University of Huddersfield Repository

Alyassin, Mohammad

Extraction of soluble fibre food ingredients within integrated biorefineries

Original Citation

Alyassin, Mohammad (2016) Extraction of soluble fibre food ingredients within integrated biorefineries. Masters thesis, University of Huddersfield.

This version is available at http://eprints.hud.ac.uk/id/eprint/32081/

The University Repository is a digital collection of the research output of the University, available on Open Access. Copyright and Moral Rights for the items on this site are retained by the individual author and/or other copyright owners. Users may access full items free of charge; copies of full text items generally can be reproduced, displayed or performed and given to third parties in any format or medium for personal research or study, educational or not-for-profit purposes without prior permission or charge, provided:

- The authors, title and full bibliographic details is credited in any copy;
- A hyperlink and/or URL is included for the original metadata page; and
- The content is not changed in any way.

For more information, including our policy and submission procedure, please contact the Repository Team at: E.mailbox@hud.ac.uk.

http://eprints.hud.ac.uk/

University of HUDDERSFIELD
Inspiring tomorrow's professionals

Extraction of Soluble Fibre Food Ingredients within Integrated Biorefineries

Mohammad Alyassin

A thesis submitted to the University of Huddersfield in partial fulfilment of the requirements for the degree of Master by Research

December 2016

Supervisor: Professor Grant Campbell

Copyright statement

I. The author of this thesis (including any appendices and/or schedules to this thesis) owns any copyright in it (the "Copyright") and s/he has given The University of Huddersfield the right to use such copyright for any administrative, promotional, educational and/or teaching purposes.

II. Copies of this thesis, