AX				Xylose				Arabinose				A/X			
	0	1	4	24	0	1	4	24	0	1	4	24	0	1	4	24
9	24.5	34.2	35.9	38.9	26.4	38.3	40.3	43.8	19.8	23.9	24.7	26.6	0.29	0.25	0.24	0.24
10	21.7	38.3	43.4	48	21.1	40.5	46.7	50.8	23.4	32.6	35.2	41	0.44	0.32	0.30	0.32
11	21	46.3	45.2	52.9	20.7	49.9	48.9	57.9	21.8	37.1	35.9	40.3	0.42	0.29	0.29	0.27
12	18.1	42.4	45	44.3	17.6	44.9	48.3	47.5	19.3	35.8	36.8	36.1	0.43	0.31	0.30	0.30
13	18.4	43.3	57.2	63	17.7	44.3	61.1	68.9	20.3	40.9	47.3	48.2	0.45	0.36	0.30	0.28
14	36.9	60	63.3	68.6	38.4	63.6	66	71.7	33.3	50.8	56.5	60.7	0.34	0.31	0.34	0.33
15	34.8	64.1	67.2	69.7	36.3	70.1	73.9	76.9	31	48.7	50.3	51.6	0.34	0.27	0.27	0.26
16	26.6	58.5	58.5	62.6	26.5	63.6	63.7	68.1	26.8	45.5	45.3	48.7	0.40	0.28	0.28	0.28
17	21.8	53.2	54.2	59.4	21.4	57.7	58.9	64.8	22.6	41.8	42.3	45.6	0.42	0.29	0.28	0.28
18	26	69.7	74.1	74.8	25.7	75.3	79.4	80.3	26.8	55.7	60.6	61	0.41	0.29	0.30	0.30
19	41.5	63.1	68.5	69	43.2	66.9	72.5	72.7	37	53.4	58.2	59.7	0.34	0.31	0.32	0.32
20	0.5	1.8	2.1	2.6	0.5	1.8	2.2	2.6	0.7	1.7	1.9	2.7	0.21	0.15	0.14	0.16
21	0.6	2	2.9	3.7	0.6	1.9	2.7	3.5	0.8	2.6	3.8	4.9	0.22	0.22	0.23	0.23
22	0.2	0.6	0.7	1.5	0.2	0.6	0.8	1.4	0	0	0	1.8	0	0	0	0.20
23	0.1	0.7	0.8	1.3	0.1	0.8	0.9	1.2	0	0	0	1.6	0	0	0	0.21
24	0.5	1.3	2	3.9	0.5	1.3	2	4.1	0.5	1.4	1.8	2.9	0.17	0.18	0.14	0.11
25	1	2.2	3.7	6.4	0.9	2.1	3.5	6.3	1.8	3.2	4.8	7	0.32	0.24	0.22	0.18

Table 7.4. Yields of solubilization (%) of AX, of xylose and arabinose, and the calculated A/X ratios, for hydrolysates from spelt bran and hull at 0 h, 1 h, 4 h and 24 h. See Table 7.1 for experiment numbers.

Experiment number/ Time (h)	DPs	Repartition of DPs %			
		0	1	4	24
9	71	20	14.6	13.4	13.3
	17	12.8	14.6	13.9	13.7
	10	13.7	15.1	14.1	16
	6	5.8	9.6	10.4	10
	2	47.7	46.1	48.1	47.1
10	70	17.4	12.1	11.2	10.2
	17	12.1	13	12.2	10.7
	9	14.1	13	13.6	15.2
	6	7.9	10.1	9.6	9.7
	2	29.3	30.7	28.6	23.5
	1	19.2	21.2	24.8	30.7
11	71	22.8	13.8	13.3	13.4
	17	12.2	13.7	14	10.7
	10	12.8	14.6	11.8	14
	6	6.5	10.1	10	8.8
	2	45.6	47.7	50.8	53.1
12	70	21.1	14.2	12.2	12.9
	17	11.9	16.9	17.7	15.8
	12	7.1	8.9	9.7	9.2
	7	8.4	12.2	12.5	12.6
	2	51.5	47.8	47.9	49.5
13	71	10.2	10.1	9.8	10
	16	6.9	8.8	8.4	8.2
	10	24.7	19.2	17.4	15.6
	2	58.3	61.9	64.5	66.2
14	71	18.7	11.9	9.4	10.9
	16	12.1	8.9	8.3	8.2
	10	14.3	11.5	10.3	9
	6	7.5	12.4	7.2	5.4
	2	47.5	55.4	64.8	66.6
15	1151	2.9	3	3.2	3.3
	73	12.1	10.4	9.4	9.3
	16	21.6	18.5	16	16.6
	7	13.1	14	14	16.3
	1	50.3	54.1	57.5	54.5
16	1157	1.7	2.5	2.9	2.7
	72	11.9	9.5	9.4	8.7
	16	20.9	19.2	18.2	16.1
	8	13.1	13.8	12	16.2
	1	52.3	54.9	57.6	56.4

17	1164	1.2	2.5	2.5	2.7
	69	10.7	8.8	8.6	8.5
	14	18.5	18.4	17.3	16
	7	12.7	15.4	14.7	16.2
	1	56.9	54.9	56.8	56.6
18	1150	0.9	1.5	1.3	1.2
	69	9	7.9	7.5	7.5
	16	17.1	14.5	13.5	13.7
	8	11.1	7.2	7.1	6.7
	1	61.9	68.9	70.6	70.9
19	1138	2.2	1.2	0.8	0.5
	68	10.3	8.2	7.6	9.3
	12	35.9	23.6	22.6	26.8
	1	51.5	66.9	69	63.4

Table 7.5.A Distribution of the degree of polymerization (DP) according to HPSEC determination in hydrolysates from spelt bran at 0 h, 1 h, 4 h and 24 h. See Table 7.1 for experiment numbers.

7.3.3.5 Hull hydrolysates

Table 7.3B displays the total content in sugar and free monomers of the hydrolysates. No rhamnose was detected (neither as polysaccharide neither as monomer) except for the experiment 23 where 0.6%±0.6% was quantified as oligo- or polysaccharide. No free galactose and mannose were detected. Whereas all enzymes released monomers of glucose from bran, only Celluclast, Shearzyme 2X and Ultraflo L released monomers of glucose from hull (exp. 21, 24, 25). After 24 h of reaction, Ultraflo L and Celluclast were the only enzymes which released monomers of xylose, respectively 6.1 and 7.0%. The hydrolysates from Ultraflo L also contained 2.2% of free arabinose and 4.4% xylobiose after 24 h of reaction (Table 7.3B). In hull hydrolysates, total arabinose content ranged from 0.9% to 3.8% and total xylose content from 4.3% to 21.9% (for 24 h reaction). Total glucose contents were 50.4% for hydrolysates from Celluclast (exp. 21) and 1.8-25.3% for all others (exp. 20, 22-25). Generally, total saccharides' concentrations were lower in hull than in bran hydrolysates. The enzymatic hydrolysate from wheat straw contained 64.9% xylose, 13.1% arabinose and 15.2% glucose (Lequart *et al.,* 1999). The strong release of glucose by Celluclast indicated the efficiency of the cellulolytic activity on hull and consequently no combination with xylanase was tested as glucose extraction was to be avoided in the present work (Table 7.3B). The hydrolysate from Belfeed (exp. 20) contained no monomers and 11.3% xylose, 1.6% arabinose and 15% glucose after 24 h. Hydrolysate from Pentopan (exp. 22), in spite of a very low content in total saccharides, displayed an interesting profile with a higher xylose than glucose content.

Experiment number	Time	Total sugars						Free monomers and xylobiose			
		Ara	Xyl	Man	Glu	Gal	A/X ratio	Ara	Xyl	Glu	Xylob
DD HULL		2.6 ± 0	16.4 ± 0.2	0.2 ± 0	1.3 ± 0	1.1 ± 0	0.16				
20	0	0.4 ± 0.4	1.9 ± 0.1	n.d.	14 ± 0.2	0.3 ± 0.3	0.70	n.d.	n.d.	n.d.	n.d.
	1	1.1 ± 0	7.3 ± 0.1	n.d.	12.4 ± 0.2	n.d.	0.16	n.d.	n.d.	n.d.	n.d.
	4	1.6 ± 0	11.3 ± 0.2	n.d.	15 ± 0.1	n.d.	0.13	n.d.	n.d.	n.d.	n.d.
	24	2 ± 0	12.2 ± 0.2	n.d.	14.4 ± 0	n.d.	0.14	n.d.	n.d.	n.d.	n.d.
21	0	0.5 ± 0.5	2.2 ± 0.2	0.5 ± 0.5	26.3 ± 0.3	0.5 ± 0.5	0.22	n.d.	n.d.	25.1 ± 0.2	n.d.
	1	1.5 ± 0.1	6.8 ± 0.1	0.7 ± 0.1	35.2 ± 0.8	0.9 ± 0.1	0.22	n.d.	3.7 ± 0	26.9 ± 0.1	n.d.
	4	2.5 ± 0.3	10.9 ± 0.4	1.2 ± 0.1	46.8 ± 0.4	1.6 ± 0.2	0.23	n.d.	6.3 ± 0	40.8 ± 0.2	n.d.
	24	2.6 ± 0.1	11.3 ± 0.4	1.1 ± 0	50.4 ± 1.2	1.5 ± 0	0.23	n.d.	7 ± 0.1	41.2 ± 0.4	n.d.
22	0	n.d.	1.5 ± 0.2	n.d.	0.8 ± 0.8	n.d.	0.47	n.d.	n.d.	n.d.	n.d.
	1	n.d.	2.1 ± 0	n.d.	0 ± 0	n.d.	0.22	n.d.	n.d.	n.d.	n.d.
	4	n.d.	5.7 ± 0.2	n.d.	0.8 ± 0.8	n.d.	0.16	n.d.	n.d.	n.d.	n.d.
	24	1.5 ± 0	7.6 ± 0.4	n.d.	1.8 ± 0.3	n.d.	0.17	n.d.	n.d.	n.d.	n.d.
23	0	n.d.	0.5 ± 0.5	n.d.	1.3 ± 0	n.d.	0	n.d.	n.d.	n.d.	n.d.
	1	n.d.	2.8 ± 0.1	n.d.	1.2 ± 0	n.d.	0	n.d.	n.d.	n.d.	n.d.
	4	n.d.	3.3 ± 0	n.d.	1.8 ± 0.1	n.d.	0	n.d.	n.d.	n.d.	n.d.
	24	0.9 ± 0	4.3 ± 0.2	n.d.	3.3 ± 0.1	n.d.	0.21	n.d.	n.d.	n.d.	n.d.
24	0	0.4 ± 0.4	2.6 ± 0.2	n.d.	11.8 ± 0.8	n.d.	0.17	n.d.	n.d.	10.2 ± 0.1	n.d.
	1	1.2 ± 0	6.5 ± 0.1	n.d.	11.7 ± 0.3	n.d.	0.18	n.d.	n.d.	10.6 ± 0.2	n.d.
	4	1.5 ± 0	10.8 ± 0.4	n.d.	11.6 ± 0.4	n.d.	0.14	n.d.	n.d.	10.6 ± 0.1	n.d.
	24	2.5 ± 0.1	21.9 ± 0.1	n.d.	13.5 ± 0	n.d.	0.11	n.d.	n.d.	11.4 ± 0	7.5 ± 0.1
25	0	1.6 ± 0.2	5.1 ± 0.1	n.d.	25.4 ± 0.6	0.8 ± 0.8	0.32	n.d.	n.d.	22.6 ± 0	n.d.
	1	2.3 ± 0	9.5 ± 0.1	n.d.	21.1 ± 0.1	0.9 ± 0.1	0.24	n.d.	n.d.	19.1 ± 0	n.d.
	4	3.3 ± 0.1	14.9 ± 0.5	n.d.	22.9 ± 0.6	1 ± 0.1	0.22	n.d.	n.d.	20.1 ± 0.1	n.d.
	24	3.8 ± 0.1	21.6 ± 0	n.d.	25.3 ± 0.3	1.2 ± 0.1	0.18	2.2 ± 0	6.1 ± 0	23.2 ± 0	4.4 ± 0

Table 7.3.B Content of total saccharides, monomers and xylobiose (%, d.m.) (± SD) in hydrolysates from spelt hull at 0 h, 1 h, 4 h and 24 h.

Yields of extraction of arabinose and xylose were very weak for hull hydrolysates, between 1.3 and 6.4% after 24 h of reaction (Table 7.4, Appendix Fig.A17 and A.18). As for bran hydrolysates, a high proportion of AXs solubilized in the hull hydrolysates after 24 h was already present initially (0 h).The increase of the yield between 1 h and 24 h ranged between 0.6 and 4.1%. Enzymes whose main activity was xylanase did not manage to extract satisfyingly the arabinoxylans from the hull (Rohalase, Pentopan, Belfeed). Celluclast which combines cellulolytic and xylanolytic activities did neither achieve a satisfying extraction yield. These differences between hydrolysates from bran and hull supposed that the accessibility of the enzymes to the arabinoxylans might be different, or that the composition might change, or that the structure might be distinct between both materials (Table 7.4). Contrary to bran hydrolysates, a positive correlation ($r=+.037$, $P=0.07$) was found between the yields of extraction and the A/X ratios. However this result should to be taken with caution as the range of yields of extraction was narrow and consequently the correlation might not be representative. A/X

ratios of the hull hydrolysates, which were lower than those of bran hydrolysates, varied between 0.11 and 0.23 (for 24 h reaction).

Spelt hull had a composition close to wheat straw (Escarnot *et al.,* 2011b). Lequart *et al.* (1999) and Rémond *et al.* (2010) hydrolysed wheat straw with an endoxylanase which released respectively 18% and 20% of the cell-wall xylan. The higher yields of extraction than in the present study may be explained by pretreatment which consisted in autoclaving (45 min, 121°C) and/or swelling of straw in water (16 h, 60°C) or soaking in aqueous ammonia (30% v/v, 3 days at room temperature). Some authors ascribed to lignin the low efficiency of endoxylanases. Sewalt *et al.* (1997) hypothesized that polysaccharides associated to lignins may well be inaccessible to endoxylanases due to the physical barrier of lignin. Or xylanases adsorption on straw lignin may limit the efficiency of xylanases (Rémond *et al.,* 2010). This phenomenon may also happen for spelt hull even if the lignin content (5.7%) is lower (Escarnot *et al.*, 2011b) than in wheat straw 10-30% (Lequart *et al.*, 1999). The lower hydrolysis efficiency of AXs from hull than from bran cannot be ascribed to the arabinose substitution, since wheat bran is more substituted by arabinose than wheat straw (Lequart *et al.*, 1999).

The range of DPs of the hull hydrolysates was very reduced, between 1 and 17 (Table 7.5B). The highest DP was 17 for Rohalase (exp. 23) and 9 for all other enzymatic preparations far much lower than those of bran hydrolysates. The highest DP of each hydrolysate decreased during the reaction, it represented initially 12.7-79% and 10.2-62.3% after 24 h. Fractions of DP 1 represented 61.1-89.2% for experiments 21, 24, 25 and 5.8-7.6% for experiments 20, 22, 23 at the end of the reaction (Table 7.5B). The majority of products of the hydrolysates were DP 1 for Celluclast, Shearzyme 2X and Ultraflo L (exp. 21, 24, 25) and almost all hydrolysates had DP lower than 10. The range of the DPs was in accordance with requirements for a prebiotic preparation for experiments 20, 22 and 23. Celluclast 1.5L showed a good efficiency to release xylose, however less than Shearzyme 2X and Ultraflo L with respective extraction yields of AXs of 3.5%, 4.1% and 6.3%, and better than the rest of enzymatic preparations.

Experiment number/ Time (h)	DPs	Repartition of DPs %			
		0	1	4	24
20	9	61.2	66.1	56.4	57
	2	38	31.2	37.1	37.3
	1	0.8	2.7	6.5	5.8
21	9	16.4	11.9	16.7	10.8
	1	83.6	88.1	83.3	89.2
22	9	79	44.2	59.1	62.3
	2	23.9	59.1	36.3	30.4
	1	0	0	4.6	7.3
23	17	59.5	37.1	19.3	49.6
	7	16	27.9	32.8	20.5
	2	24	27.5	37.7	22.4
	1	0.6	7.6	10.2	7.6
24	9	26.1	25.4	3.4	14.9
	2	20.8	24.5	26.3	24
	1	53.1	50.1	70.3	61.1
25	9	12.7	16.2	12.2	10.2
	2	10.8	21	26.3	25.3
	1	76.5	62.8	61.5	64.5

Table 7.5.B Distribution of the degree of polymerization (DP) according to HPSEC determination in hydrolysates from spelt hull at 0 h, 1 h, 4 h and 24 h.

Physical pretreatments, such as extrusion (Figueroa-Espinoza *et al.,* 2004) or hydrothermal (Lequart *et al.,* 1999) should be considered in order to possibly increase the rate of solubilization of AXs from hull as it appeared that a fraction of the AXs remained inaccessible to the enzymes.

7.3.4 Conclusion

To our knowledge, this is the first study on the enzymatic hydrolysis of AXs from spelt bran and hull. The xylanolytic activity of the tested commercial enzyme preparations was highly variable and the side activities had a strong impact on the profiles of the hydrolysates. The contents of free xylose and glucose were heterogeneous among the hydrolysates. The solubilization of AXs from spelt bran was at least as efficient as that of wheat bran. However, the purity of the hydrolysates should be improved, through a decrease of the glucose content. The concentrations of free xylose and glucose remained too high in some hydrolysates and should be improved. The range of DPs of the bran hydrolysates from endoxylanase combined with Celluclast should be reduced in order to eliminate high DPs. Concerning hull, the hydrolysates were less concentrated in saccharides than those from bran and the efficiency of the hydrolysis remained low. However the DPs of the hydrolysates corresponded well to the DPs required for prebiotic preparations, which was not the case for hydrolysates from bran.

If yields of extraction from spelt hull could be improved, spelt hull could be used as a source of different prebiotics. If it is not the case, the hydrolysis of AXs could be made simultaneously for bran

and hull in order to combine high yield and interesting profile. Physical treatment should be tested to improve the accessibility of the enzymes to the substrate, especially for hull.

7.3.5 Acknowledgments

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7.3.6 References

Andersson R., Eliasson C., Selenare M., Kamal-Eldin A., Aman P., 2003. Effect of endo-xylanase-containing enzyme preparations and laccase on the solubility of rye bran arabinoxylan. *Journal of the Science of Food and Agriculture*, 83, 617-623.

Antoine C., Peyron S., Mabille F., Lapierre C., Bouchet B., Abecassis J., Rouau X., 2003. Individual contribution of grain outer layers and their cell wall structure to the mechanical propertes of wheat bran. *Journal of Agricultural and Food Chemistry*, 51, 2026-2033.

Bacic A., Harris P. J., Stone B.A., 1988. Structure and function of plant cell walls. *The Biochemistry of Plants,* 14, 297-370.

Barron C., Surget A., Rouau X., 2007. Relative amounts of tissues in mature wheat (Triticum aestivum L.) grain and their carbohydrate and phenolic acid composition. *Journal of Cereal Science*, 45, 88-96.

Beaugrand J., Crônier D., Debeire P., Chabbert B., 2004a. Arabinoxylan andhydroxycinnamate content of wheat bran in relation to endoxylanase susceptiblity. *Journal of Cereal Science,* 40, 223-230.

Beaugrand J., Chambat G., Wong V. W. K., Goubet F., Remond C., Paes G., Benamrouche S., Debeire P., O'Donohue M., Chabbert B., 2004b. Impact and efficiency of GH10 and GH11 thermostable endoxylanases on wheat bran and alkali-extractable arabinoxylans. *Carbohydrate Research*, 339, 2529-2540.

Beaugrand J., Reis D., Guillon F., Debeire P., Chabbert B., 2004c. Xylanase-mediated hydrolysis of wheat bran: evidence for subcellular heterogeneity of cell walls. *International Journal of Plant Science*, 165, 553-563.

Benamrouche S., Crônier D., Debeire P. and Chabbert B., 2002. A chemical and histological study on the effect of ((1->4)-β-endo-xylanase treatment on wheat bran. *Journal of Cereal Science*, 36, 253-260.

Bonafaccia G., Galli V., Francisci R., Mair V., Skrabanja V., Kreft, I., 2000. Characteristics of spelt wheat products and nutritional value of spelt wheat-based bread. *Food Chemistry*, 68, 437-441.

Brillouet J.M. and Joseleau J.P., 1987. Investigation of the structure of a heteroxylan from the outer pericarp (beeswing bran) of the wheat kernel. *Carbohydrate Research*, 159, 106-126.

Campbell K.G., 1997. Spelt: agronomy, genetics and breeding. *Plant Breeding Reviews* 15, 187-213.

Courtin C.M., Swennen K., Verjans P., Delcour J.A., 2009. Heat and pH stability of prebiotic arabinoxylooligosaccharides and fructooligosaccharides. *Food Chemistry*, 112, 831-837.

Direction générale de la Statistique et de l'Information économique, SPF Economie, P.M.E., Classes moyennes et Energie, Belgium, 2010.
http://statbel.fgov.be/fr/modules/publications/statistiques/economie/estimation_de_la_production_des_cultures_agricoles_annee_de_recolte_2009.jsp

Edwards S., Chaplin M.F., Blackwood A.D., Dettmar P.W., 2003. Primary structure of arabinoxylans of ispaghula husk and wheat bran. *Proceedings of the Nutrition Society*, 62, 217-222.

Englyst H.N. and Cummings J.H., 1984. Simplified method for the measurement of total non-starch polysaccharides by gas-liquid chromatography of constituent sugars as alditols acetates. *Analyst*, 109, 937-942.

Escarnot E., Agneessens R., Wathelet B., Paquot M., 2010. Quantitative and qualitative study of spelt and wheat fibres in varying milling fractions. *Food Chemistry*, 122, 857-863.

Escarnot E., Aguedo M., Agneessens R., Wathelet B., Paquot M., 2011a. Extraction and characterization of water-extractable and water-unextractablearabinoxylans from spelt bran: Study of the hydrolysis conditions formonosaccharides analysis. *Journal of Cereal Science*, 53, 45-52.

Escarnot E., Aguedo M., Paquot M., 2011b. Characterization of hemicellulosic fractions from spelt hull extracted by different methods. *Carbohydrate Polymers*, 85, 419-428.

Figueroa-Espinoza M. C., Poulsen C., Soe J. B., Zargahi M. R., Rouau X., 2004. Enzymatic solubilization of arabinoxylans from native, extruded, and high-shear-treated rye bran by different endo-xylanases and other hydrolyzing enzymes. *Journal of Agricultural and Food Chemistry*, 52, 4240-4249.

Gebruers K., Dornez E., Boros D., Fras A., Dynkowska W., Bedo Z., Rakszegi M., Delcour J. A., Courtin C. M., 2008. Variation in the content of dietary fiber and components thereof in wheats in the HEALTHGRAIN diversity screen. *Journal of Agricultural and Food Chemistry*, 56, 9740-9749.

Granström T.B., Izumori K., Leisola M., 2007. A rare sugar xylitol. Part II: biotechnological production and future applications of xylitol. *Applied Microbiology and Biotechnology*, 74, 273-276.

Hahn-Hägerdal B., Kahrumaa K., Fonseca C., Spencer-Martins I., Gorwa-Grauslund M.F., 2007. Towards industrial pentose-fermenting yeast strains. *Applied Microbiology and Biotechnology*, 74, 937-953.

Hucl P., Abdel-Aal E.M.S., Sosulski F.W., 1995. Specialty wheats. Page 40-47 in:Proc. Crop opportunities III conference. University of Saskatchewan, SK, Canada.

Iiyama K., Lam T.B.T., Stone B.A., 1994. Covalent cross-links in the cell wall. *Plant Physiology,* 104, 315-320.

Lequart C., Nuzillard J.-M., Kurek B., Debeire P., 1999. Hydrolysis of wheat bran and straw by an endoxylanase: production and structural characterization of cinnamoyl-oligosaccharides. *Carbohydrate Research*, 319, 102-111.

Maes C. and Delcour J.A., 2002. Structural characterisation of water-extractable and water-unextractable arabinoxylans in wheat bran. *Journal of Cereal Science,* 35, 315-326.

Maes C., Vangeneugden B., Delcour J.A., 2004. Relative activity of two endoxylanases towards water-unextractable arabinoxylans in wheat bran. *Journal of Cereal Science*, 39, 181-186.

Moore J., Cheng Z., Su L., Yu L.L., 2006. Effects of solid-state enzymatic treatments on the antioxidant properties of wheat bran. *Journal of Agricultural and Food Chemistry*, 54, 9032-9045.

Pala H., Mota M., Gama F.M., 2007. Enzymatic depolymerisation of cellulose. *Carbohydrate Polymers*, 68, 101-108.

Pastell H., Tuomainen P., Virkki L., Tenkanen M., 2008. Step-wise enzymatic preparation and structural characterization of singly and doubly substituted arabinoxylo-oligosaccharides with non-reducing end terminal branches. *Carbohydrate Research*, 343, 3049-3057.

Pomeranz Y., 1988. Chemical composition of kernel structure. In: Pomeranz, Y. (Ed), Wheat and chemistry technology. AACC, St Paul, Minnesota, U.S.A., pp 97-158.

Ranhotra G. S. and Gelroth J. A., 1995. Baking and nutritional qualities of a spelt wheat sample. *Lebensmittel-Wissenschaft und Technologie*, 28, 118-122.

Rantanen H., Virkki L., Toumainen P., Kabel M., Schols H., Tenkanen M., 2007. Preparation of arabinoxylobiose from rye xylan using family 10 *Aspergillus aculeatus* endo-1,4-β-D-xylanase. *Carbohydrate Polymers*, 68, 350–359.

Rémond C., Aubry N., Crônier D., Noël S., Martel F., Roge B., Rakotoarivonina H., Debeire P., Chabbert B., 2010. Combination of ammonia and xylanase pretreatments: Impact on enzymatic xylan and cellulose recovery from wheat straw. *Bioresource Technology*, 101, 6712-6717.

Ruibal-Mendieta N. L., Rozenberg R., Delacroix D., Petitjean G., Dekeyser A., Baccelli C., Marques C., Delzenne N.M., Meurens M., Habib-Jiwan J.-L., Quetin-Leclercq J., 2004a. Spelt (Triticum spelta L.) and winter wheat (Triticum aestivum L.) wholemeals have similar sterols profiles, as determined by quantitative liquid chromatography and mass spectrometry analysis. *Journal of Agricultural and Food Chemistry*, 52, 4802-4807.

Ruibal-Mendieta N. L., Delacroix D., Mignolet E., Pycke J.-M., Marques C., Rozenberg R., Petitjean G., Habib-Jiwan J.-L., Meurens M., Quetin-Leclercq J., Delzenne N.M., Larondelle Y., 2005. Spelt (Triticum aestivum ssp. Spelta) as a source of breadmaking flours and bran naturally enriched in oleic acid and minerals but not phytic acid. *Journal of Agricultural and Food Chemistry*, 53, 2751-2759.

Saulnier L., Guillon F., Sado P.-E., Rouau X., 2007. Plant cell wall polysaccharides in storage organs : xylans (food applications). In Comprehensive glycoscience. From chemistry to systems biology. Analysis of glycans. *Polysaccharide Functional Properties*. Vol 2, 653-687.

Schober T. J., Bean S. R., Kuhn M., 2006. Gluten proteins from spelt (Triticum aestivum ssp. spelta) cultivars: A rheological and size-exclusion highperformance liquid chromatography study. *Journal of Cereal Science,* 44, 161–173.

Schooneveld-Bergmans M. E. F., Beldman G., Voragen A.G.J., 1999. Structural features of (glucurono)arabinoxylans extracted from wheat bran by barium hydroxide. *Journal of Cereal Science*, 29, 63-75.

Sewalt V.J.H., Beauchemin K.A., Rode L.M., Acharya S., Baron V.S., 1997.. Lignin impact on fiber degradation. IV. Enzymatic saccharification and *in vitro* digestibility of alfalfa and grasses following selective solvent delignification. *Bioresource Technology*, 61, 199-206.

Sorensen H.R., Meyer A.S., Pedersen S., 2003. Enzymatic hydrolysis of water-soluble wheat arabinoxylan. 1. Synergy between α-L-arabinofuranosidases, endo-1,4-β-xylanases, and β-xylosidases activities. *Biotechnology and Bioengineering*, 81, 726-731.

Sorensen H.R., Pedersen S., Vikso-Nielsen A., Meyer A.S., 2005. Efficiencies of designed enzyme combinations in releasing arabinose and xylose fom wheat arabinoxylan in an industrial ethanol fermentation residue. *Enzyme and Microbial Technology*, 36, 773-784.

Sorensen H.R., Pedersen S., Meyer A.S., 2007. Synergistic enzyme mechanisms and effects of sequential enzyme additions on degradation of water insoluble wheat arabinoxylan. *Enzyme and Microbial Technology*, 40, 908-918.

Vafiadi C., Topakas E., Christakopoulos P., 2008. Preparation of multipurpose cross-linked enzyme aggregates and their application to production of alkyl ferulates. *Journal of Molecular Catalysis B: Enzymatic*, 54, 35-41.

Van Craeyveld V., Holopainen U., Selinheimo E., Poutanen K., Delcour J.A., Courtin C.M., 2009a. Extensive dry ball milling of wheat and rye bran leads to in situ production of arabinoxylan oligosaccharides through nanoscale fragmentation. *Journal of Agricultural and Food Chemistry*, 57, 8467-8473.

Van Craeyveld V., Delcour J.A., Courtin C.M., 2009b. Extractability and chemical and enzymic degradation of psyllium (Plantago ovata Forsk) seed husk arabinoxylans. *Food Chemistry*, 112, 812-819.

Van Craeyveld V., Dornez E., Holopainen U., Selinheimo E., Poutanen K. Delcour J.A., Courtin, C.M., 2010. Wheat bran AX properties and choice of xylanase affect enzymic production of wheat bran-derived arabinoxylan-oligosaccharides. *Cereal Chemistry*, 87, 283-291.

Willför S., Pranovich A., Tamminen T., Puls J., Laine C., Suurnäkki A., Saake B., Uotila K., Simolin H., Hemming J., Holmbom B., 2009. Carbohydrate analysis of plant materials with uronic acid-containing polysaccharides - A comparison between different hydrolysis and subsequent chromatographic analytical techniques. *Industrial Crops and Products*, 29, 571-580.

7.3.7 Supplement

For the sake of completeness, controls of enzymatic hydrolysis were prepared. The objective was to check that liberation of AXs was due to the enzymatic activity and not to a natural liberation of AXs in the buffer with rising temperatures. Table 7.6 shows the various experiments with the controls and the corresponding enzymatic hydrolysis.

Buffer	pH	Temperature °C	Experiment number and letter			
			Bran	Control	Hull	Control
NaAc	5	55	9	A	20	F
NaAc	5	60	10	B	21	G
NaP	6,3	40	-	-	-	-
NaAc	5	55	11	A	22	F
NaP	5,5	55	12	C	23	H
NaAc	4,7	40	-	-	-	-
NaAc	4,7	40	13	D	24	I
NaAc	4,7	40	14	D	25	I
NaAc	5	55	15	A	-	-
NaAc	5	55	16	A	-	-
NaP	5,5	55	17	C	-	-
NaAc	5	45	18	E	-	-
NaAc	5	45	19	E	-	-

Table 7.6. Conditions of reaction: buffer, pH, temperature °C, and experiment number for spelt bran, spelt hull and experiment letter for their respective controls

In the bran controls, rhamnose and mannose content was negligible (<0.8%) and xylose (1.2-3%), arabinose (0.6-1.5%) and galactose (1.2-2.0%) content was low (Table 7.7). Glucose was very high, between 34 and 55.1 %, depending on the buffer; on average it was 45.6% for buffer NaAc pH 5, 53.4% for buffer NaAC pH 4.7 and 34.4% for buffer NaP pH 5.5. Temperature did not seem to have an impact, with sets A, B and E having similar glucose content but different working temperatures. In the hull controls, sugar content was nil except for F at 24 h and H at 24 h, where xylose content was 0.6% and 0.4%, respectively.

Experiment letter	Time (h)	Total sugars					Free monomers
		Ara	Xyl	Glu	Gal	A/X ratio	Glu
A	0	0.9 ± 0.1	1.3 ± 0.1	46.3 ± 1.1	1.5 ± 0.1	0.67	5.7 ± 0.1
	1	0.9 ± 0.1	1.5 ± 0	45.4 ± 0.7	1.4 ± 0	0.6	5.9 ± 0.1
	4	1 ± 0	1.8 ± 0.1	46.9 ± 0.5	1.5 ± 0.1	0.57	5.8 ± 0
	24	1.5 ± 0.1	2.7 ± 0	43.9 ± 0.4	1.4 ± 0	0.54	5.5 ± 0
B	0	0.9 ± 0.1	1.3 ± 0.1	46.3 ± 1.1	1.5 ± 0.1	0.67	5.7 ± 0.1
	1	1 ± 0.1	1.6 ± 0.1	48.3 ± 1.1	1.8 ± 0	0.65	5.3 ± 0.1
	4	1 ± 0	1.6 ± 0.1	47.6 ± 2.8	1.7 ± 0.1	0.62	5.4 ± 0.1
	24	1.2 ± 0.1	2 ± 0	43.8 ± 0.1	1.6 ± 0	0.61	5.1 ± 0
C	0	1 ± 0.2	1.3 ± 0.2	34 ± 0.6	1.5 ± 0.2	0.77	4.9 ± 0.1
	1	0.8 ± 0	1.2 ± 0	35 ± 0.2	1.2 ± 0	0.65	4.8 ± 0.2
	4	0.9 ± 0.1	1.4 ± 0	34.8 ± 0.8	1.3 ± 0	0.65	3.6 ± 0.1
	24	1.5 ± 0	2.6 ± 0	33.6 ± 0.2	1.4 ± 0.1	0.61	3.7 ± 0
D	0	0.8 ± 0.1	1.4 ± 0.2	52.1 ± 0.6	1.9 ± 0.3	0.62	6.4 ± 0
	1	0.6 ± 0	1.3 ± 0	55.1 ± 4.3	1.4 ± 0	0.49	6 ± 0.1
	4	0.7 ± 0	1.6 ± 0	54.7 ± 4.9	1.4 ± 0.1	0.44	6.2 ± 0.1
	24	1.3 ± 0	3 ± 0.1	51.7 ± 3.4	1.5 ± 0	0.42	6.1 ± 0
E	0	0.9 ± 0.1	1.3 ± 0.1	46.3 ± 1.1	1.5 ± 0.1	0.67	5.7 ± 0.1
	1	1.2 ± 0.3	1.5 ± 0.2	44.7 ± 2.3	2 ± 0.3	0.8	5.6 ± 0.2
	4	0.9 ± 0.1	1.6 ± 0.1	41.9 ± 0.1	1.5 ± 0.1	0.56	5.9 ± 0.1
	24	1.5 ± 0	3 ± 0.1	45.5 ± 1.7	1.7 ± 0.1	0.49	5.2 ± 0.3
F	0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	-	n.a.
	1	0 ± 0	0 ± 0	0 ± 0	0 ± 0	-	n.a.
	4	0 ± 0	0 ± 0	0 ± 0	0 ± 0	-	n.a.
	24	0 ± 0	0.6 ± 0.6	0 ± 0	0 ± 0	0	n.a.
G	0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	-	n.a.
	1	0 ± 0	0 ± 0	0 ± 0	0 ± 0	-	n.a.
	4	0 ± 0	0 ± 0	0 ± 0	0 ± 0	-	n.a.
	24	0 ± 0	0 ± 0	0 ± 0	0 ± 0	-	n.a.
H	0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	-	n.a.
	1	0 ± 0	0 ± 0	0 ± 0	0 ± 0	-	n.a.
	4	0 ± 0	0 ± 0	0 ± 0	0 ± 0	-	n.a.
	24	0 ± 0	0.4 ± 0.4	0 ± 0	0 ± 0	0	n.a.
I	0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	-	n.a.
	1	0 ± 0	0 ± 0	0 ± 0	0 ± 0	-	n.a.
	4	0 ± 0	0 ± 0	0 ± 0	0 ± 0	-	n.a.
	24	0 ± 0	0 ± 0	0 ± 0	0 ± 0	-	n.a.

Table 7.7. Total saccharide and monomers content (%, d.m.) (± SD) in the controls at 0 h, 1 h, 4 h and 24 h; (n.a.: not analyzed). See Table 7.6 for experiment numbers and letters

As there were no or very low sugar amount in the hull controls, free sugars were measured only in the bran controls. The free sugar content was below 1%, except for glucose which was 3.6-6.4%, a low value compared with the total content.

The yield from AX solubilization remained below 2.6% for the bran controls, but there was an increase towards the end of the reaction (Table 7.8). For the hull controls, the yield was below 0.1%.

These yields were lower than those obtained with enzymatic hydrolysates, indicating that enzymes were effective.

The high glucose content in the controls from the beginning of the reaction showed that not only were the enzymes effective, but also that there was a natural liberation of glucose.

Experiment letter/ Time (h)	AX				Xylose				Arabinose				A/X			
	0	1	4	24	0	1	4	24	0	1	4	24	0	1	4	24
A	1.2	1.4	1.6	2.6	1	1.2	1.4	2.3	1.7	1.9	2	3.2	0.67	0.60	0.57	0.54
B	1.2	1.6	1.6	2.2	1	1.4	1.4	1.9	1.7	2.3	2.2	2.9	0.67	0.65	0.62	0.61
C	1.1	1	1.3	2.4	0.9	0.9	1.1	2	1.7	1.5	1.7	3.1	0.77	0.65	0.65	0.61
D	1.1	1.1	1.4	2.6	0.9	1	1.3	2.6	1.4	1.3	1.5	2.8	0.62	0.49	0.44	0.42
E	1.2	1.2	1.3	2.2	1	1	1.1	2.1	1.7	2	1.6	2.6	0.67	0.80	0.56	0.49
F	0	0	0	0.1	0	0	0	0.1	0	0	0	0	-	-	-	0
G	0	0	0	0	0	0	0	0	0	0	0	0	-	-	-	-
H	0	0	0	0.1	0	0	0	0.1	0	0	0	0	-	-	-	0
I	0	0	0	0	0	0	0	0	0	0	0	0	-	-	-	-

Table 7.8. Extraction yield (%) of AX and of xylose and arabinose, and the calculated A/X ratios for the controls at 0 h, 1 h, 4 h and 24 h. See Table 7.6 for experiment numbers and letters.

8 CHAPTER 8

General discussion

In the context of the overall objective to find ways of developing spelt by-products from milling, this thesis focused on fibers, which are concentrated in the bran and hull. As noted earlier, among the fibers present in these fractions, hemicelluloses are the most important quantitatively and have many applications. The extraction and characterization of hemicelluloses were therefore essential and constituted the core of the thesis work. The work involved characterizing the available material, testing several methods and improving them, which enabled us to make valid comparisons and provide a diverse range of products.
Although the chapters here address the questions and provide answers in line with the thesis objectives, there was some work done prior to the experiments that has not been described elsewhere here. This work related to the selection of milling methods, the selection of methods for quantifiying the fibers which are a special feature of the bran, the assessment of hydrolysis conditions for the monosaccharide analysis, and the discussion of the purification method.

With regard to milling method, classical milling (0.5-1mm) tends to be the most commonly cited method in the literature. At the beginning of the work, experiments were conducted using this type of milling, but the results were poor. It was therefore decided to go for finer milling, using micronization. The Alpine mill offers two types of micronization: jet-milling and impact milling. The first was chosen because the milling was finer. This micronization was then retained for all extractions in this work. Granulometry among sets of bran is almost identical providing a good basis for comparing spelt bran and spelt hull extractions. It was shown that micronization had an impact on the material and that there were some differences between classical milling and micronization. The reduction of the particle size of various fiber-rich plant materials is known to alter the structure, surface area and functional properties of the particles (Hemery *et al.,* 2011a). Chau *et al.* (2007) and Wu *et al.* (2007) have shown that different treatments (ball-milling, jet milling, high-pressure micronization) of the insoluble fibers of carrot and fruits led to a redistribution of fibers from insoluble to soluble, improved their physicochemical properties and had a favorable effect on the health and intestinal function of hamsters. Wu *et al.* (2007) and Chou *et al.* (2008) showed that the micronization of these insoluble fibers improved their ability to reduce the concentration of serum triglycerides, serum cholesterol and liver lipids, when fed to hamsters. This demonstrates that particle size is an important factor affecting the characteristics and physiological functions of insoluble fibers. Van Craeyveld *et al.* (2009a) showed that an extensive (120 h) laboratory-scale ball-mill treatment increased the level of wheat bran WE-AX from 4% (untreated bran) to 61% of the wheat bran AX. When the particle size is reduced, the accessible surface area is increased, giving the enzymes a larger contact area for accessing carbohydrates (Stewart and Slavin, 2009). In this thesis, the destarching, deproteination and enzymatic hydrolysis of AXs must have been made easier by using micronization.

After the preparation of the material, the selection of methods for quantifying fibers was discussed. This can be a controversial topic because the choice of method depends on the point of view of the scientist. The 'plant-rich diet approach' and the 'indigestibility approach' relate to the two main methods: the NSP method and the enzymatic-gravimetric group of methods (AOAC 985.29 & 991.43) (Englyst *et al.,* 2007). For this thesis, we chose an enzymatic-gravimetric methods in order to be able to make a comparison with other scientific investigations that use this type of method more frequently. In this group, the method reported by Lee *et al*. (1992) (AOAC 991.43) measures insoluble and soluble fibers, while the one described by Van Soest and Wine (1967), combined with an α-amylase treatment introduced by Schaller (1977) (AOAC 32-20), measures hemicelluloses, cellulose and lignin. We chose these two methods because they provide reliable results and are the best known and most widely used methods. This will enable comparisons to be made with other studies in the literature. The first method is commonly used in food analysis, and the second in forage analysis for feed. In this study, soluble and insoluble fibers were measured and added. In view of the very low amount of soluble fibers in the material available and the difficulty of extracting and precipitating these fibers, it was better and easier to measure the total fiber and insoluble fiber amounts and, from that deduce the soluble fiber amount. For the hemicelluloses, cellulose and lignin, the α-amylase treatment was very important with these kind of samples because the presence of starch could influence the way the Van Soest and Wine (1967) solutions worked. The comparison of methods for measuring the insoluble fibers (Lee *et al.,* 1992) and the neutral detergent fibers (Van Soest and Wine, 1967) showed that these measures are very similar and can substitute for each other in an initial step for cereals and for fractions such as whole grain flour, bran and whole spikelet flour.

To ascertain the traceability and purity of the bran and hull and to have a large enough quantity from the same production batch, the spikelets had to be dehulled and grains milled using a special scale. An industrial scale would not have guaranteed the purity, and a smaller scale would have been too difficult. Using traditional milling (Moulin de Hollange, Belgium), the spikelets were squashed between two stones and the grains were put through a milling machine, but separation of hull and grain was coarse. The starch content in the spelt bran was high (41.2%), which is very rare for bran whether spelt or wheat. In similar studies, the starch content of wheat bran was 8.8% (Hollman and Lindhauer, 2005), 16.7% starch (Maes and Delcour, 2002) and 15-25% (Beaugrand *et al.,* 2004a). The monosaccharide analysis of the spelt bran indicated a glucose content of 49.9%. The challenge then was to remove the starch in order to obtain material from which the purest AXs possible could be extracted. Enzymatic destarching was chosen, which was successful thanks to a new combination of enzymes, long reaction time and optimal working conditions for the enzymes. The proteins also had to be removed from the bran and the same strategy as that used for the starch was applied. Spelt whole grain flour is generally richer in protein than wheat whole grain flour; the literature indicates 10.9-17.5% for wheat and 9.8-25.5% for spelt (Escarnot *et al*., 2012). In this study, the protein content was

18.9% and the deproteination was therefore likely to be more efficient for spelt than for wheat. A new combination of enzymes was used to cut protein into peptides and, through filtration, they were successfully removed.

When the extractions of AXs started, a question arose about the hydrolysis conditions of the monosaccharide analysis. The hydrolysis conditions described in the literature for wheat vary; for example, 2M TFA 2h at 100°C for solids; 2M TFA 1h at 100°C for liquids (Maes and Delcour, 2001); 1M H_2SO_4 2h 105°C (Hollman and Lindhauer, 2005); 72% H_2SO_4 1M 3h 100°C and 1M H_2SO_4 3h 100°C (Schooneveld-Bergmans *et al.,* 1999); 1M H_2SO_4 2h 100°C for liquids; and 13M H_2SO_4 30 min 25°C and 1M H_2SO_4 2h 100°C for solids (Ordaz-ortiz and Saulnier, 2005; Barron *et al.,* 2007; Antoine *et al.,* 2003). It was difficult to select a method as none had ever been applied before to spelt fractions. Two acids were tested at several concentrations. It was decided to choose the method that produced the highest sugar content (arabinose and xylose), but the A/X ratio could also have been chosen as a parameter for choice of method. The variation in the A/X ratio shows that hydrolysis is selective, and therefore choice cannot be based on this parameter. It is important to note, however, that the percentages and A/X ratios depend on the method, and comparing data from the literature should be done with caution. Grouping the various fractions could be criticized for lack of precision or specificity, but the aim was to choose methods that could be used for other experiments where extraction systems might differ. The hydrolysis recommendations for three kinds of fractions do constitute a satisfactory solution in terms of precision and laboratory convenience. The choice of only one temperature for the hydrolysis could also be criticized, but again it does offer laboratory convenience. In most of the literature, one method is used for liquids and another for solids (Maes and Delcour, 2001; Schooneveld-Bergmans *et al*., 1999; Ordaz-ortiz and Saulnier, 2005; Barron *et al.,* 2007; Antoine *et al.,* 2003).

The purity of the obtained fractions is an essential aspect of the chemical extraction and enzymatic solubilization of AXs. Ethanol precipitation and dialysis were tested with this objective in mind. With regard to sugars, it appeared that more glucose, galactose and mannose were enclosed in the fraction with precipitation than with dialysis. With regard to ashes, the content was lower with dialysis than with precipitation, but the cut-off had to be carefully selected. Ultrafiltration was not tested, but it remains an effective tool for separating fractions of hemicelluloses with different MW, like the graded precipitation technique used by Maes and Delcour (2002). Swennen *et al.,* (2005) compared two methods and concluded that fractionation using graded ethanol precipitation or ultrafiltration membranes with different molecular-mass cut-off points yielded fractions that differed in both degree of polymerization and degree of substitution. The ultrafiltration fractions were more heterogeneous than those obtained using ethanol graded precipitation, but AXOS fractions with a similar degree of polymerization and substitution could be obtained with both methods.

In addition to process development and the quantitative and qualitative analysis conducted in the laboratory, more consideration needs to be given in the laboratory to the field production of AXs. The extraction yield of AXs can be as good for spelt bran as for wheat bran, but differences at field level and in terms of the morphology of the spelt spike need to be taken into account in order to answer the initial question more fully. The production of AXs was evaluated per hectare depending on the species, the by-product and the method used. The mean yield of wheat was 8,864 kg/ha (Herman, 2005, 2006, 2007b, 2008b, 2009b; Herman and Jacquemin, 2010; Herman *et al.,* 2011), which was higher than for spelt (7,287 kg/ha) (Herman, 2007a, 2008a, 2009a; Hermand *et al.,* 2010), giving a difference of 1,577 kg/ha. The wheat bran production (1,578 kg/ha), was higher than that for spelt (1,052 kg/ha). Hull production also needs to be taken into account; it was 1,720 kg/ha (Table 8.1).

Data	Wheat	Spelt
Yield grain/spikelet**	8,864	7,287
Yield grain**	8,864	5,567
Ratio bran/grain*	17.8	18.9
Ratio hull/spikelet**	-	23.6
Bran production	1,578	1,052
Hull production	-	1,720

* from the trial mentioned in Chapter 4
** from Herman and Couvreur (2005), Herman (2006, 2007a,b, 2008a,b, 2009a,b, 2010a), Herman and Jacquemin (2010b), Herman et al. (2011)

Table 8.1. Yield (kg/ha), ratio bran/grain (%), ratio hull/spikelet (%), bran production and hull production (kg/ha) for wheat and spelt.

The extraction of WE-AX, described in Chapter 5 for the bran and Chapter 6 for the hull, resulted in 12 kg/ha WE-AXs being produced from spelt (bran and hull) and 23 kg/ha from wheat (bran only) (Table 8.2). This difference was due to the intial AX content of the bran and to the difference of bran production per hectare. The extraction of WU-AXs from bran using alkaline methods for wheat and spelt, produced 148 and 47 kg/ha, respectively. Again, the initial AX content of the bran and the bran production per hectare explained the difference. When the production from the hull was added, 1 ha spelt could provide 110-176 kg of WU-AXs, depending on the process used for the hull. This figure demonstrates that spelt production using chemical processes can compete with the process for wheat. With regard to enzymatic solubilization, the highest production reached with spelt bran was 72 kg/ha of WU-AX and AXOS, while wheat bran provided 174 kg/ha of AXOS using the most efficient enzymatic method. This difference was mainly due to the initial AX content of the bran and to bran production per hectare. The solubilization of AXs from the hull was not satisfactory, with a maximum of 20 kg/ha of AXOS; the total WU-AX and AXOS production from spelt using enzymes was 92

kg/ha, which was lower than that for wheat. The efficiency of extraction and solubilization for spelt bran remained in the same range as those for wheat bran in literature. The difference of AX production per hectare could be explained by two main factors: the initial AX content of the bran and the bran production per hectare, which is influenced by the yield per hectare and the morphology of the spike.

Material	Method	Product extracted	AXs' yield g/100g of material	MW or DP	A/X	AXs' production kg/ha	Reference
Spelt bran	-	WE-AX	0.65	6,7 and 28 kDa	0.47	7	Escarnot *et al.*, 2011a
Wheat bran	-	WE-AX	1.45	5 and 20 kDa	0.58	23	Maes and Delcour, 2002
Spelt bran	H_2O_2 2%; pH 11.5; 4h twice; 60°C	WU-AX	4.43	8,1 and 310; 7,4 and 415 kDa	0.60 ; 0.65	47	Escarnot *et al.*, 2011a
Wheat bran	H_2O_2 2%; pH 11.5; 4h twice; 60°C	WU-AX	9.42	5-10 and 100-120 kDa	0.82	149	Maes and Delcour, 2002
Wheat bran	H_2O_2 2%; pH 11; 4h; 40°C	WU-AX	9.33	100-110 kDa	0.8	147	Hollman and Lindhauer, 2005
Spelt hull	-	WE-AX	0.27	5,4 kDa	0.55	5	Escarnot *et al.*, 2011b
Spelt hull	NaOH; 16h; 45°C	WU-AX	7.5	9,3 and 177 kDa	0.18	129	Escarnot *et al.*, 2011b
Spelt hull	H_2O_2 2%; pH 11.5; 4h twice; 60°C	WU-AX	3.65	6,8-7,8 and 230-275 kDa	0.30-0.40	63	Escarnot *et al.*, 2011b
Spelt hull	H_2O_2 2%; pH 11.5 ; 24h; 50°C	WU-AX	4.55	9,2 and 273 kDa	0.36	83	Escarnot *et al.*, 2011b
Spelt bran	Belfeed; 24h	AXOS and WU-AX	3.58	DP 2-71	0.24	38	Escarnot *et al.*, 2012
Spelt bran	Celluclast + Shearzyme 2X; 24h	AXOS and WU-AX	6.88	DP 1-1150	0.3	72	Escarnot *et al.*, 2012
Wheat bran	Endo-β-(1-4)-xylanase *Bacillus sp.*; 24h	AXOS	5.7	745-1669 Da	0.30-0.63	79	Lequart *et al.*, 1999
Wheat bran	(1-4)-β-endo-xylanase *Thermobacillus xylanilyticus*; 24h	AXOS	12.6	DP 2-5	0.21	174	Benamrouche *et al.*, 2002
Wheat bran	Xylanase *Hypocrea jecorina* ; 24h	AXOS	10.73	DP 2,3-3,2	0.29	169	Van Craeyveld *et al.*, 2010
Spelt hull	Rohalase; 24h	AXOS	0.24	DP 2-17	0.21	3	Escarnot *et al.*, 2012
Spelt hull	Ultraflo; 24h	AXOS	1.14	DP 2-9	0.18	20	Escarnot *et al.*, 2012

Table 8.2. Material, method, product extracted, yield of AX/100g of material, MW or DP, A/X ratio, production AX kg/ha and reference of several processes.

The objective of the thesis was to focus on fibers, the major components of the studied by-products, but the usefulness of the whole process could be improved by considering other components of interest that are currently neglected in the processes described. Some compounds are removed at the start of the processes, but could be extracted in other ways from the by-products and become usable. For example, in this study, the starch and proteins were cut by enzymes and their respective components (dextrins, glucose and peptides) were removed through washing. This means the starch and proteins were no longer usable in themselves, although they could have many uses. This is true for both spelt bran and hull, but in terms of the amount of starch and proteins would be more interesting for spelt bran. For spelt hull, it would be more efficient to concentrate on cellulose and lignins (Fig. 8.1).

Starch contributes greatly to the textural properties of many foods and is widely used in food and industrial applications as a thickener, colloidal stabilizer, gelling agent, bulking agent and water retention agent (Singh *et al.,* 2007). Some modifications enable it to also be used as a binder, film former, texturizer, fat replacer, emulsion stabilizer and viscozifier (Singh *et al.,* 2007). Resistant starch is also used in food applications because it has the functionality of dietary fiber (Premavalli *et al.,* 2006). Other applications of starch include using it to form edible or biodegradable films (Mali et al., 2006) or as a filler to produce reinforced plastics (Evangelista *et al.,* 1991). For these applications, it is important to extract starch in its native form or with weak degradation. Wheat gluten is extensively used in both food and non-food applications (cosmetic and hair products, detergents, rubber and polymer products) (Bietz et Lookhart, 1996; Magnuson, 1985). It is industrially prepared from flour during gluten-starch separation (Van der Borght *et al.,* 2005). Wheat gluten plastics can be used in composite materials, films for agricultural use or as molded objects (Pommet *et al.,* 2003; Domenek *et al.,* 2004).

Swennen *et al.,* (2005) used the method reported by McRitchie (1985), which enabled gluten, prime starch, a water-soluble fraction and a squeegee fraction to be fractionated. Recently, a dry/wet milling process was tested for extracting of starch and gluten from wheat. Whole wheat kernels were crushed dry between smooth rolls, prior to wet disintegration in excess water (Steeneken and Helmens, 2009). Using a wet-milling process with 70% ethanol and successive filtrations, 90% of starch from wheat bran, as well as three other fractions (protein concentrate, coarse fiber concentrate and fine fiber concentrate), were obtained (Xie *et al.,* 2008).

Another common approach involves pearling wheat grains, which produces bran-rich pearlings and endosperm-rich pearlings. The bran-rich pearlings were separated by air classification to obtain monosaccharides and functional foods (through enzymatic hydrolysis), ferulic acid and arabinoxylan (through water or solvent extraction), and germ-rich fractions. Hemery *et al.* (2011b) tested electrostatic separation in order to obtain purified fractions from wheat bran. The charge of the particles was influenced by their biochemical composition: particles rich in highly branched and cross-

linked AXs (pericarp) were separated from particles rich in β-glucan, ferulic acid and para-coumaric acid (aleurone cell walls).

With regard to the hull, which is part of the available lignocellulosic biomass, it is a rich source of cellulose and contains lignin. Cellulosic biomass can be processed into bioenergy (biofuels such as bioethanol, biodiesel, heat, power) and bioproducts (such as biopolymers, biolubricants, biosurfactants and biosolvents) (Wertz *et al.,* 2010). Lignins are interesting as a renewable bioresource that serves as basis of various products; commercial lignins are sought after because of their dispersing, binding, complexing and emulsifying properties; lignins also serve as polyols in the production of various polymers and can be used as a raw material for many value-added products, such as vanillin, ferulic acid, coumaric acid, guaiacol and catechol (Buranov and Mazza, 2008).

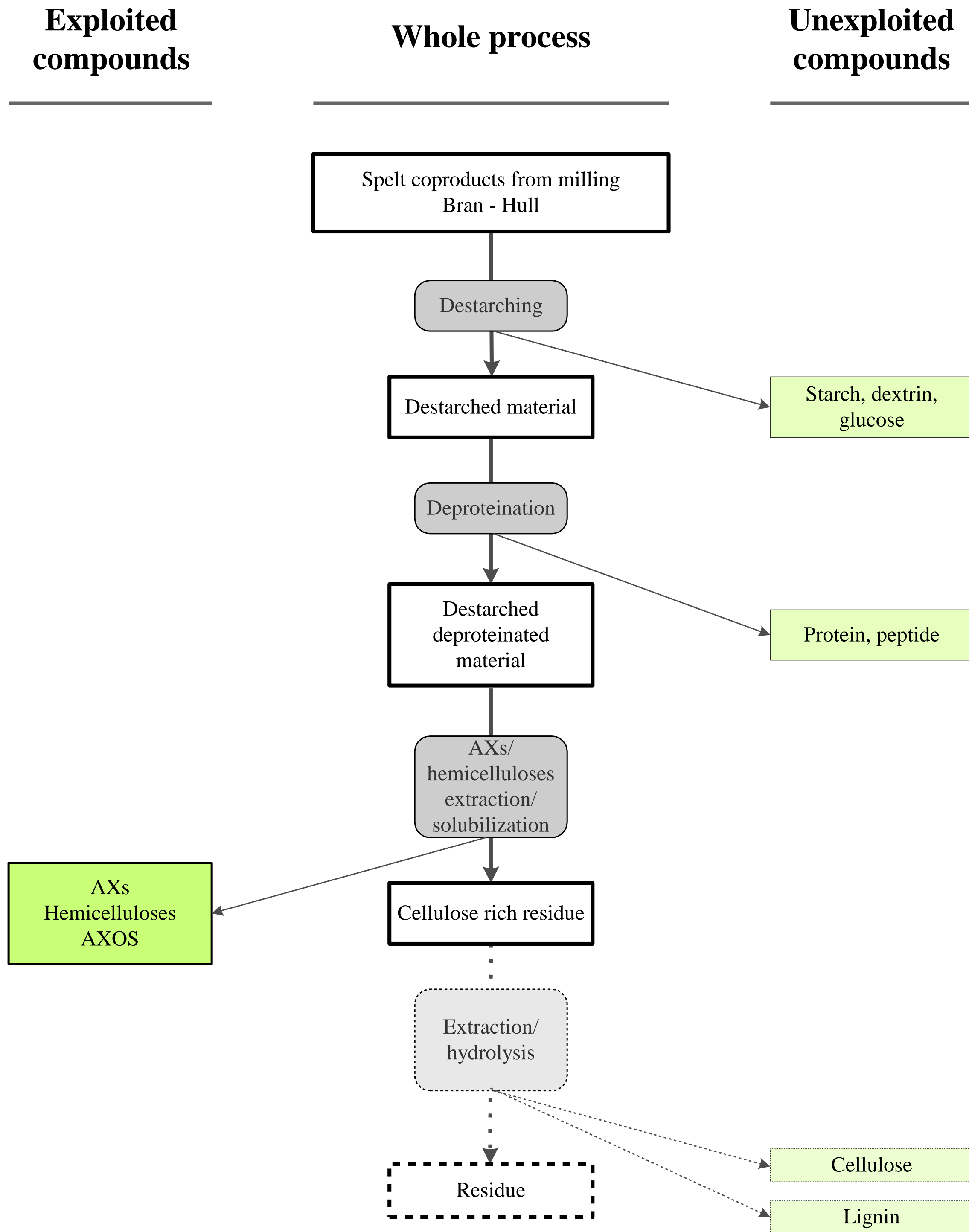

Figure 8.1. Diagrammatic representation of the thesis showing process, exploited and unexploited compounds

9 CHAPTER 9

Conclusions and prospects

This thesis, focusing on cereals and chemistry, achieved its objectives of contributing to scientific knowledge on materials, methods and compounds and suggesting ways of better exploiting spelt spikelets based on hemicelluloses.

The characterization of spelt bran and hull based on their content of different types of fibers indicated that they are rich sources of hemicelluloses. The bran was studied before the hull because wheat bran has been heavily researched and that experience could be transferred to spelt bran. However, it then seemed that the hull contained more hemicelluloses than bran, and could be an interesting material to explore. The methodology applied to wheat bran (alkaline peroxide hydrogen) was successfully transferred to spelt bran, with similar yields of extraction and higher molecular weight being obtained compared with wheat, indicating that the AXs were better protected. This methodology was less efficient on hull hemicelluloses, but other chemical processes (sodium and potassium hydroxide) resulted in high yields of extraction with high molecular weights being obtained. The use of another type of chemical method (organic acids) showed that alkaline conditions protected the structure of the hemicelluloses and made it easier to extract them without strong degradation. Althought the organic acids degraded the molecules, this method gave the highest yields from spelt hull of all experiments in this study. The hemicelluloses were in solution and the molecular weights were low, which could be useful for some applications. For the hull, many types of chemicals, conditions and final product forms were tested, resulting in a great diversity of products and yields. Enzymatic solubilization was an interesting alternative to chemical extraction from an environmental point of view. The enzymatic process worked as well on spelt bran as it did on wheat bran in terms of the yields from solubilization. AXOS production from spelt bran was possible and efficient, but quality needed to be improved (glucose content and range of DP). AXOS production from spelt hull was very difficult. Even when the range of DP exceeded the requirements of the AXOS profile, the yields from solubilization were far lower than those for spelt bran, and the glucose content was too high. Differences depending on commercial enzymatic preparation and type of enzyme used were observed, with a notable increase in glucose content and range of DP when cellulase was included.

The extractions of AXs from spelt bran and of hemicelluloses from spelt hull using chemical methods was efficient and provided different molecules. The solubilization of AXs from spelt bran using enzymatic methods was satisfactory in terms of yield, but not as good as the chemical extractions in terms of the quality of the hydrolysate. The enzymatic hydrolysis of AXs from spelt hull was not efficient in terms of yield, but the quality was not entirely satisfactory. The alkaline methods were better for obtaining long chains of hemicelluloses with precipitate, while acidic and enzymatic methods were better for obtaining short chains of hemicellulloses, preferentially under solution.

This thesis has opened up new possibilities for processing spelt by-products that are efficient and could lead to more research, a scaling-up of the laboratory process, market studies, partnerships with manufacturers, and so on. Some methods, however, still need to be improved to increase the profitability of the whole process (from field to product) and to determine the qualitative differences between spelt and wheat. This thesis has provided the data to move closer to a final product if more in-depth research can be conducted.

From an industrial perspective, an initial goal would be to increase the extraction efficiency and obtain different profiles. A mixture of bran and hull could be submitted to enzymatic or chemical treatment. This method has already been tested with a mixture of wheat straw and wheat bran using a twin-screw extruder (Marechal *et al.*, 2004). Although micronization was used in this study, other physical methods were not tested. Soft physical treatments such as micro-wave could also have a beneficial effect on hemicelluloses extraction. For example, Palm and Zacchi (2003) extracted hemicellulosic oligosaccharides from spruce, achieving a yield of 70% (treatment: 200°C for 5 min). They obtained 12.5g of oligosaccharides per 100 g of dry wood. Rose and Inglett (2010) extracted 70% of insoluble AXs in a two-stage hydrothermal process using microwave. Stronger treatments, such as steam explosion, could have different effects on hemicelluloses, depending on the strength. Under relatively mild conditions, it is possible to hydrolyse the hemicellulosic fraction and obtain monomers and oligomers of hemicelluloses (Sun *et al.,* 2005b); this process could be particularly useful for xylose production. Another approach could involve producing xylose (Sorensen *et al.,* 2005), which is a susbtrate for the bioproduction of several chemicals, including xylitol (Canilha et al., 2006). Xylitol is a valuable sweetener that is non-cariogenic (Mäkinen *et al.,* 1998) and possibly anti-cariogenic (Miyasawa *et al.,* 2003); it has a sweetness similar to that of sucrose but contains fewer calories (Pepper and Olinger, 1988) and is passively absorbed from the digestive tract, independent of insulin and without elevation of blood glucose, which warrants its use in diabetic foods (Touster, 1974). Instead of looking for HMW with chemical procedures and LMW with enzymatic procedures, a flux of xylose monomers could be sought. Various chemical and enzymatic treatments, excluding steam explosion, that have been tested on wheat straw could be tested on spelt hull. For example, Canilha *et al.* (2006) used sulphuric acid; Liavoga *et al.* (2007) investigated sulphuric acid treatment and hydrothermal combined with xylanase treatment; and Zhuang *et al.* (2009) tested formic acid and hydrochloric acid.

Another objective directly connected to the work done for this thesis relates to the solubilization of AXs through enzymatic methods. The creation of AXOS and their full characterization should be a future objective. The production of AXOS has been achieved on wheat bran and has been described by Swennen *et al.* (2006b). AXOS show some prebiotic properties and constitute an excellent waof enhancing the use of spelt by-product. Prebiotics are food ingredients that cannot be digested by the enzymes of the upper gastrointestinal tract in healthy individuals and are fermented selectively by

some types of intestinal bacteria in the large intestine (Gibson and Roberfroid, 1995). The ingestion of prebiotics causes a shift in the composition of the intestinal bacterial population (generally an increase of *Lactobacillus* and *Bifidobacterium* species) that is associated with improved overall health (Macfarlane *et al.,* 2006; Swennen *et al.,* 2006a). The fermentation of prebiotics by colonic bacteria leads to the production of unbranched short-chain fatty acids that have been shown to exert positive health effects in the colon. The preparation of XOS causes significant positive changes in the intestine or colon (Van Craeyeveld *et al.,* 2008). Van Craeyeveld *et al.* (2008) reported that an AXOS preparation with an average DP of 5 and an average degree of arabinose substitution of 0.27 showed, in rats, the best combination of desirable effects on gut health characteristics, and fructo-oligo-saccharide and inulin had similar bifidogenic effects. Another application of AXOS is in feed; they have been shown to improve feed conversion when added to the animal diets (Courtin *et al.,* 2008a), while uncleaved AX is known to hamper efficient feed utilization (Choct and Annison, 1992). AXOS have also been shown to stimulate the growth of beneficial microflora components in the ceco-colonic compartment of the gastrointestinal tract (Yamada *et al.,* 1993; Courtin *et al.,* 2008a, b), whereas AXs promote fermentation in the ileum (Choct *et al.,* 1996). Eeckhaut *et al. (*2008) concluded that the dietary addition of AXOS provides dose-dependent protection against oral infections from *Salmonella enteritidis* in poultry (which causes gastroenteritis, a major problem in animal husbandry and in human medicine; Hall *et al.,* 2005). The effects of AXOS from spelt bran could be evaluated in the light of what has been done with AXOS from wheat bran. Identifying differences between AXs from wheat and spelt could justify the extraction of AXs or the creation of AXOS from spelt by-products.

With this objective, it would be worth conducting a deeper characterization of the obtained fractions using techniques such as nuclear magnetic resonance (NMR) to see the branching and the position of the residues on the backbone of xylose (Hoffman et al., 1991; Kormelink et al., 1993). More recently, other techniques using matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry (MS), as well as electrospray ionization (ESI) mass spectrometry using a Q-TOF mass analyzer (ESI-Q-TOF) or an ion trap (IT) mass analyser, have been used to characterize a complex oligosaccharide mixture obtained by the enzymatic digestion of AX from wheat seeds (Matamoros Fernandez *et al.,* 2003; Quéméner *et al.,* 2006). These methods could also be used to characterize the structure of the hemicelluloses from different materials (such as bran, hul and straw) and from different species, especially spelt and wheat.

Compounds linked to fibers, such as phenolic acids, could be investigated because they are known to have beneficial effects on human health. The content of several phenolic acids has been evaluated by Li *et al.,* (2008) within the framework of the Healthgrain project but the number of spelt genotypes used (5) was limited. The mean content was 579 µg/g d.m. (382-726) for spelt and 664 µg/g d.m. (326-1171) for winter wheat. Ferulic acid, for example, was reported to have many physiological functions, including antioxidant, antimicrobial, anti-inflammatory, anti-thrombosis and anti-cancer

activities. It also protects against coronary disease, reduces cholesterol and increases sperm viability (Ou and Kwok, 2004). The mean content of ferulic acid was 365 µg/g d.m. (223-502) for spelt and 395 µg/g d.m. (181-742) for wheat (Li *et al.,* 2008).

List of references

AACC, 2001. The definition of dietary fiber. *Cereal Foods World*, 46,112-126.

Abdel-Aal E.S.M., Hucl P., Sosulski F.W., 1995. Compositional and nutritional characteristics of spring einkorn and spelt wheats. *Cereal Chemistry*, 72, 621-624.

Abdel-Aal E.S.M., Salama D.A., Hucl P., Solsulski F.W., Cao, W., 1996. Electrophoretic characterization of spring spelt wheat gliadins. *Journal of Agricultural and Food Chemistry*, 44, 2117-2123.

Abdel-Aal E., Solsulski F.W., Hucl P., 1998a. Origins, characteristics and potentials of ancient wheats. *Cereal Foods World*, 43, 708-715.

Abdel-Aal E.S.M., Hucl P., Sosulski F.W., 1998b. Food uses for ancient wheats. *Cereal Foods World*, 43, 763-767.

Abdel-Aal E.-S.M., Hucl P. and Sosulski F.W., 1999a. Optimizing the bread formulation for soft spelt wheat. *Cereal Foods World*, 44, 7, 480-483.

Abdel-Aal E.-S.M., Hucl P. and Sosulski F.W., 1999b. Starches from primitive wheats. II Thermal and structural properties. Annual meeting Abstract 225, AACC International St Paul MN, http://www.aaccnet.org/meetings/99mtg/abstracts/acabc27.htm, consulted on 05/11/2010.

Abdel-Aal E., and Hucl P., 2002. Amino acid composition and in vitro protein digestibility of selected ancient wheats and their end products. *Journal of Food Composition and Analysis*, 15, 737-747.

Abdel-Aal E. and Hucl P. 2005. Spelt: a specialty wheat for emerging food uses. In Specialty grains for food and feed. Abdel-Aal E. and Wood P. American Association of Cereal Chemists. St-Paul, Minnesota. p 109-141.

An X.L., Li Q.Y., Yan Y.M., Xiao Y.H., Hsam S.L.K., Zeller F.J., 2005. Genetic diversity of European spelt wheat (*Triticum aestivum* ssp *spelta* L. em. Thell.) revealed by glutenin subunit variations at the Glu-A and Glu-3 loci. *Euphytica*, 146, 3, 193-201.

Anderson N.E., Clydesdale F.M., 1980. An analysis of the dietary fiber content of a standard wheat bran. *Journal of Food Science*, 45, 336-340.

Andersson R., Eliasson C., Selenare M., Kamal-Eldin A., Aman P., 2003. Effect of endo-xylanase-containing enzyme preparations and laccase on the solubility of rye bran arabinoxylan. *Journal of the Science of Food and Agriculture*, 83, 617-623.

Andrews A.C., 1964. The genetic origin of spelt ad related wheats. *Der Zuchter: Zeitschrift fur theorische und angewandte genetic*, 34, 1, 17-22.

Antoine C., Peyron S., Mabille F., Lapierre C., Bouchet B., Abecassis J., Rouau X., 2003. Individual contribution of grain outer layers and their cell wall structure to the mechanical propertes of wheat bran. *Journal of Agricultural and Food Chemistry*, 51, 2026-2033.

Asp H.G., Bjorck I ., Nyman M., 1993. Physiological effect of cereal dietary fibre. *Carbohydrate Polymers*, 21, 183-187.

Bacic A., Stone B.A., 1981a. Chemistry and organization of aleurone cell wall components from wheat and barley. *Australian Journal of Plant Physiology,* 8, 475-495.

Bacic A. and Stone B. A., 1981b. Isolation and ultrastructure of aleurone cell walls from wheat and barley. *Australian Journal of Plant Physiology*, 8, 453-475.

Bacic A., Harris P. J., Stone B.A., 1988. Structure and function of plant cell walls. *The Biochemistry of Plants*, 14, 297-370.

Baghurst P.A., Baghurst K.I., Record S.J., 1996. Dietary fibre, non-starch polysaccharides and resistant starch- a review. *Food Australia*, 48, S3-S35.

Barron C., Surget A., Rouau X., 2007. Relative amounts of tissues in mature wheat (Triticum aestivum L.) grain and their carbohydrate and phenolic acid composition. *Journal of Cereal Science*, 45, 88-96.

Bataillon M., Mathaly P., Nunes Cardinali A.-P., Duchiron F., 1998. Extraction and purification of arabinoxylan from destarched wheat bran in a pilot scale. *Industrial Crops and Products*, 8, 37-43.

Beaugrand J., Crônier D., Debeire P., Chabbert B., 2004a. Arabinoxylan andhydroxycinnamate content of wheat bran in relation to endoxylanase susceptiblity. *Journal of Cereal Science*, 40, 223-230.

Beaugrand J., Chambat G., Wong V. W. K., Goubet F., Remond C., Paes G., Benamrouche S., Debeire P., O'Donohue M., Chabbert B., 2004b. Impact and efficiency of GH10 and GH11 thermostable endoxylanases on wheat bran and alkali-extractable arabinoxylans. *Carbohydrate Research*, 339, 2529-2540.

Beaugrand J., Reis D., Guillon F., Debeire P., Chabbert B., 2004c. Xylanase-mediated hydrolysis of wheat bran: evidence for subcellular heterogeneity of cell walls. *International Journal of Plant Science*, 165, 553-563.

Beaugrand J. (2004). Bases cytologiques et moléculaires de la dégradation enzymatique du son de blé tendre. Laboratoire INRA, UNIR-614 Fractionnement des AgroRessources et Emballages, Reims Champagne-Ardenne, 172p.

Belitz H.-D. and Grosch W., 1999. Food chemistry. Second edition. Springer-Verlag, Berlin Heidelberg, p 631-636.

Belton P.S. and Taylor J.R.N., 2002. Pseudocereals and less common cereals. Grain properties and utilization potential. Springer-Verlag, Berlin Heidelberg, V-VII.

Benamrouche S., Crônier D., Debeire P. and Chabbert B., 2002. A chemical and histological study on the effect of ((1->4)-β-endo-xylanase treatment on wheat bran. *Journal of Cereal Science*, 36, 253-260.

Bergmans M.E.F., Beldman G., Gruppen H., Voragen A.G.J., 1996. Optimisation of the selective extraction of (glucurono)arabinoxylans from wheat bran: use of barium and calcium hydroxide solution at elevated temperatures. *Journal of Cereal Science*, 23, 235-245.

Bietz J.A. and Lookhart G.L., 1996. Properties and non-food potential of gluten. *Cereal Foods World*, 41, 376-382.

Blakeney A.B., Harris P.J., Henry RJ., Stone B.A., 1983. A simple and rapid preparation of alditol acetates for monosaccharide analysis. *Carbohydrate Research,* 113, 291-299.

Bonafaccia G., Galli V., Francisci R., Mair V., Skrabanja V., Kreft I., 2000. Characteristics of spelt wheat products and nutritional value of spelt wheat-based bread. *Food Chemistry*, 68, 437-441.

Brillouet J.-M. and Mercier C., 1981. Fractionation of wheat bran carbohydrates. *Journal of the Science of Food and Agriculture*, 32, 243-251.

Brillouet J.M. and Joseleau J.P., 1987. Investigation of the structure of a heteroxylan from the outer pericarp (beeswing bran) of the wheat kernel. *Carbohydrate Research*, 159, 106-126.

Buranov A.U. and Mazza G., 2008. Lignin in straw of herbaceous crops. *Industrial Crops and Products*, 28, 327-359.

Caballero L., Martin L.M., Alvarez J.B. 2004. Genetic variability of the low-molecular-weight glutenin subunits in spelt wheat (*Triticum aestivum* ssp. *spelta* L. em. Thell.). *Theoretical and Applied Genetics*, 108, 914-919.

Cai Z.-S., Paszner L., 1988. Salt catalysed wood bonding with hemicellulose. *Holzforschung*, 42, 11-20.

Campbell K.G., 1997. Spelt: agronomy, genetics, and breeding. *Plant Breeding Reviews*, 15, 187-213.

Canilha L., Carvalho W., Almeida e Silva J..B., 2006. Xylitol bioproduction from wheat straw: hemicellulose hydrolysis and hydrolyzate fermentation. *Journal of the Science of Food and Agriculture*, 86, 1371-1376.

Carré B. and Brillouet J.-M., 1986. Yield and composition of cell wall residues isolated from various feedstuffs used for non-ruminant farm animals. *Journal of the Science of Food and Agriculture*, 37, 341-351.

Carvalheiro F., Duarte L.C., Medeiros R., Girio F.M., 2007. Xylitol production by Debaryomyces hansenii in brewery spelt grain dilute-acid hydrolysate : effect of supplementation. *Biotechnology Letters*, 29, 1887-1891.

Chau C.F., Wang Y.T. ; Wen Y.L., 2007. Different micronization methods significantly improve the functionality of carrot insoluble fibre. *Food Chemistry*, 100, 1402-1408.

Chen W.-J. L. and Anderson J.W., 1981. Soluble and insoluble plant fiber in selected cereals and vegetables. *The American Journal of Clinical Nutrition*, 34, 1077-1082.

Chesson A., 1995. Dietary fiber. In A. Stephen, Food polysaccharides and their applications, pp 547-551. New-York, Marcel Dekker.

Choct M. and Annison G.. 1992. Anti-nutritive effect of wheat pentosans in broiler chickens: Roles of viscosity and gut microflora. *British Poultry Science,* 33, 821–834.

Choct M., Hughes R. J, Wang J., Bedford M. R., Morgan A. J., Annison G., 1996. Increased small intestinal fermentation is partly responsible for the anti-nutritive activity of non-starch polysaccharides in chickens. *British Poultry Science,* 37, 609–621.

Chou S.-Y., Chien P.-J., Chau C.-F., 2008. Particle size reduction effectively enhances the cholesterol-lowering activities of carrot insoluble fiber and cellulose. *Journal of Agricultural and Food Chemistry*, 56, 10994–10998.

Chung O.K. and Ohm J.-B., 2000. Cereal lipids. In Handbook of cereal science and techonology. Second edition. Kulp K. and Ponte J.G.Jr., Marcel Dekker, NewYork, 417-477.

Chung O.K., Ohm J.-B., Ram M.S., Park S.-H., Howitt C.A., 2009. Wheat lipids in Wheat Chemistry and Technology. Fourth Edition. St Paul, Minnesota, AACC, p 363-399.

Clamot G., 1984. Genetic variability of the protein and lysine content of spelt (*Triticum spelta*). *Zeitschrift für Pflanzenzüchtung*, 93, 106-114.

Clamot G., 1978. Disponibilités et perspectives variétales en épeautre. In *Colloque d'information scientifique, L'épeautre,* Libramont-Chevigny, le 10 mars 1978 (pp 14-24). Royaume de Belgique, Centre de Recherches Agronomiques de l'Etat.

Codianni P., Ronga G., Di Fonzo N., Troccoli A., 1996. Performance of selected strains of 'Farro' (*Triticum monococcum* L., *Triticum dicoccum* Schübler, *Triticum spelta* L.) and Durum Wheat (*Triticum durum* Desf. cv. Trinakria) in the difficult flat environment of southern Italy. *Journal of Agronomy and Crop Science*, 176, 15-21.

Courtin C. M. and Delcour J., 2002. Arabinoxylans and endoxylanases in wheat flour bread-making. *Journal of Cereal Science*, 35, 225-243.

Courtin C.M., Roelants A., Delcour J.A., 1999. Fractionation-Reconstitution experiments provide insights into the role of endoxylanases in bread-making. *Journal of Agricultural and Food Chemistry*, 47, 1870-1877.

Courtin C.M., Broekaert W.F., Swennen K., Lescroart O., Onagbesan O., Buyse J., Decuypere E., Van de Wiele T., Marzorati M., 2008a. Dietary inclusion of wheat bran arabinoxylooligosaccharides induces beneficial nutritional effects in chickens. *Cereal Chemistry*, 85, 607–13.

Courtin C. M., Swennen K., Broekaert W. F., Swennen Q., Buyse J., Decuypere E., Michiels C. W., De Ketelaere B., and Delcour J. A., 2008b. Effects of dietary inclusion of xylooligosaccharides, arabinoxylooligosaccharides and soluble arabinoxylan on the microbial composition of cecal contents of chickens. *Journal of the Science of Food and Agriculture*, 88, 2517-2522.

Courtin C.M., Swennen K., Verjans P., Delcour J.A., 2009. Heat and pH stability of prebiotic arabinoxylooligosaccharides and fructooligosaccharides. *Food Chemistry*, 112, 831-837.

Cubadda R. and Marconi E., 1996. Technological and nutritional aspects in emmer and spelt. In Hulled wheats. Paludosi S., Hammer K., Heller J. Proceedings of the first international workshop on hulled wheats, 21-22 July 1995, Castelvechio Pascoli, Italy. IPGRI, Rome, 203-212.

Cubadda R. and Marconi E., 2002. Spelt wheat. In Pseudocereals and less common cereals. Grains properties and utilization potential. Belton P.S. et Taylor J.R.N. Springer-Verlag, Berlin, Allemagne. p 153-175.

Da Silva L.P. and Santorio Ciocca M.L., 2005. Total, insoluble and soluble dietary fiber values measured by enzymatic-gravimetric method in cereal grains. *Journal of Food Composition and Analysis*, 18, 113-120.

Da Silva D.D.V., de Mancilha I.M., Da Silva S.S., Felipe M.D.D. 2007. Improvement of biotechnological xylitol production by glucose during cultive of Candida guilliermondii in sugarcane bagasse hydrolysate. *Brazilian Archives of Biology and Technology*, 50, 207-215.

Davin L.B., Lewis N.G., 2005. Lignin primary structures and dirigent sites. *Current Opinion in Biotechnology,* 16, 407-415.

Delcour J. A., Van Win H., Grobet P., 1999. Distribution and structural variation of arabinoxylans in common wheat mill streams. *Journal of Agricultural and Food Chemistry*, 47, 271-275.

Delcour J.A. and Hoseney R.C., 2010. Principles of cereal science and technology. Third Edition. AACC, St Paul, Minnesota: 270 p.

DGSIE, Direction générale Statistique et Information économique, Belgium, 2011. http://statbel.fgov.be/fr/statistiques/chiffres/economie/agriculture/exploitations/index.jsp, consulted on 23/03/2011.

Direction générale de la Statistique et de l'Information économique, SPF Economie, P.M.E., Classes moyennes et Energie, Belgium, 2010.
http://statbel.fgov.be/fr/modules/publications/statistiques/economie/estimation_de_la_production_des_cultures_agricoles_annee_de_recolte_2009.jsp

Domenek S.; Feuilloley P.; Gratraud J.; Morel M. H.; Guilbert S., 2004. Biodegradability of wheat gluten based bioplastics. *Chemosphere*, 54, 4, 551–559.

Doner L.W., Hicks K.B., 1997. Isolation of hemicellulose from corn fiber by alkaline hydrogen peroxide extraction. *Cereal Chemistry*, 74, 2, 176-181.

Duarte L.C., Carvalheiro F., Lopes S., Marques S., Parajo J.C., Girio F.M., 2004. Comparison of two posthydrolysis processes of brewery's spent grain autohydrolysis liquor to produce a pentose containing culture medium. *Applied Biochemistry and Biotechnology*, 113-116, 1041-1058.

Duarte L.C., Carvalheiro F., Lopes S., Neves I., Girio F.M., 2008. Yeast biomass production in brewery's spent grains hemicellulosic hydrolyzate. *Applied Biochemistry and Biotechnology*, 148,119-129.

Dupont M.S. and Selvendran R.R., 1987. Hemicellulosic polymers from the cell walls of beeswing wheat bran : part I, polymers solubilised by alkali at 2°. *Carbohydrate Research*, 163, 99-113.

Dupont F.M. and Alltenbach S.B., 2003. Molecular and biochemical impacts of environmental factors on wheat grain development and protein synthesis. *Journal of Cereal Science*, 38, 133-146.

Dvoracek V., Curn V., Moudry J., 2002. Evaluation of amino acid content and composition in spelt wheat varieties. *Cereal Research Communications*, 30, 187-193.

Ebringerova A., Hromadkova Z., Heinze T., 2005. Hemicellulose in Polysaccharides 1: structure, characterization and use. *Advances in Polymer Science*, 186, 1-67. Springer-Verlag, Berlin, Germany.

Edwards S., Chaplin M.F., Blackwood A.D., Dettmar P.W., 2003. Primary structure of arabinoxylans of ispaghula husk and wheat bran. *Proceedings of the Nutrition Society*, 62, 217-222.

Eeckhaut V., Van Immerseel F., Dewulf J., Pasmans F., Haesebrouck F., Ducatelle R., Courtin C.M., Delcour J.A., Broekaert W.F., 2008. Arabinoxylooligosaccharides from wheat bran inhibit salmonella colonization in broiler chickens. *Poultry Science*, 87, 2329–2334.

Englyst H.N. and Cummings J.H., 1984. Simplified method for the measurement of total non-starch polysaccharides by gas-liquid chromatography of constituent sugars as alditols acetates. *Analyst*, 109, 937-942.

Englyst K.N., Liu S., Englyst H.N., 2007. Nutritional characterization and measurement of dietary carbohydrates. *European Journal of Clinical Nutrition*, 61 S1, S19-S39.

Escarnot E., Agneessens R., Wathelet B., Paquot M., 2010. Quantitative and qualitative study of spelt and wheat fibres in varying milling fractions. *Food Chemistry*, 122, 857-863.

Escarnot E., Aguedo M., Agneessens R., Wathelet B., Paquot M., 2011a. Extraction and characterization of water-extractable and water-unextractable arabinoxylans from spelt bran: Study of the hydrolysis conditions for monosaccharides analysis. *Journal of Cereal Science*, 53, 45-52.

Escarnot E., Aguedo M., Paquot M., 2011b. Characterization of hemicellulosic fractions from spelt hull extracted by different methods. *Carbohydrate Polymers*, 85, 419-428.

Escarnot E., Jacquemin J.-M., Agneessens R., Paquot M., 2012. Comparative review of the content and profiles of macronutrients in spelt and wheat. *Biotechnology, Agriculture, Society and Environment*, in press.

Evangelista R. L., Nikolov Z. L., Sung W., Jane J., Gelina R. J. (1991). Effect of compounding and starch modification on properties of starch-filled low density polyethylene. *Industrial & Engineering Chemistry Research*, 30, 1841–1846.

Fang J.M., Sun R.C., Salisbury D., Fowler P., Tomkinson J.T., 1999. Comparative study of hemicelluloses from wheat straw by alkali and hydrogen peroxide extractions. *Polymer degradation and Stability*, 66, 423-432.

FAO, 1998. Carbohydrates in human nutrition. FAO Food and nutrition paper – 66. Report of a Joint FAO/WHO Expert Consultation. Rome, 14-18 April 1997. Reprinted 1998. http://www.fao.org/docrep/W8079E/W8079E00.htm, consulted on 11/01/07.

FAOSTAT, 2011a. Production. http://faostat.fao.org/site/567/default.aspx#ancor, consulted on 23/03/2011.

FAOSTAT, 2011b. Food Balance Sheets, http://faostat.fao.org/site/368/default.aspx#ancor, consulted on 23/03/2011.

Federal Statistical Office Germany, 2011. Request by mail, response by Doris Schmeling on 12/04/2011.

Federmann G.R., Goecke E.U., Steiner A.M., 1992. Research note: detection of adulteration of flour spelt (*Triticum spelta* L.) with flour wheat (*Triticum aestivum* L. emend, Fiori and Paol.) by electrophoresis. *Plant Var. Seeds*, 5, 123-125.

Feillet P., 2000. Le grain de blé, composition et utilisation. Paris, France : Institut National de la Recherche Agronomique, 308 p.

Ferguson L.R. and Harris P.J., 1999. Protection against cancer by wheat bran: role of dietary fibre and phytochemicals. *European Journal of Cancer Prevention*, 8, 17-25.

Figueroa-Espinoza M. C., Poulsen C., Soe J. B., Zargahi M. R., Rouau X., 2004. Enzymatic solubilization of arabinoxylans from native, extruded, and high-shear-treated rye bran by different endo-xylanases and other hydrolyzing enzymes. *Journal of Agricultural and Food Chemistry*, 52, 4240-4249.

Fincher G.B., Sawyer W.H., Stone B.A., 1974. Chemical and physical properties of an arabinogalactan-peptide from wheat endosperm. *The Biochemical Journal*, 139, 535-545.

Folch J., Lees M., Stanley G.H.S., 1957. A simple method for the isolation and purification of total lipids from animal tissues. *Journal of Biochemical Chemistry*, 226, 497-509.

Frison E.A., Smith I.F., Johns T., Cherfas J., Eyzaguirre P., 2006. Agricutural biodiversity, nutrition, and health: making a difference to hunger and nutrition in the developing world. *Food and Nutrition Bulletin*, 27, 167-179.

Gebruers K., Dornez E., Boros D., Fras A., Dynkowska W., Bedo Z., Rakszegi M., Delcour J.A., Courtin C.M., 2008. Variation in the content of dietary fiber and components thereof in wheats in the healthgrain diversity screen. *Journal of Agricultural and Food Chemistry*, 56, 9740-9749.

Gebruers K., Dornez E., Bedo Z., Rakszegi M., Courtin C.M, Delcour J.A., 2010. Variability in xylanase and xylanase inhibition activities in different creals in the healthgrain diversity screen and contribution of environnemnt and genotype to this variability in common wheat. *Journal of Agricultural and Food Chemistry*, 58, 9362-9371.

Gibson G.R., Roberfroid M.B., 1995. Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. *Journal of Nutrition*, 125, 1401–12.

Girio F.M., Fonseca C., Carvalheiro F., Duarte L.C., Marques S., Bogel-Lukasik R., 2010. Hemicelluloses for fuel ethanol: a review. *Bioresource Technology*, 101, 4775-4800.

Gomez M., Ronda F., Blano C.A., Caballero P.A., Apesteguia A., 2003. Effect of dietary fibre on dough rheology and bread quality. *European Food Research and Technology*, 216, 51-56.

Gould J.M., 1984. Alkaline peroxide delignification of agricultural residues to enhance enzymatic saccharification. *Biotechnology and Bioengineering*, 26, 1, 46-52.

Gräber S. and Kuhn, M., 1992. Evaluation of the baking quality of different varieties of spelt. *Getreide, Mehl u Brot*, 46, 102-108.

Granström T.B., Izumori K., Leisola M., 2007. A rare sugar xylitol. Part II: biotechnological production and future applications of xylitol. *Applied Microbiology and Biotechnology*, 74, 273-276.

Gray J., 2003. Carbohydrates: nutritional and health aspects. ILSI Europ Concise Monograph Series. Brussels, 30 p.

Gray J., 2006. Dietary Fibre. Definition and analysis, physiology, health. ILSI Europ Concise Monograph Series. Brussels, 36 p.

Grela E.R., 1996. Nutrient composition and content of anti-nutritional factors in spelt (*Triticum spelta* L.) cultivars. *Journal of the Science of Food and Agriculture*, 71, 399-404.

Gruppen H., Marseille J.P., Voragen A.G., Hamer R.J., Pilnik W., 1989. Mild-isolation of water-insoluble cell wall material from wheat flour: composition of fractions obtained with emphasis on non-starch polysaccharides. *Journal of Cereal Science*, 9, 247-260.

Hahn-Hägerdal B., Kahrumaa K., Fonseca C., Spencer-Martins I., Gorwa-Grauslund M.F., 2007. Towards industrial pentose-fermenting yeast strains. *Applied Microbiology and Biotechnology*, 74, 937-953.

Hall G., Kirk M. D., Becker B., Gregory J. E., Unicomb L., Millard G., Stafford R., and Lalor K., 2005. Estimating foodborne gastroenteritis. *Australia. Emerg. Infect. Dis.,* 11, 1257–1264.

Han G., Deng J., Zhang S., Bicho P., Wu Q., 2010. Effect of steam explosion treatment on characteristics of wheat straw. *Industrial Crops and Products*, 31, 28-33.

Harsch S., Günther T., Kling Ch.I., Rozynek B., Hesemann C.U., 1997. Characterization of spelt (*Triticum spelta* L.) forms by gel electrophoretic analyses of seed storage proteins. I The gliadins. *Theoretical and Appied Genetics*, 94, 52-60.

Hemery Y., Chaurand M., Holopainen U., Lampi A.-M., Lehtinen P., Piironen V., Sadoudi A., Rouau X., 2011a. Potential dry fractionation of wheat bran for the development of food ingredients, part I: Influence of ultra-fine grinding. *Journal of Cereal Science*, 53, 1-8.

Hemery Y., Holopainen U., Lampi A.-M., Lehtinen P., Nurmi T., Piironen V., Edelmann M., Rouau X., 2011b. Potential dry fractionation of wheat bran for the development of food ingredients, part II: Electrostatic separation of particles. *Journal of Cereal Science*, 53, 9-18.

Heredia A., Jimenez A., Guillen R.Z., 1995. Composition of plant cell walls. *Lebensmittel Unters Forsch*, 200, 24-31.

Herman J.L., 2006. Essais variétaux froment d'hiver. Essais valeur culturale et d'utilisation. *Comité pour l'élaboration du catalogue national des variétés des espèces de plantes agricoles,* (28p). Gembloux, Centre Wallon de Recherches Agronomiques.

Herman J.L., 2007a. Essais variétaux épeautre. Essais valeur culturale et d'utilisation. *Comité pour l'élaboration du catalogue national des variétés des espèces de plantes agricoles,* (8p). Gembloux, Centre Wallon de Recherches Agronomiques.

Herman J.L., 2007b. Essais variétaux froment d'hiver. Essais valeur culturale et d'utilisation. *Comité pour l'élaboration du catalogue national des variétés des espèces de plantes agricoles,* (24p). Gembloux, Centre Wallon de Recherches Agronomiques.

Herman J.L., 2008a. Essais variétaux épeautre. Essais valeur culturale et d'utilisation. *Comité pour l'élaboration du catalogue national des variétés des espèces de plantes agricoles,* (12p). Gembloux, Centre Wallon de Recherches Agronomiques.

Herman J.L., 2008b. Essais variétaux froment d'hiver. Essais valeur culturale et d'utilisation. *Comité pour l'élaboration du catalogue national des variétés des espèces de plantes agricoles,* (23p). Gembloux, Centre Wallon de Recherches Agronomiques.

Herman J.L., 2009a. Essais variétaux épeautre. Essais valeur culturale et d'utilisation. *Comité pour l'élaboration du catalogue national des variétés des espèces de plantes agricoles,* (8p). Gembloux, Centre Wallon de Recherches Agronomiques.

Herman J.L., 2009b. Essais variétaux froment d'hiver. Essais valeur culturale et d'utilisation. *Comité pour l'élaboration du catalogue national des variétés des espèces de plantes agricoles,* (23p). Gembloux, Centre Wallon de Recherches Agronomiques.

Herman J.L. and Couvreur L., 2005. Essais variétaux froment d'hiver. Essais valeur culturale et d'utilisation. *Comité pour l'élaboration du catalogue national des variétés des espèces de plantes agricoles,* (25p). Gembloux, Centre Wallon de Recherches Agronomiques.

Herman J.L. and Jacquemin G., 2010. Essais variétaux froment d'hiver. Essais valeur culturale et d'utilisation. *Comité pour l'élaboration du catalogue national des variétés des espèces de plantes agricoles,* (24p). Gembloux, Centre Wallon de Recherches Agronomiques.

Herman J.L., Sinnaeve G., Gofflot S., Jacquemin G., 2010. Essais variétaux épeautre. Essais valeur culturale et d'utilisation. *Comité pour l'élaboration du catalogue national des variétés des espèces de plantes agricoles,* (8p). Gembloux, Centre Wallon de Recherches Agronomiques.

Herman J.L., Pannecoucque J., Sinnaeve G., Gofflot S., Jacquemin G., 2011. Essais variétaux épeautre. Essais valeur culturale et d'utilisation. *Comité pour l'élaboration du catalogue national des variétés des espèces de plantes agricoles,* (8p). Gembloux, Centre Wallon de Recherches Agronomiques.

Hoffman R.A., Leeflang B.R., Debarse M.M.J., 1991. Characterization by H-1-NMR spectroscopy of oligosaccharides, derived from arabinoxylans of white endosperm of wheat, that contain the elements --> 4)[α-L-Araf-(1 --> 3)]-β-D-Xylp-(1 --> or --> 4)[α-L-Araf-(1 --> 2)][α-L-Araf-(1 --> 3)]-β-D-Xylp-(1 -->. *Carbohydrate Research*, 221, 63-81.

Hoije A., Grondahl M., Tommeraas K., Gatenholm P., 2005. Isolation and characterization of physicochemical and material properties of arabinoxylans from barley husks. *Carbohydrate Polymers*, 61, 266-275.

Hollmann J., Lindhauer M.G., 2005. Pilot-scale isolation of glucuronoarabinoxylans from wheat bran. *Carbohydrate Polymers*, 59, 225-230.

Hoseney C.R., 1994a. Proteins of cereals. In: Principles of cereal science and technology. Second edition. American Asociation of Cereal Chemists. St-Paul, Minnesota, p 65-79.

Hoseney C.R., 1994b. Dry milling of cereal. In : Principles of cereal science and technology. Second edition. American Asociation of cereal chemists. St-Paul, Minnesota, p 125-145.

Hoseney C.R., 1994c. Gluten proteins. In: Principles of cereal science and technology. Second edition. American Association of Cereal Chemists. St-Paul, Minnesota, p 197-211.

Hromadkova Z. and Ebringerova A., 2003. Ultrasonic extraction of plant materials-investigation of hemicellulose release from buckwheat hulls. *Ultrasonics Sonochemistry*, 10, 127-133.

Hucl P., Abdel-Aal E.M.S., Sosulski F.W., 1995. Specialty wheats. Page 40-47 in:Proc. Crop opportunities III conference. University of Saskatchewan, SK, Canada.

INRA, 2007. Alimentation des bovins, ovins et caprins. Besoins des animaux - Valeur des aliments. *Tables INRA 2007*. Editions Quae, Versailles, 307p.

Iafelice G., Verardo V., Marconi E., Caboni M.F., 2009. Characterization of total, free and esterified phytosterols in tetraploid and hexaploid wheats. *Journal of Agricultural and Food Chemistry*, 57, 2267-2273.

Iiyama K., Lam T.B.T., Stone B.A., 1994. Covalent cross-links in the cell wall. *Plant Physiology*, 104, 315-320.

Izydorczyk M.S. and Biliaderis C.G., 1995. Cereal arabinoxylans: advances in structure and physicochemical properties. *Carbohydrate Polymers*, 28, 33-48.

Jiang L.-N., Shao M., Liu L., Li C.-X., 2007. Effects of genotypes and environments on pentosan content and viscosity in wheat grains (in Chinese/English abstract). *Journal of Henan Agricutlural Science*, 1, 17-19

Jin .X., Ren J.L., Peng F., Xu F., Zhou G.Y., Sun R.C., Kennedy J.F., 2009. Comparative characterization of degraded and non-degradative hemicelluloses from barley straw and maize stems: composition, structure, and thermal propetrties. *Carbohydrate Polymers*, 78, 609-619.

Johns T.E., Eyzaguirre P.B., 2006. Linking biodiversity, diet and health in policy and practice. *Proceedings of the Nutrition Society*, 65, 182-189.

Jones J.A., 2006. Grain-based foods and health. *Cereal Foods World*, 51, 108-113.

Jorgensen J.R., Olsen C.C., Christiansen S., 1997. Cultivation and quality assessment of spelt (*Triticum spelta* L.) compared with winter wheat (*Triticum aestivum* L.). In: Minutes from COST 814 programme, Copenhagen, Denmark, 31-37.

Kabel M.A., Kortenoeven L., Schols H.A., Voragen A.G.J., 2002a. In vitro fermentability of differently substituted xylooligosaccharides. *Journal of Agricultural and Food Chemistry*, 50, 6205-6210.

Kabel M.A., Carvalheiro F., Garrote G., Avgerinos E., Koukios E., Parajo J.C., Girio F.M., Schols H.A., Voragen A.G.J., 2002b. Hydrothermally treated xylan rich by-products yield different classes of xylo-oligosaccharides. *Carbohydrate Polymers*, 50, 47-56.

Kabel M.A., Van den Borne H., Vincken J.-P., Voragen A.G.J., Schols H.A., 2007. Structural differences of xylans affect their interaction with cellulose. *Carbohydrate Polymers*, 69, 94-105.

Kasarda D.D. and D'Ovidio P., 1999. Deduced amino acid sequence of an α-gliadin gene from spelt wheat (spelta) includes sequences active in celiac disease. *Cereal Chemistry*, 76 (4), 548-551.

Key TJ., Spencer E.A., 2007. Carbohdyrates and cancer: an overview of the epidemiological evidence. European *Journal of Clinical Nutrition*, 61, S1, S112-S121.

Kies C. and Fox H.M., 1970. Determination of the first limiting amino acid of wheat and triticale grain for humans. *Cereal Chemistry*, 47, 615-625.

Koenig A., Wieser H., Koehler P., 2009. Distinguishing wheat and spelt using typical protein markers. Proceedings of the 10th International Gluten Workshop 7-9 September 2009, Clermont-Ferrand, France, p 142-145.

Koh-Banerjee P., Franz M., Sampson L., Liu S., Jacobs Jr D.R., Spiegelman D., Willett W., Rimm E., 2004. Changes in whole-grain, bran, and cereal fibre consumption in relation to 8-year weight gain among men. *American Journal of Clinical Nutrition*, 80, 1237-1245.

Kormelink F.J.M., Hoffmann, R.A., Gruppen, H., 1993. Characterization by H-1-NMR spectroscopy of oligosaccharides, derived from alkali-exractable wheat-flour arabinoxylans by digestion with endo-(1 --> 4)-β-D-xylanase III from Aspergillus awamori. *Carbohydrate Research*, 249, 369-382.

Kritchevsky D., 2001. Dietary fibre in health and disease. In McCleary B.V. and Prosky L., *Advanced dietary Fibre Technology*, (pp 149-161). Oxford: Blackwell Science.

Lagrain B., Goderis B., Brijs K., Delcour J.A., 2010. Molecular basis of processing wheat gluten towards biobased materials. *Biomacromolecules*, 11, 533-541.

Larsson S.C., Giovanucci E., Bergkvist L., Wolk A., 2005. Whole grain consumption and risk of colorectal cancer: a population-based cohort of 60000 women. *British Journal of Cancer*, 92, 1803-1807.

Lebesi D.M., Tzia C., 2011. Effect of the Addition of Different Dietary Fiber and Edible Cereal Bran Sources on the Baking and Sensory Characteristics of Cupcakes. *Food and Bioprocess Technology*, 4, 710–722.

Lecomte P., Boreux M., Agneessens R., Beckers Y., Dekeyser A., 1996. Caractérisation de la composition chimique et de la valeur de l'épeautre (*Triticum spelta*) en alimentation des ruminants. *Rencontres Recherche Ruminants*, 3, 112.

Lee J.W. and Stenvert N.L., 1973. Conditioning studies on Australian wheat. IV. Compositional varieties in the bran layers of wheat and their relation to milling. *Journal of the Science of Food and Agriculture*, 24, 1565-1569.

Lee S.C., Prosky L., De Vries J.W., 1992. Determination of total, soluble, and insoluble dietary fiber in foods – Enzymatic-gravimetric method, MES-TRIS buffer : collaborative study. *Journal of AOAC International*, 75 (3): 395-416.

Lequart C., Nuzillard J.-M., Kurek B., Debeire P., 1999. Hydrolysis of wheat bran and straw by an endoxylanase: production and structural characterization of cinnamoyl-oligosaccharides. *Carbohydrate Research*, 319, 102-111.

Lempereur I., Rouau X., Abecassis J., 1997. Genetic and agronomic variation in arabinoxylan and ferulic acid contents of durum wheat (Triticum durum L.) grain and its milling fractions. *Journal of Cereal Science*, 25, 103-110.

Li K.C., Azadi P., Collins R., Tolan J., Kim J.S., Eriksson K.E.L., 2000. Relationships between activities of xylanases and xylan-structures. *Enzyme Microbioly and Technology,* 27, 89-94.

Li C.-X., Qiu Z.-B., Jiang L.-N., Zhang X., 2002. Research on content of pentosans in wheat grain in different ecological environment (in chinese/English abstract). *Acta Agriculturae Boreali Sin*, 17, 1-4.

Li L., Shewry P.R., Ward J.L., 2008. Phenolic acids in wheat varieties in the Healthgrain diversity screen. *Journal of Agricultural and Food Chemistry*, 56, 9732-9739.

Li S., Morris C.F., Bettge A.D., 2009. Genotype and environment variation for arabinoxylans in hard winter and spring wheats of the U.S. Pacific Northwest. *Cereal Chemistry*, 86, 88-95.

Liavoga A.B., Bian Y., Seib P.A., 2007. Release of D-Xylose from Wheat Straw by Acid and Xylanase Hydrolysis and Purification of Xylitol. *Journal of Agricultural and Food Chemistry*, 55, 7758–7766.

Loje H., Moller B., Laustsen A.M., Hansen A., 2003. Chemical composition, functional properties and sensory profiling of einkorn (*Triticum monococcum* L.). *Journal of Cereal Science*, 37, 231-240.

Loosveld A.-M. A., Grobet P.J., Delcour J.A., 1997. Contents and structure features of water extractable arabinogalactan in wheat flour fractions. *Journal of Agricultural and Food Chemistry*, 45, 1998-2002.

Lundqvist J., Teleman A., Junel L., Zacchi G., Dalman O., Tjernel F., 2002. Isolation and characterization of galactoglucomannan from spruce (Picea abies). *Carbohydrate Polymers*, 48, 1, 29-39.

Macfarlane S., Macfarlane G.T., Cummings J.H., 2006. Review article: prebiotics in the gastrointestinal tract. *Alimentary Pharmacology and Therapeutics,* 24, 701–714.

Maes C. and Delcour J.A., 2001. Alkaline hydrogen peroxide extraction of wheat bran non-starch polysaccharides. *Journal of Cereal Science* 34, 29-35.

Maes C. and Delcour J.A., 2002. Structural characterisation of water-extractable and water-unextractable arabinoxylans in wheat bran. *Journal of Cereal Science*, 35, 315-326.

Maes C., 2004. Structural features of wheat (Triticum aestivum) bran arabinoxylan fractions extracted with endoxylanases or alkaline media. Dissertationes de agricultura. Doctoraatsproefschrift. Katholieke Universiteit Leuven, Belgium, 173 p.

Maes C., Vangeneugden B., Delcour J. A., 2004. Relative activity of two endoxylanases towards water-unextractable arabinoxylans in wheat bran. *Journal of Cereal Science*, 39: 181-186.

Magnuson K.M., 1985. Uses and functionality of vital wheat gluten. *Cereal Foods World*, 30, 179-181.

Mäkinen K. K.; Chiego D. J.; Allen P.; Bennet C.; Isotupa K. P.; Tiesko J.; Makinen P. L., 1998. Physical, chemical and histological changes in dentin caries lesion of primary teeth induced by regular use of polyol chewing-gums. *Acta Odontologica Scandinavica,* 56, 148–156.

Mali S., Grossman M.V.E., Garcia M.A., Martino M.N., Zaritzky N.E., 2006. Effects of controlled storage on thermal, mechanical and barrier properties of plasticized films from different starch sources. *Journal of Food Engineering*, 75, 453–460.

Mandalari G., Faulds C. B., Sancho A. I., Saija A., Bisignano G., LoCurto R., Waldron K. W., 2005. Fractionation and characterisation of arabinoxylans from brewers' spent grain and wheat bran. *Journal of Cereal Science*, 42, 205-212.

Mankarios A.T., Christopher F., Jones G., Jarvis M.C., Threlfall D.R., Friend J., 1979. Hydrolysis of plant polysaccharides and GLC analysis of their constituent neutral sugars. *Phytochemistry,* 18, 419-422.

Mann J., 2007. Dietary carbohydrate: relationship to cardiovascular disease and disorders of carbohydrate metabolism. *European Journal of Clinical Nutrition*, 61, S1, S100-S111.

Marconi E., Carcea M., Graziano M., Cubadda R., 1999. Kernel properties and pasta-making quality of five European spelt wheat (*Triticum spelta* L.) cultivars. *Cereal Chemistry*, 76 (1), 25-29.

Marconi E., Carcea M., Schiavone M., Cubadda R., 2002. Spelt (*Triticum spelta* L.) pasta quality: combined effect of flour properties and drying conditions. *Cereal Chemistry*, 79 (5), 634-639.

Marechal P., Jorda J., Pontalier P.Y., Rigal, L., 2004. Twin screw extrusion and ultrafiltration for xylan production from wheat in "Hemicelluloses : Science and Technology Book" Gatenholm P. and Tenhanen M., Edition ACC, Washington, p 38-51.

Mares D.J., Stone B.A., 1973. Studies on wheat endosperm I. Chemical composition and ultrastructure of the cell walls. *Australian Journal of Biological Sciences*, 26, 793-812.

Massaux C., Sindic M., Lenartz J., Sinnaeve G., Bodson B., Falisse A., Dardenne P., Deroanne C., 2008. Variations in physicochemical and functional properties of starches extracted from European soft wheat (*Triticum aestivum* L.): The importance to preserve the varietal identity. *Carbohydrate Polymers*, 71, 32-41.

Matamoros Fernandez L. E.; Obel N.; Scheller H. V.; Roepstorff P. Characterization of plant oligosaccharides by matrix-assisted laser desorption/ionization and electrospray mass spectrometry. *Journal of Mass Spectrometry*, 2003, 38, 427–437.

Matuz J., Bartok T., Morocz-Salamon K., Bona L., 2000a. Structure and potential allergenic character of cereal proteins. I. Protein content and amino acid composition. *Cereal Research Communications*, 28 (3), 263-270.

McDougall R.M., Yakymyshyn L. Walker K., Thursten O.G., 1978. The effect of wheat bran on serum lipoproteins and biliary lipids. *Canadian Journal of Surgery*, 21, 433-435.

Mielenz J.R., 2001. Ethanol production from biomass: technology and commercialization status. *Current Opinions in Microbiology*,4, 324-329.

Miyasawa H. I. Y.; Mayanagi H.; Takahashi N., 2003. Xylitol inhibition of anaerobic acid production by streptococcus mutans at various pH levels. *Oral Microbiology and Immunology*, 18, 215–219.

Moonen J.H.E, Zeven, A.C., 1985. Association between high molecular weight subunits of glutenin and bread-making quality in wheat Lines derived from backcrosses between *T. aestivum* and *T. speltoides*. *Journal of Cereal Science*, 3, 97-101.

Moore M.A., Beom Park C., Tsuda H., 1998. Soluble and insoluble fiber influences on cancer development. *Critical Review Oncology and Hematology*, 27, 229-242.

Moore J., Cheng Z., Su L., Yu L.L., 2006. Effects of solid-state enzymatic treatments on the antioxidant properties of wheat bran. *Journal of Agricultural and Food Chemistry*, 54, 9032-9045.

Moudry J., Dvoracek V., 1999. Chemical composition of grain of different spelt (*Triticum spelta* L.) varieties. *Rostlinna Vyroba*, 45 (12), 533-538.

Moure A., Gullon P., Dominguez H., Parajo J.C. Advances in the manufacture, purification and applications of xylooligosccharides as food additives and nutraceuticals. *Process Biochemistry*, 41, 1913-1923

Muramatsu M., 1963. Dosage effect of the spelta gene q of hexaploid wheat. *Genetics,* 48: 469-482.

Nabarlatz D., Montané D., Kardosova A., Bekesova S., Hribalova V., Ebringerova A., 2007. Almond shell xylo-oligosaccharides exhibiting immunostimulatory activity. *Carbohydrate Research*, 342, 1122-1128.

Nesbitt M. and Samuel D., 1996. From staple crop to extinction? The archaeology and history of the hulled wheats. In: Hulled wheats: Proc. First. Int. Workshop on hulled wheats. Padulosi S. et Heller J. International Plant Genetic Resources Institute, Rome, p 41-100.

Nesbitt M., McBurney R.P.H., Broin M., Beetje H.J., 2010. Linking biodiversity, food and nutrition: The importance of plant identification and nomenclature. *Journal of Food Composition and Analysis*, 23, 486-498.

Nurmi T., Nyström L., Edelmann M., Lampi A.-M., Piironen V., 2008. Phytosterols in wheat genotypes in the healthgrain diversity screen. *Journal of Agricultural and Food Chemistry*, 56, 9710-9715.

Office fédéral de la statistique Suisse, 2008. http://www.bfs.admin.ch/bfs/portal/fr/index.html

Office fédéral de la statistique, Suisse, 2011.
http://www.bfs.admin.ch/bfs/portal/fr/index/themen/07.html, consulted on 23/03/2011.

Oliveira J.A., 2001. North Spanish emmer and spelt wheat landraces: agronomical and grain quality characteristics evaluation. *Plant Genetic Resources Newsletter,* 125, 16-20.

Ou S. and Kwok K.-C., 2004. Ferulic acid: pharmaceutical functions, preparation and applications in foods. *Journal of the Science of Food and Agriculture*, 84, 1261–1269.

Ordaz-Ortiz J.-J., Saulnier L., 2005; Structural variability of arabinoxylans from wheat flour. Comparison of water-extractable and xylanase-extractable arabinoxylans. *Journal of Cereal Science*, 42, 1, 119-125.

Pala H., Mota M., Gama F.M., 2007. Enzymatic depolymerisation of cellulose. *Carbohydrate Polymers*, 68, 101-108.

Palm M. and Zacchi G., 2003. Extraction of Hemicellulosic Oligosaccharides from Spruce Using Microwave Oven or Steam Treatment. *Biomacromolecules*, 4, 617-623.

Pastell H., Tuomainen P., Virkki L., Tenkanen M., 2008. Step-wise enzymatic preparation and structural characterization of singly and doubly substituted arabinoxylo-oligosaccharides with non-reducing end terminal branches. *Carbohydrate Research*, 343, 3049-3057.

Paszner L. Jeong C., Quinde A., Awrdel-Karim S., 1993. Two-stage, continuous hydrolysis of wood by the acid catalysed organosolv saccharification (ACOS) process. *Energy Biomass Wastes*, 16, 629-64.

Pepper T and Olinger PM, Xylitol in sugar-free confections. *Food Technology*, 42, 98–106 (1988).

Percival J., 1921. The wheat plant. A monograph. Duckworth and Co. London, p 325-334.

Persson T., Ren J. L., Joelsson E., Jönsson A.-S, 2009. Fractionation of wheat and barley straw to access high-molecular-mass hemicelluloses prior to ethanol production. *Bioresource Technology*, 100, 3906-3913.

Pessoa A. Jr, Mancilha I.M., Sato S., 1996. Cultivation of Candida tropicalis in sugar cane hemicellulosic hydrolyzate for microbial protein production. *Journal Biotechnology*, 51:83-88.

Piergiovanni A.R., Laghetti G., Perrino P., 1996. Characteristics of meal from hulled wheats (*Triticum dicoccon* Schrank and *T. spelta* L.): an evaluation of selected accessions. *Cereal Chemistry*, 73 (6), 732-735.

Piergiovanni A.R., and Volpe, N., 2003. Capillary electrophoresis of gliadins as a tool in the discrimination and characterization of hulled wheats (*Triticum dicoccon* Schrank and *T. spelta* L.). *Cereal Chemistry*, 80, 269-273.

Planchon V., 2005. Traitement des valeurs aberrantes : concepts actuels et tendances générales. *Biotechnologie, Agronomie, Société et Environnement*, 9 (1), 19-34.

Pogna N.F., Autran J.C., Mellini F., Lafiandra D., Feillet P., 1990. Chromosome 1B-encoded gliadins and glutenins subunits in durum wheat: genetics and relationship to gluten strength. *Journal of Cereal Science*, 11, 15-34.

Poutanen K., 2006. Grain fibre: how to deliver the health benefits. L42. *Dietary fibre 2006 Conference*, June 12-14, 2006, Helsinki, Finland.

Pomeranz Y., 1988. Chemical composition of kernel structure. In: Pomeranz, Y. (Ed), Wheat and chemistry technology, vol I, 3rd edition. AACC, St Paul, Minnesota, U.S.A., p 97-158.

Pommet M.; Redl A.; Morel M. H.; Domenek S.; Guilbert S. Thermoplastic processing of protein-based bioplastics: Chemical engineering aspects of mixing, extrusion and hot molding. *Macromolecular Symposium,* 2003, 197, 207–217.

Premavalli K.S., Roopa S., Bawa A.S., 2006. Resistant starch- A functional dietary fiber. *Indian Food Industry*, 25, 40-45.

Prosky L., Asp G.N., Scheweizer T.F., de Vries J.W., Furda I., 1992. Determination of insoluble and soluble dietary fiber in foods and food products: collaborative study. *Journal of the Association of Analytical Chemistry International*, 75, 360-367.

Pruska-Kedzior A., Kedzior Z., Klockiewicz-Kaminska E., 2008. Comparison of viscoelastic properties of gluten from spelt and common wheat. *European Food Research Technology*, 227, 199-207.

Puchart V., Biely, P., 2008. Simultaneous production of endo-beta-1,4-xylanase and branched xylooligosaccharides by Thermomyces lanuginosus. *Journal of Biotechnology*, 137, 34-43.

Quéméner B., Ordaz-Ortiz J.J., Saulnier L., 2006. Structural characterization of underivatized arabino-xylo-oligosaccharides by negative-ion electrospray mass spectrometry. *Carbohydrate Research*, 341, 1834–1847.

Radic H., Günther T., Kling Ch.I., Hesemann C.U., 1997. Characterization of spelt (*Triticum spelta* L.) forms by gel electrophoretic analyses of seed storage proteins. II The glutenins. *Theoretical and Applied Genetics*, 94, 882-886.

Radic-Miehle H., Saam C., Huls R., Kling Ch.I., Hesemann C.U., 1998. Characterization of spelt (*Triticum spelta* L.) forms by gel-electrophoretic analyses of seed storage proteins. III. Comparative analyses of spelt and central European winter wheat (*Triticum aestivum* L.) cultivars by SDS-PAGE and acid-PAGE. *Theoretical and Applied Genetics*, 97, 1340-1346.

Ralet M.-C., Thibault J.-F., Della Valle G., 1990. Influence of extrusion-cooking on the physico-chemical properties of wheat bran. *Journal of Cereal Science*, 11, 249-259.

Ranhotra G.S., Gelroth J.A., Glaser B.K., Lorenz K.J., 1995. Baking and nutritional qualities of spelt wheat sample. *Lebensmittel-Wissenschaft und Technologie*, 28, 118-122.

Ranhotra G.S., Gelroth J.A., Glaser B.K., Stallknecht G.F., 1996a. Nutritional profile of three spelt wheat cultivars grown at five different locations. *Cereal Chemistry*, 73 (5), 533-535.

Ranhotra G.S., Gelroth J.A., Glaser B.K., Lorenz K.J., 1996b. Nutrient composition of spelt wheat. *Journal of Food Composition and Analysis*, 9, 81-84

Rantanen H., Virkki L., Toumainen P., Kabel M., Schols H., Tenkanen M., 2007. Preparation of arabinoxylobiose from rye xylan using family 10 *Aspergillus aculeatus* endo-1,4-β-D-xylanase. *Carbohydrate Polymers*, 68, 350–359.

Rémond C., Aubry N., Crônier D., Noël S., Martel F., Roge B., Rakotoarivonina H., Debeire P., Chabbert B., 2010. Combination of ammonia and xylanase pretreatments: Impact on enzymatic xylan and cellulose recovery from wheat straw. *Bioresource Technology*, 101, 6712-6717.

Ring S.G. and Selvendran R.R., 1980. Isolation and analysis of cell wall material from beeswing wheat bran (Triticum aestivum). *Phytochemistry*, 19, 1723-1730.

Rivas B., Torre P., Dominguez J.M., Converti A., Parajo J.C., 2006. Purification of xylitol obtaind by fermentation of corncob hydrolysates. *Journal of Agricultural and Food Chemistry*, 54, 4430-4435.

Roos A.A., Persson T., Krawczyk H., Zacchi G., Stalbrand H., 2009. Extraction of water-soluble hemicelluloses from barley husks. *Bioresource Technology*, 100, 763-769.

Rose D.J. and Inglett G.E., 2010. Production of feruloylated arabinoxylo-oligosaccharides from maize (Zea mays) bran by microwave-assisted autohydrolysis. *Food Chemistry*, 119, 1613–1618.

Rosell C.M., Santos E., Collar C., 2006. Mixing properties of fibre-enriched wheat bread doughs: a response surface methodology study. *European Food Research and Technology*, 223, 333-340.

Ruibal-Mendieta N.L., 2004. Lipids and minerals in spelt (*Triticum aestivum* ssp. *spelta*) and common wheat (*T.aestivum* ssp. *vulgare*). Chemical and nutritional distinction between both cereals. Thèse de doctorat. Faculté d'ingénierie biologique, agronomique et environnementale. Université catholique de Louvain.

Ruibal-Mendieta N.L., Delacroix D.L., Meurens M., 2002. A comparative of analysis of free, bound and total lipid content on spelt and winter wheat wholemeal. *Journal of Cereal Science*, 35, 337-342.

Ruibal-Mendieta N.L., Rozenberg R., Delacroix D.L., Petitjean G., Dekeyser A., Baccelli C., Marques C., Delzenne N.M., Meurens M., Habib-Jiwan J.-L., Quetin-Leclercq J., 2004a. Spelt (*Triticum spelta* L.) and winter wheat (*Triticum aestivum* L.) wholemeals have similar sterols profiles, as determined by quantitative liquid chromatography and mass spectrometry analysis. *Journal of Agricultural and Food Chemistry*, 52, 4802-4807.

Ruibal-Mendieta N.L., Dekeyser, A., Delacroix D.L., Mignolet E., Larondelle Y., Meurens M., 2004b. The oleate/palmitate ratio allows the distinction between wholemeals of spelt (*Triticum spelta* L.) and winter wheat (*T. aestivum* L.). *Journal of Cereal Science* 39, 413-415.

Ruibal-Mendieta N.L., Delacroix D.L., Mignolet E., Pycke J.-M., Marques C., Rozenberg R., Petitjean G., Habib-Jiwan J.-L., Meurens M., Quetin-Leclercq J., Delzenne N.M., Larondelle Y., 2005. Spelt (*Triticum aestivum* ssp. *spelta*) as a source of breadmaking flours and bran naturally enriched in oleic acid and minerals but not phytic acid. *Journal of Agricultural and Food Chemistry*, 53, 2571-2759.

Saha B.C., 2000. α-L-arabinofuranosidases: Biochemistry, molecular biology and application in biotechnology. *Biotechnology Advances*, 18, 403-423.

Saha B.C., 2003. Hemicellulose bioconversion. *Journal of Indian Microbiology and Biotechnology,* 30, 279-291.

Salomonsson A.-C., Theander O., Westerlund E., 1984. Chemical characterization of some Swedish cereal whole meal and bran fractions. *Swedish Journal of Agricultural Research*, 14, 111-117.

Saulnier L., Guillon F., Sado P.-E., Rouau X., 2007. Plant cell wall polysaccharides in storage organs : xylans (food applications). In: Kamerling, J.-P., Boons, G.-J., Lee, Y.C., Suzuki, A., Taniguchi, N., Voragen, A.G.J. (Eds), Comprehensive glycoscience. From chemistry to systems biology. Analysis of glycans. Polysaccharide functional properties, Vol 2., Elsevier, Oxford, pp. 653-687.

Sauvant D., Perez J.-M., Tran G., 2002. Tables de composition et de valeur nutritive des matières premières destinées aux animaux d'élevage. Ed. INRA, Paris, 80-81, 258-259.

Schaller D., 1977. Analysis of dietary fiber. *Food Product Development*, 11, 70-72.

Scheller H.V., Ulvskov P., 2010. Hemicelluloses. *Annual Review of Plant Biology*, 61, 263-289.

Schmid J.E., Winzeler M. and Winzeler, H., 1994. Analysis of disease resistance and quality characters of F1 hybrids of crosses between wheat (*Triticum aestivum* L.) and spelt (*Triticum spelta* L.). *Euphytica*, 75, 105-110.

Schober T.J, Clarke C.I., Kuhn M., 2002. Characterization of functional properties of gluten proteins in spelt cultivars using rheological and quality factor measurements. *Cereal Chemistry*, 79, 408-417.

Schober T.J., Bean S.R., Kuhn M., 2006. Gluten proteins from spelt (*Triticum aestivum* ssp. *spelta*) cultivars: a rheological and size-exclusion high-performance liquid chromatography study. *Journal of Cereal Science*, 44, 161-173.

Schooneveld-Bergmans M. E. F., Hopman A.M.C.P., Beldman G., Voragen A.C.J., 1998. Extraction and partial characterization of feruloylated glucuronoarabinoxylans from wheat bran. *Carbohydrate Polymers*, 35, 39-47.

Schooneveld-Bergmans M. E. F., Beldman G., Voragen A.G.J., 1999. Structural features of (glucurono)arabinoxylans extracted from wheat bran by barium hydroxide. *Journal of Cereal Science*, 29, 63-75.

Scoones I., Melnyk M., Pretty J.N., 1992. The Hidden Harvest: Wild Foods and Agricultural Systems, a Literature Review and Annotated Bibliography. International Institute for Environment and Development, London.

Selvendran R.R., Ring S.G., O'Neill M.A., DuPont M.S., 1980. Composition of cell wall material from wheat bran used in clinical feeding trials. *Chemistry and Industry*, 11, 885-888.

Sewalt V.J.H., Beauchemin K.A., Rode L.M., Acharya S., Baron V.S., 1997.. Lignin impact on fiber degradation. IV. Enzymatic saccharification and *in vitro* digestibility of alfalfa and grasses following selective solvent delignification. *Bioresource Technology*, 61, 199-206.

Shallom D. and Shoham Y., 2003. Microbial hemicellulases. *Current Opinion Microbiology*, 6, 219-228.

Shewry P.R., Tatham A.S., Barro F., Barcelo P., Lazzeri P., 1995. Biotechnology of breadmaking: unraveling and manipulating the multi-protein gluten complex. *Bio/Technology*, 13, 1185-1190.

Shiiba K., Yamada H., Hara H., Okada K., Nagao S., 1993. Purification and characterization of two arabinoxylans from wheat bran. *Cereal Chemistry*, 70, 209-214.

Shin H., Guebitz G., Cavaco-Paulo A., 2001. "In situ" enzymatically prepared polymers for wool coloration. *Macromolecular Materials and Engineering*, 286, 691-694.

Singh J., Kaur L., McCarthy O.J., 2007. Factors influencing the physico-chemical, morphological, thermal and rheological properties of some chemically modified starches for food applications – A review. *Food Hydrocolloids*, 21, 1-22.

Sorensen H.R., Meyer A.S., Pedersen S., 2003. Enzymatic hydrolysis of water-soluble wheat arabinoxylan. 1. Synergy between α-L-arabinofuranosidases, endo-1,4-β-xylanases, and β-xylosidases activities. *Biotechnology and Bioengineering*, 81, 726-731.

Sorensen H.R., Pedersen S., Vikso-Nielsen A., Meyer A.S., 2005. Efficiencies of designed enzyme combinations in releasing arabinose and xylose fom wheat arabinoxylan in an industrial ethanol fermentation residue. *Enzyme and Microbial Technology*, 36, 773-784.

Sorensen H.R., Pedersen, S., Meyer A.S., 2007. Synergistic enzyme mechanisms and effects of sequential enzyme additions on degradation of water insoluble wheat arabinoxylan. *Enzyme and Microbial Technology*, 40, 908-918.

Southgate D.A.T., 1995. Analytical Strategies. In : Belton, P.S. (Eds.), Dietary fibre Analysis, Royal Society of Chemistry, Cambridge, pp. 34-35.

Spigno G., Pizzorno T., De Faveri D.M., 2008. Cellulose and hemicelluloses recovery from grape stalks. *Bioresource Technology*, 99: 4329-4337.

Statistisches Bundesamt Deutschland, 2008. http://www.destatis.de

Steeneken P.A.M. and Helmens H.J., 2009. Laboratory-scale dry/wet milling process for the extraction of starch and gluten from wheat. *Starch*, 61, 389-397.

Stewart M.L., Slavin J.L., 2009. Particle size and fraction of wheat bran influence short-chain fatty acid production in vitro. *British Journal of Nutrition*, 102, 1404-1407.

Stoldt W., 1952. Vorschlag zur Vereinheitlichung der Fettbestimmung in Lebensmitteln. *Fette und Seifen*, 54, 206-207.

Stone B. and Morell M.K., 2009. Carbohydrates. In: Khan K. and Shewry P.R. (Eds.), Wheat Chemistry and Technology. Fourth edition. AACC, St Paul, Minnesota, USA, p 323.

Strechlow W., Hertzka G., Wueffen W, 1991. The dietary properties of spelt in the treatment of chronical diseases. *Proceeding, 2,* Hohenheimer Dinkelkolooquium Verlag, Stuttgart, 243-259.

Sun R.C., Fang J.M., Rowlands P., Bolton J., 1998. Physicochemical and thermal characterization of wheat straw hemicelluloses and cellulose. *Journal of Agricultural Chemistry*, 46, 2804-2809.

Sun R.C., Tomkinson J., Wang Y.X., Xioa B., 2000. Physico-chemical and structural characterization of hemicelluloses from wheat straw by alkaline peroxide extraction. *Polymer*, 41, 2647-2656.

Sun Y. and Cheng J., 2002. Hydrolysis of lignocellulosic materials for ethanol production: a review. *Bioresource Technology*, 83, 1-11.

Sun R. and Hughes S., 1998. Fractional extraction and physico-chemical characterization of hemicelluloses and cellulose from sugar beet pulp. *Carbohydrate Polymers*, 36, 293-299.

Sun R.C., Lawther J.M., Banks W.B., 1995. Influence of alkaline pre-treatments on the cell wall components of wheat straw. *Industrial Crops and Products*, 4, 127-145.

Sun R.C., Lawther J.M., Banks W.B., 1996. Fractional and structural characterization of wheat straw hemicelluloses. *Carbohydrate Polymers*, 29, 325-331.

Sun R.C., Fang J.M., Rowlands P., Bolton J., 1998. Physicochemical and thermal characterization of wheat straw hemicelluloses and cellulose. *Journal of Agricultural Chemistry*, 46, 2804-2809.

Sun R.C., Tomkinson J., Wang Y.X., Xioa B., 2000. Physico-chemical and structural characterization of hemicelluloses from wheat straw by alkaline peroxide extraction. *Polymer*, 41, 2647-2656.

Sun R.C., Sun X.F., Zhang S.H., 2001. Quantitative determination of hydroxycinnamic acids in wheat, rice, rye, and barley straws, maize stems, oil plam frond fiber, and fast-growing poplar wood. *Journal of Agricultural and Food Chemistry*, 49, 5122-5129.

Sun R.C., Wang X.Y., Sun X.F., Sun J.X., 2002. Physicochemical and thermal characterisation of residual hemicelluloses isolated by TAED activated peroxide from ultrasonic irradiated and alkali organosolv pre-treated wheat straw. *Polymer Degradation and Stability*, 78, 295-303.

Sun X.F., Sun R.C., Fowler P., Baird M.S., 2004a. Isolation and characterisation of cellulose obtained by a two-stage treatment with organosolv and cyanamide activated hydrogen peroxide from wheat straw. *Carbohydrate Polymers*, 55, 379-391.

Sun J.X., Sun X.F., Sun R.C., Su Y.Q., 2004b. Fractional extraction and structural characterization of sugarcane bagasse hemicelluloses. *Carbohydrate Polymers*, 56, 195-204.

Sun X.F., Xu F., Zhao H., Sun R.C., Fowler P., Baird M.S., 2005a. Physicochemical characterisation of residual hemicelluloses isolated with cyanamide-activated hydrogen peroxide from organosolv pre-treated wheat straw. *Bioresource Technology*, 96, 1342-1349.

Sun X. F., Xu F., Sun R. C., Geng Z. C., Fowler P., Baird M. S., 2005b. Characteristics of degraded hemicellulosic polymers obtained from steam exploded wheat straw. *Carbohydrate Polymers*, 60, 15-26.

Swennen K., Courtin C. M., Van der Bruggen B., Vandecasteele C., Delcour J. A., 2005. Ultrafiltration and ethanol precipitation for isolation of arabinoxylooligosaccharides with different structures. *Carbohydrate Polymers*, 62, 283-292.

Swennen K, Courtin CM, Delcour JA., 2006a. Non-digestible oligosaccharides with prebiotic properties. *Critical Review of Food Science and Nutrition,* 46, 459–71.

Swennen K., Courtin C. M., Lindemans G.C.J.E., Delcour J. A., 2006b. Large-scale production and characterisation of wheat bran arabinoxylooligosaccharides. *Journal of the Science of Food and Agriculture*, 86, 1722-1731.

Thomsen M.H., Thygesen A., Thomsen A.B., 2008. Hydrothermal treatment of wheat straw at pilot plant scale using a three-step reactor system aiming at high hemicelllulose recovery, high cellulose digestibility and low lignin hydrolysis. *Bioresource Technology*, 99, 4221-4228.

Touster O. The Metabolism of Polyols. In Sugars in Nutrition; Sipple, H. L., McNutt, K. W. W., Eds.; Academic Press: St. Louis, MO, 1974; pp 229–237.

Tuohy K.M., Kolida S., Gibson G.R., 2004. Use of probiotics and prebiotics for improving gut health. *Agro Food Industry High-Tech,* 15, 33-35.

Vafiadi C., Topakas E., Christakopoulos P., 2008. Preparation of multipurpose cross-linked enzyme aggregates and their application to production of alkyl ferulates. *Journal of Molecular Catalysis: Enzymatic,* 54, 35-41.

Van Craeyveld V., Swennen K., Dornez E., Van de Wiele T., Marzorati M., Verstraete W., Delaedt Y., Onagbesan O., Decuypere E., Buyse J., De Ketelaere B., Broekaert W. F., Delcour J. A., Courtin C. M., 2008. Structurally different wheat-derived arabinoxylooligosaccharide have different prebiotic and fermentation properties in rats. *The Journal of Nutrition*, 138, 2348-2355.

Van Craeyveld V., Holopainen U., Selinheimo E., Poutanen K., Delcour J.A., Courtin C.M., 2009a. Extensive dry ball milling of wheat and rye bran leads to in situ production of arabinoxylan oligosaccharides through nanoscale fragmentation. *Journal of Agricultural and Food Chemistry*, 57, 8467-8473.

Van Craeyveld V., Delcour J.A., Courtin C.M., 2009b. Extractability and chemical and enzymic degradation of psyllium (Plantago ovata Forsk) seed husk arabinoxylans. *Food Chemistry*, 112, 812-819.

Van Craeyveld V., Dornez E., Holopainen U., Selinheimo E., Poutanen K. Delcour J.A., Courtin C.M., 2010. Wheat bran AX properties and choice of xylanase affect enzymic production of wheat bran-derived arabinoxylan-oligosaccharides. *Cereal Chemistry*, 87, 283-291.

Van Dam R.M., Seidell J.C., 2007. Carbohydrate intake and obesity. *European Journal of Clinical Nutrition*, 61, S1, S75-S99.

Van Der Borght A.; Goesaert H.; Veraverbeke W. S.; Delcour J. A. Fractionation of wheat and wheat flour into starch and gluten: Overview of the main processes and the factors involved. *Journal of Cereal Science*, 2005, 41 (3), 221–237.

Van Soest P.J., 1963. Use of detergents in the analysis of fibrous feeds. II. A rapid method for the determination of fiber and lignin. *Journal of the Association of Official Analytical Chemists*, 46 (5), 829-835.

Van Soest P.J. and Wine R.H., 1967. Use of detergents in the analysis of fibrous feeds. IV. Determination of plant cell-wall constituents. *Journal of the Association of Analytical Chemists*, 50, 50-55.

Vetter J.L., Ingredients for increasing the fiber content of grainbased foods, in Cereal Polysaccharides in Technology and Nutrition, 1984. Ed by Rasper VF, The American Association of Cereal Chemists, St Paul, MN pp 127-138.

Vilmorin-Andrieux & Cie, 1880. Les meilleurs blés. Description et culture des principales variétés de froment d'hiver et de printemps. Eds : Vilmorin-Andrieux & Cie, Paris, pp 147, 151. http://www.museum.agropolis.fr/pages/documents/bles_vilmorin/index.htm, consulted on 17/02/2012.

Von Büren M., Lüthy J. and Hübner P., 2000. A spelt-specific γ-gliadin gene: discovery and detection. *Theoretical and Applied Genetics*, 100, 271-279.

Von Büren M., Stadler M., Lüthy J., 2001. Detection of wheat adulteration of spelt flour and products by PCR. *European Food Research Technology*, 212, 234-239.

Werpy T., Petersen G., Aden A., Bozell J., Holladay J., White J., Manheim A., Elliot D., Lasure L., Jones S., Gerber M., Ibsen K., Lumberg L., Kelley S. 2004. Top value added chemicals from biomass. Volume 1 Results of screening for potential candidates from sugars and synthesis gas. US Department of Energy (DOE), Oak Ridge, TN.

Wieser H., 2000. Comparative investigations of gluten proteins from different wheat species. *European Food Research Technology*, 211, 262-268.

Wilson J.D., Bechtel D.B., Wilson G.W.T., Seib P.A. 2008. Bread quality of spelt wheat and its starch. *Cereal Chemistry*, 85, 5, 629-638.

Wertz J.-L., Bedue O., Mercier J.P., 2010. Cellulose Science and Technology. EPFL Press, Lausanne, Switzerland, 364 p.

Willför S., Pranovich A., Tamminen T., Puls J., Laine C., Suurnäkki A., Saake B., Uotila K., Simolin H., Hemming J., Holmbom B., 2009. Carbohydrate analysis of plant materials with uronic acid-containing polysaccharides - A comparison between different hydrolysis and subsequent chromatographic analytical techniques. *Industrial Crops and Products*, 29, 571-580.

Wu S. C.; Chien P. J.; Lee M. H.; Chau C. F., 2007. Particle size reduction effectively enhances the intestinal health-promotion ability of an orange insoluble fiber in hamsters. *Journal of Food Science*, 72, S618–S621.

Xiao B., Sun X. F., Sun R.C., 2001. Chemical, structural, and thermal characterizations of alkali-soluble lignins and hemicelluloses, and cellulose from maize stems, rye straw, and rice straw. *Polymer Degradation and Stability*, 74, 307-319.

Xie X.S., Cui S.W., Li W., Tsao R., 2008. Isolation and characterization of wheat bran starch. *Food Research International*, 41, 882-887.

Xu F., Liu C.F., Geng Z.C., Sun J.X., Sun R.C., Hei B.H., Lin L., Wu S.B., Je J., 2006a. Characterisation of degraded organosolv hemicelluloses from wheat straw. *Polymer Degradation and Stability*, 91, 1880-1886.

Xu F., Sun J.-X., Liu C.F., Sun R.C., 2006b. Comparative study of alkali- and acidic organic solvent-soluble hemicellulosic polysaccharides from sugarcane bagasse. *Carbohydrate Polymers*, 341, 253-261.

Yamada H., Itoh K., Morishita Y., Taniguchi H.. 1993. Structure and properties of oligosaccharides from wheat bran. *Cereal Foods World*, 38, 490–492.

Yan Y., Hsam S.L.K., Yu J.Z., Jiang Y., Ohtsuka I., Zeller F.J., 2003. HMW and LMW glutenins alleles among putative tetraploid and hexaploid European spelt wheat (*Triticum spelta* L.) progenitors. *Theoretical and Applied Genetics*, 107, 1321-1330.

Zielinski H., Ceglinska A., Michalska A., 2008. Bioactive compounds in spelt bread. *European Food Research Technology*, 226, 537-544.

Zhuang J., Liu Y., Wu Z., Sun Y., Lin L. 2009. Hydrolysis of wheat straw hemicellulose and detoxification of the hydrolysate for xylitol production. *Bioresources*, 4, 674-686.

Zorb C., Betsche T., Langenkamper G., Zapp J., Seifert M., 2007. Free sugars in spelt wholemeal and flour. *Journal of Applied Botany and Food Quality*, 81, 172-174.

Appendix

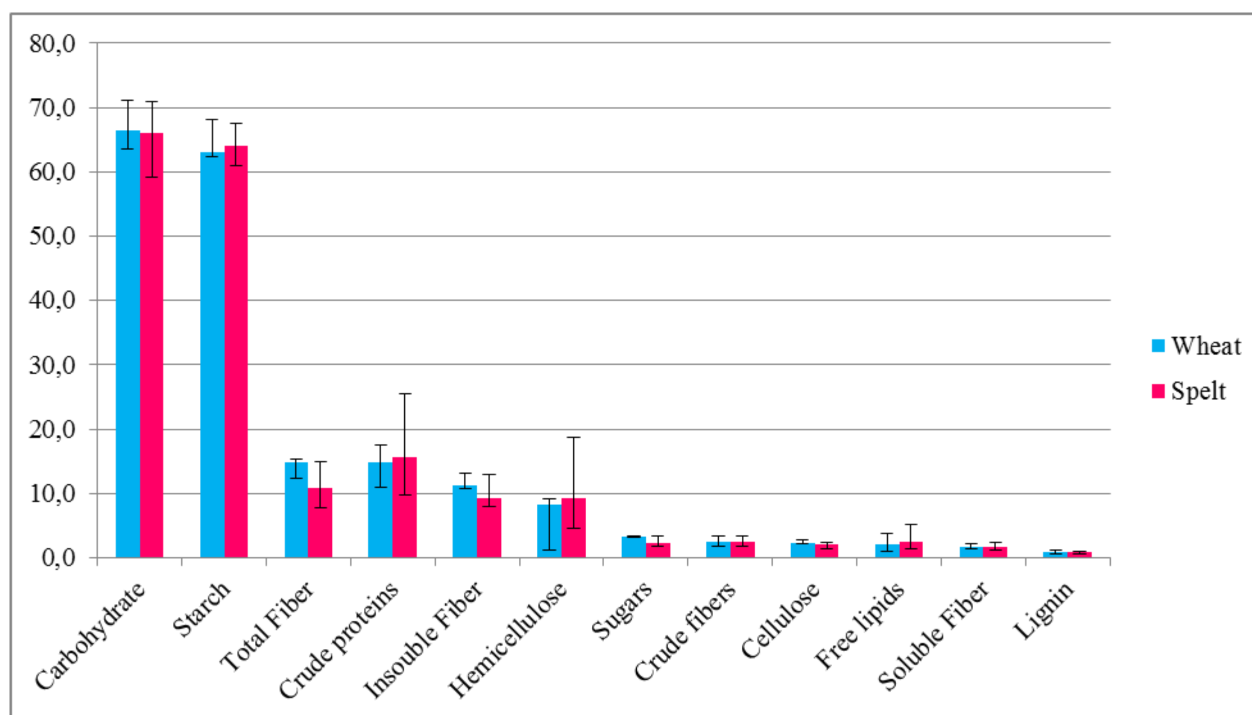

Figure A.1. Macronutrient content (weighted mean, minimum and maximum; %, d.m.) of wheat and spelt whole grain reported by different studies.

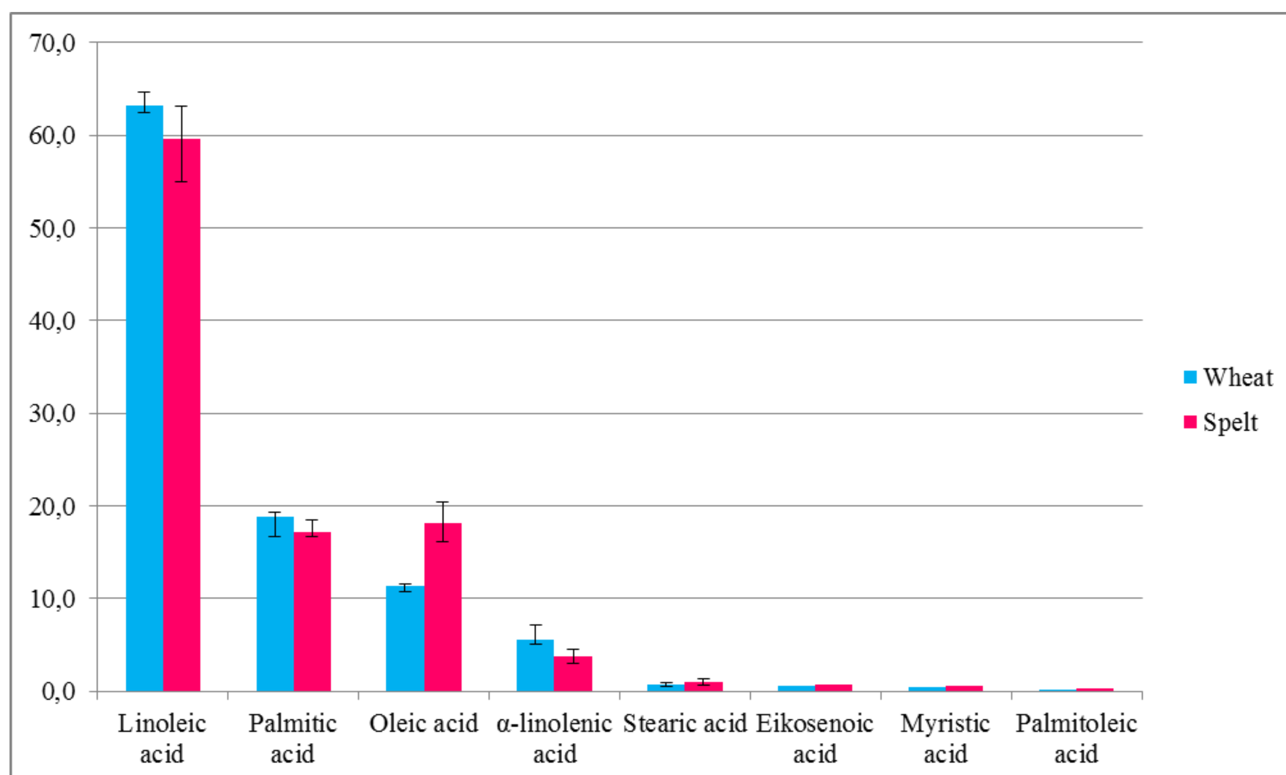

Figure A.2. Fatty acids distribution (%) of wheat and spelt whole grain reported by different studies.

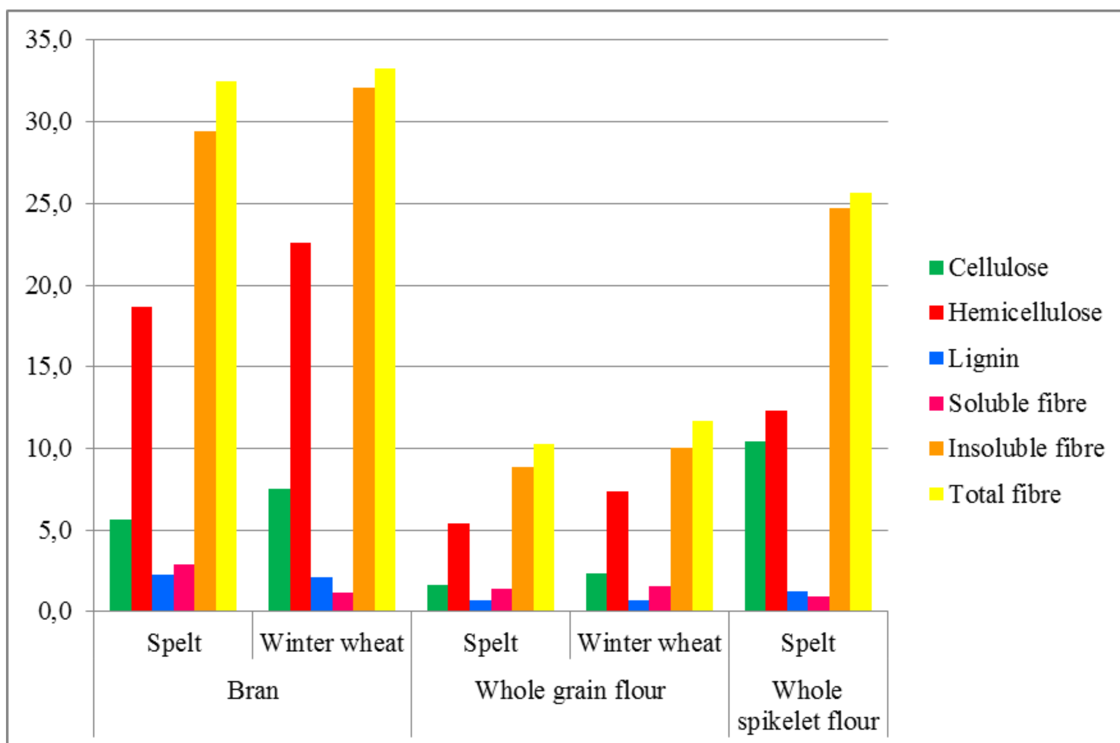

Figure A.3. Spelt and wheat fiber content (%, d.m.) by milling fractions.

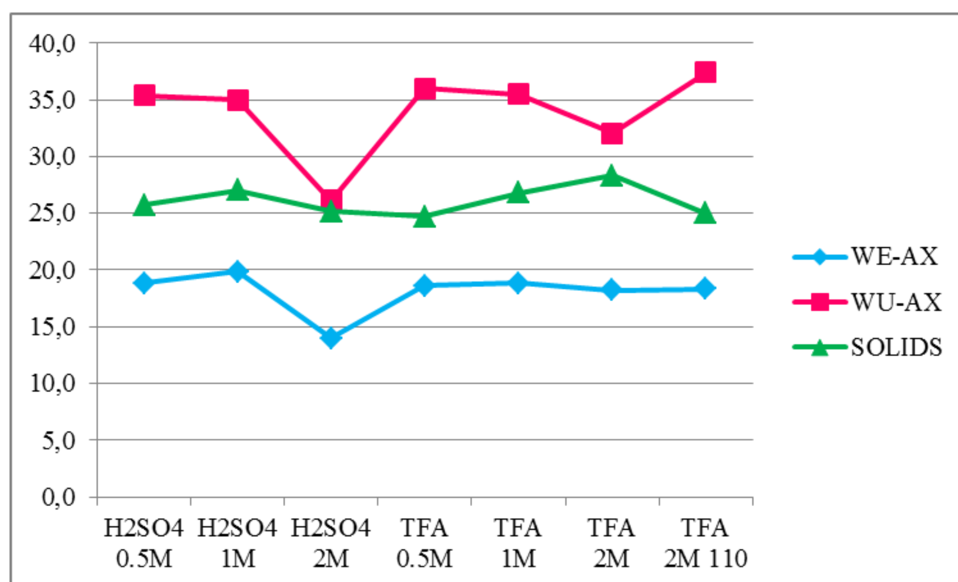

Figure A.4. A+X content (%, d.m.) of three groups of fraction, WU-AX, WE-AX and solids, according to different hydrolysis methods.

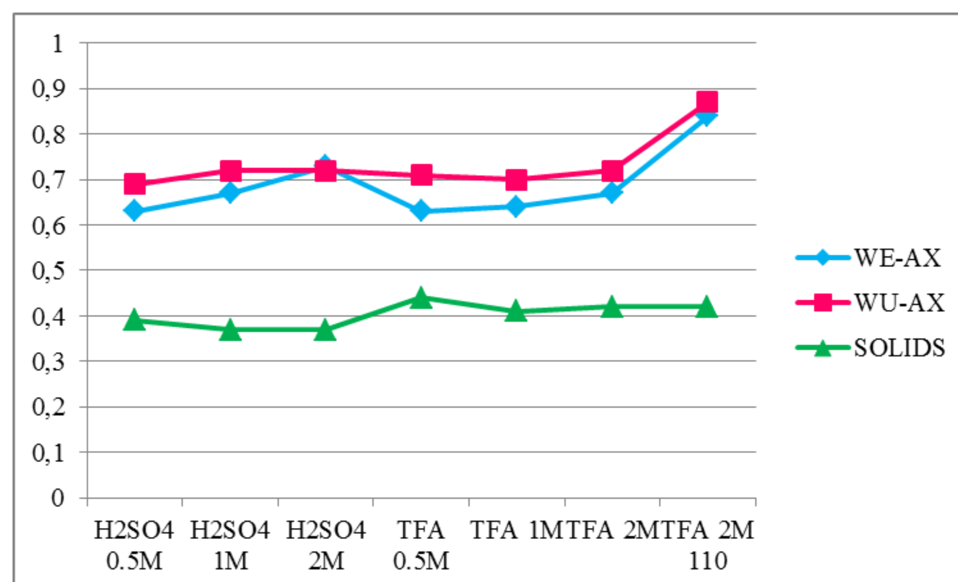

Figure A.5. A/X ratio of three groups of fraction, WU-AX, WE-AX and solids, according to different hydrolysis methods.

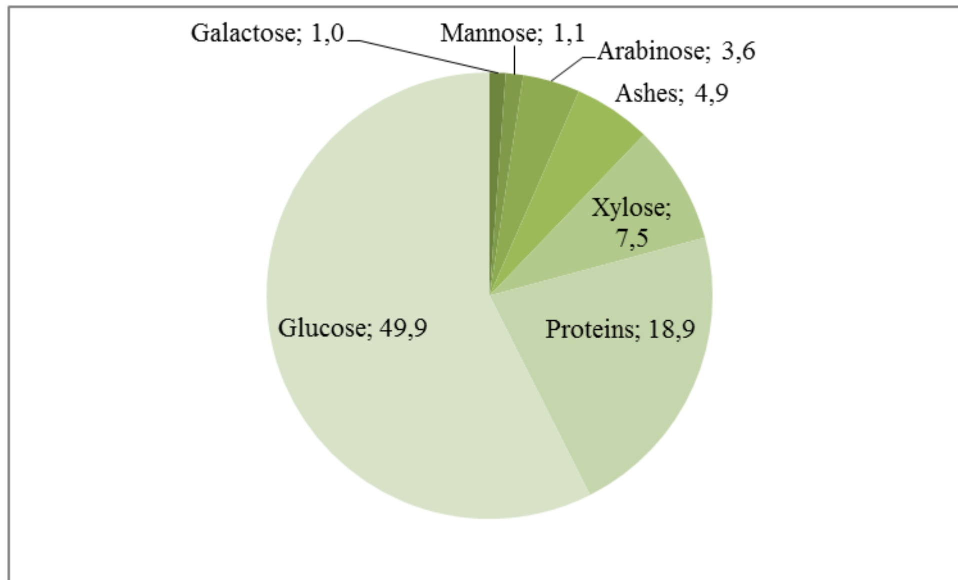

Figure A.6. Composition of the spelt bran (%, d.m.).

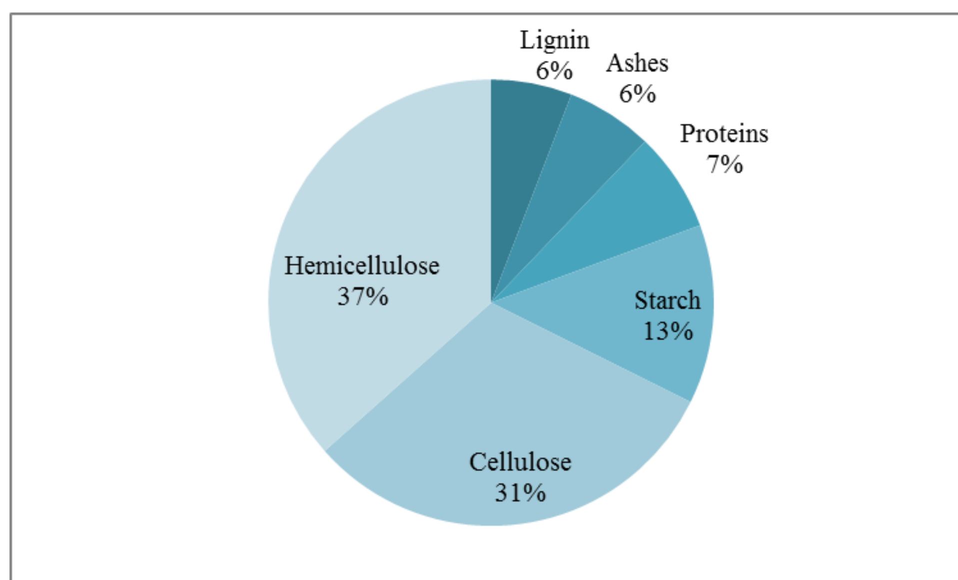

Figure A.7. Composition of the micronized hull (%, d.m.) (hemicellulose, cellulose, lignin, ashes, starch and proteins).

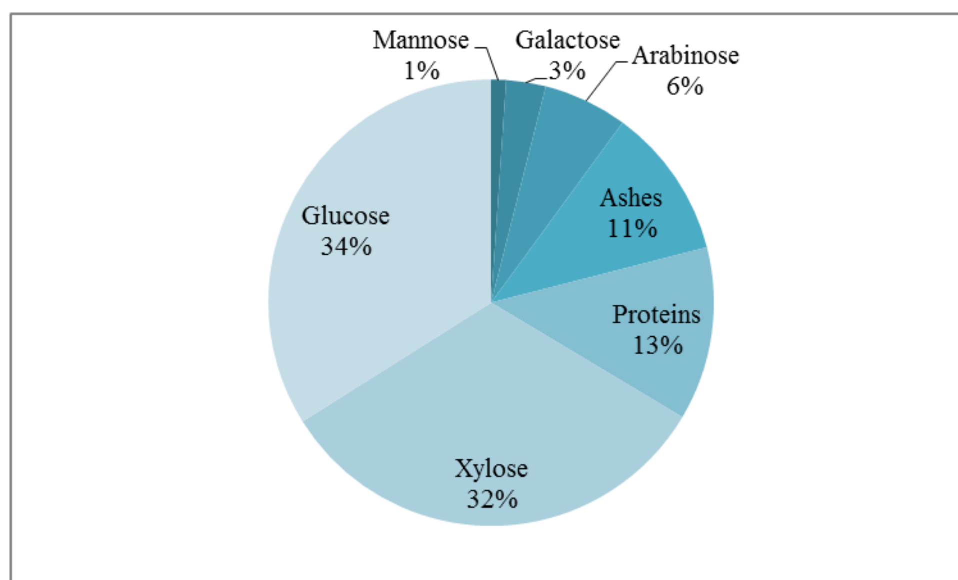

Figure A.8. Composition of the micronized hull (%, d.m.) (sugars, ashes, proteins).

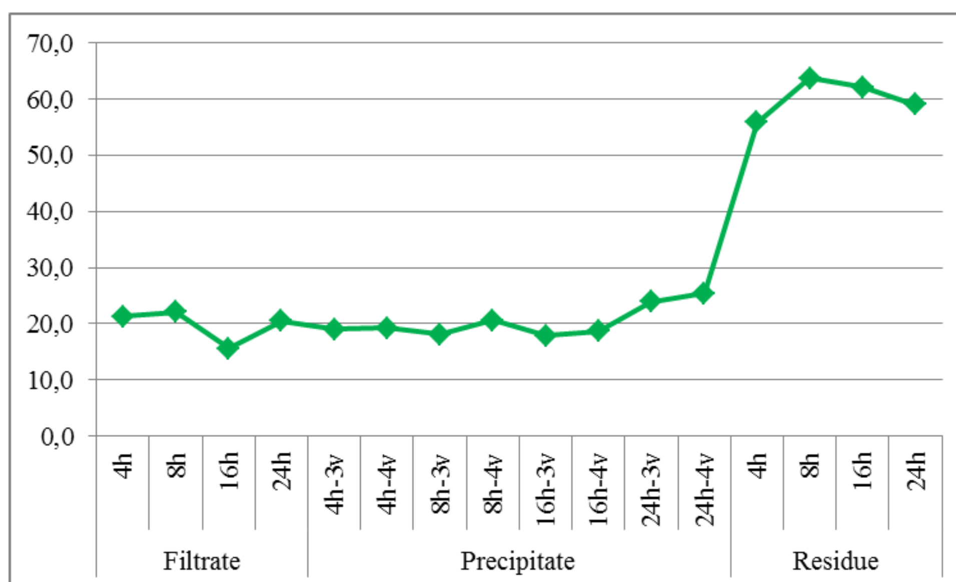

Figure A.9. Yield of extraction of AX (%) of the different fractions extracted with the second method (one extraction with H_2O_2).

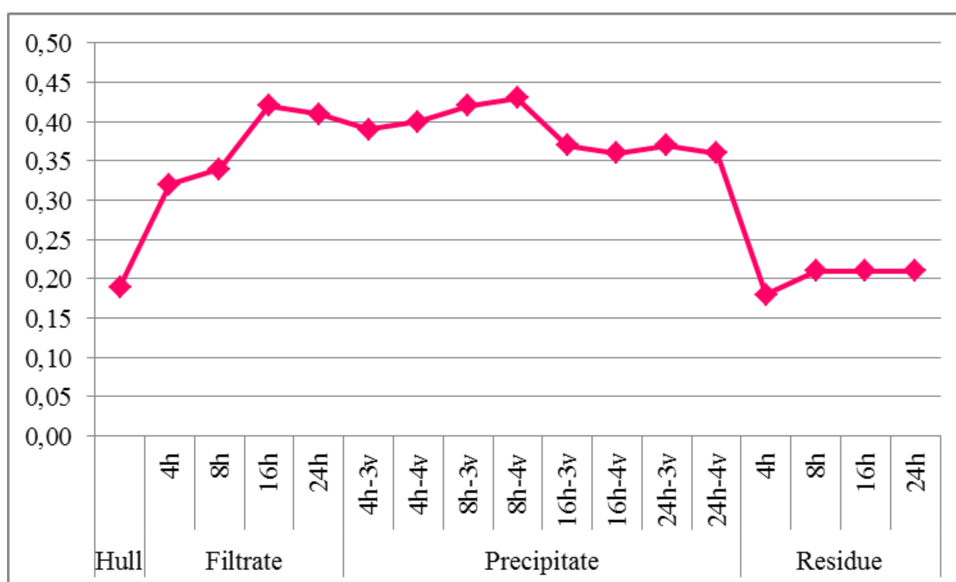

Figure A.10. A/X ratio of the different fractions extracted with the second method (one extraction with H_2O_2).

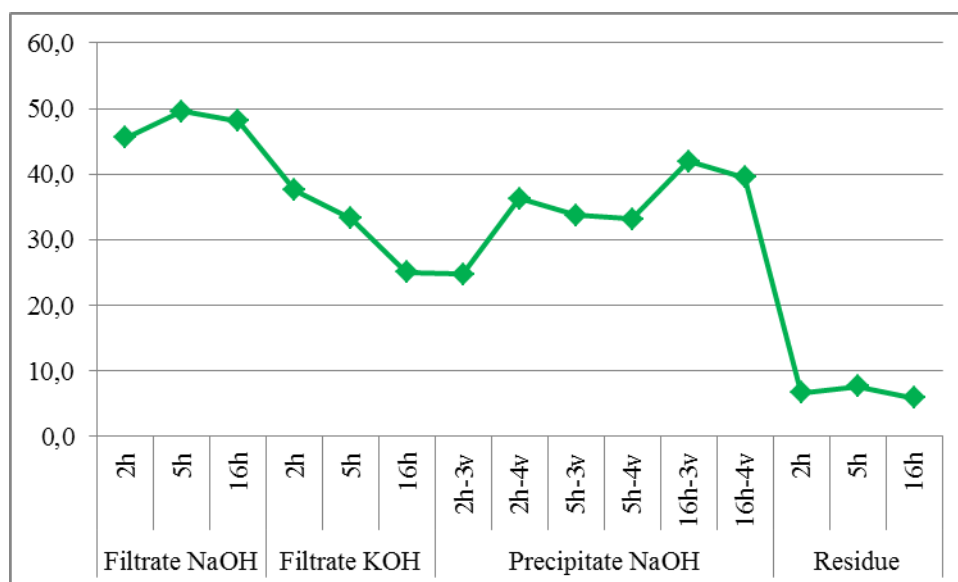

Figure A.11. Yield of extraction of AX (%) of the different fractions extracted with the third method (successive NaOH and $NaClO_2$-KOH extractions).

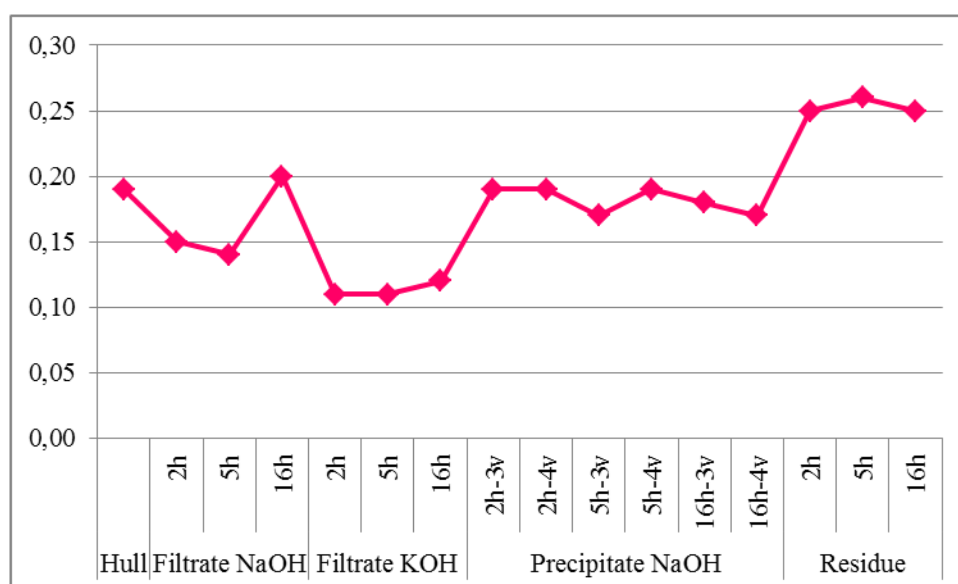

Figure A.12. A/X ratio of the different fractions extracted with the third method (successive NaOH and $NaClO_2$-KOH extractions).

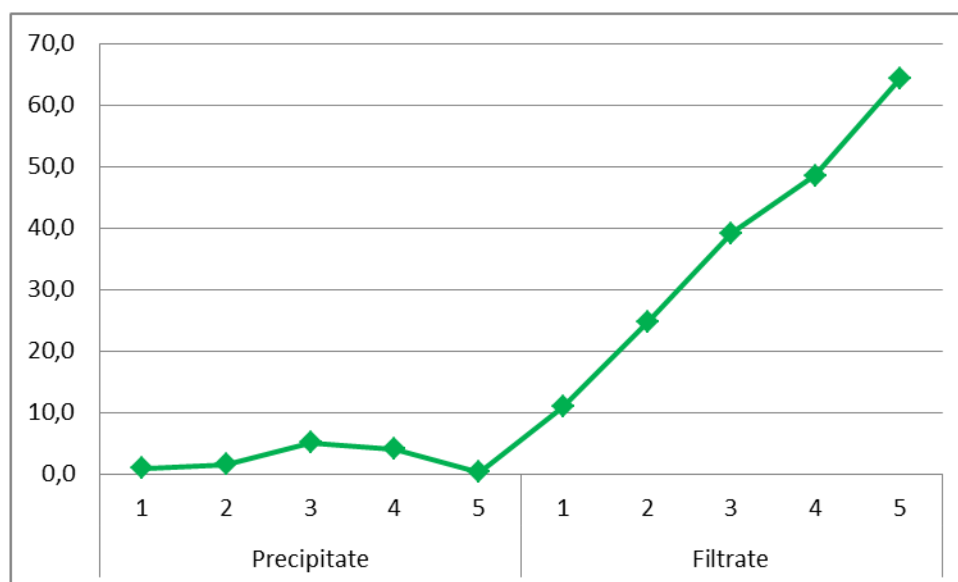

Figure A.13. Yield of extraction of AX (%) of the different fractions extracted with the fourth method (acidic extractions) (see Table 6.5 for experiment numbers).

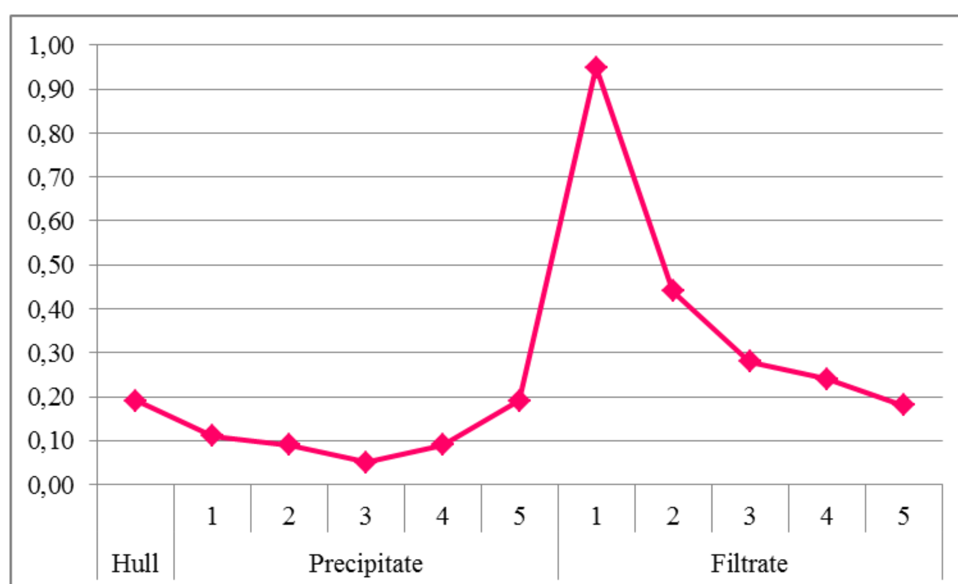

Figure A.14. A/X ratio of the different fractions extracted with the fourth method (acidic extractions) (see Table 6.5 for experiment numbers).

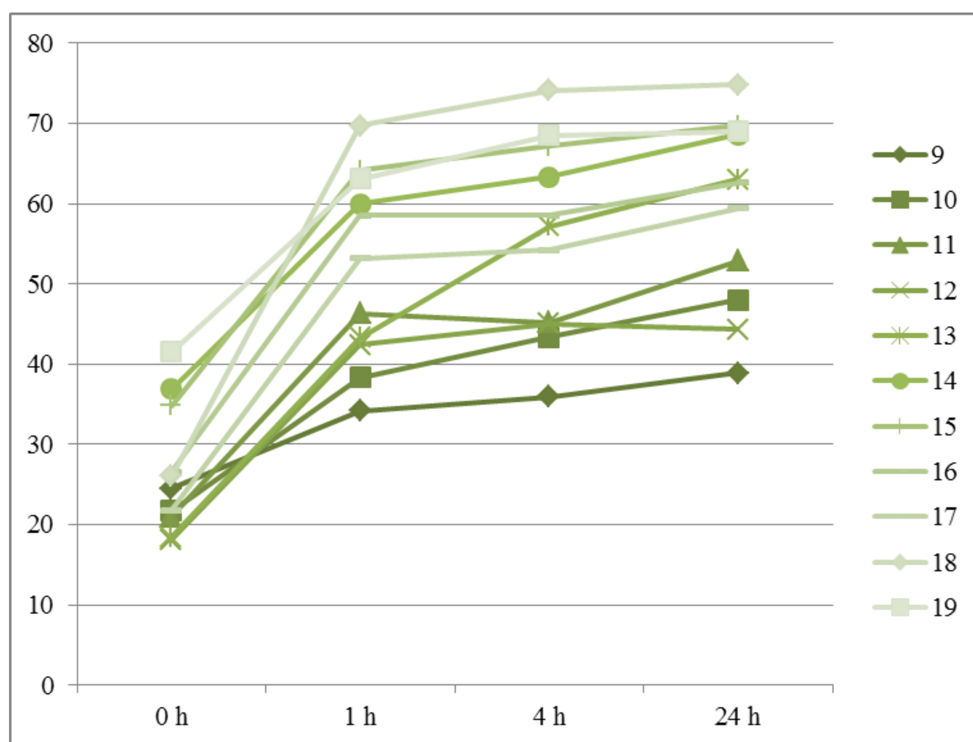

Figure A.15. Yield of solubilization of AX (%) for hydrolysates from spelt bran at 0 h, 1 h, 4 h and 24 h. See Table 7.1 for experiment numbers.

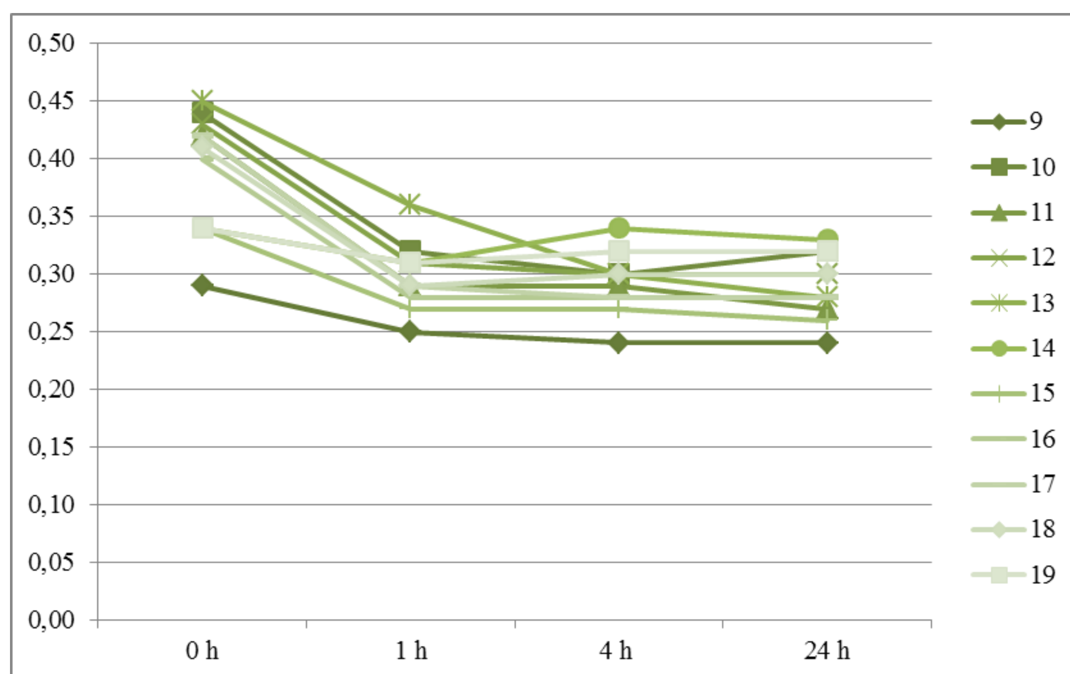

Figure A.16. Calculated A/X ratio for hydrolysates from spelt bran at 0 h, 1 h, 4 h and 24 h. See Table 7.1 for experiment numbers.

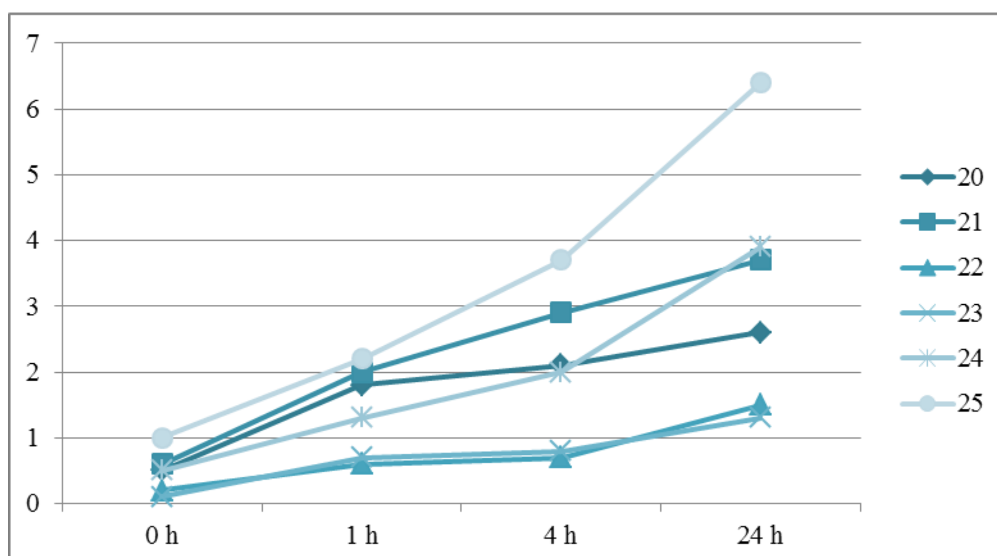

Figure A.17. Yield of solubilization of AX (%) for hydrolysates from spelt hull at 0 h, 1 h, 4 h and 24 h. See Table 7.1 for experiment numbers.

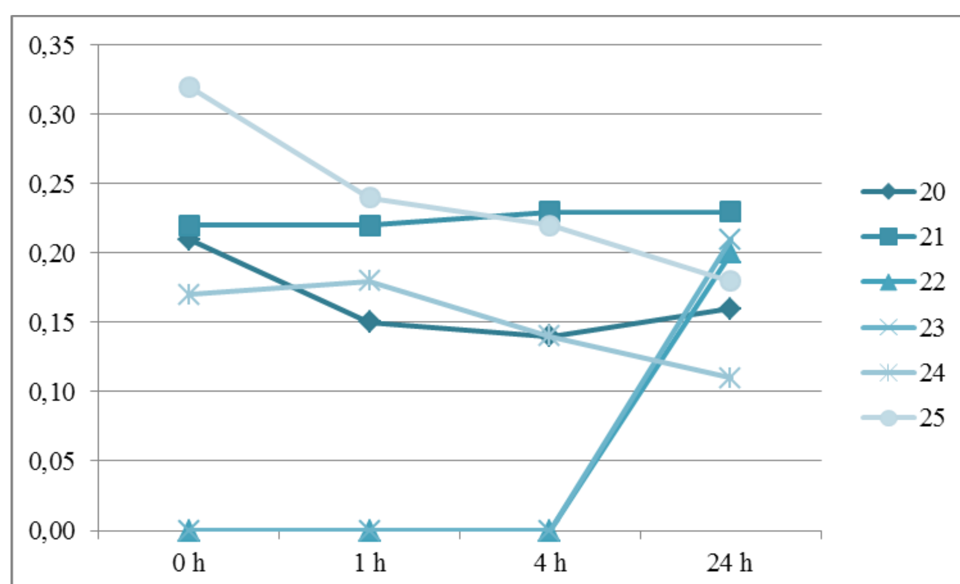

Figure A.18. Calculated A/X ratio for hydrolysates from spelt hull at 0 h, 1 h, 4 h and 24 h. See Table 7.1 for experiment numbers.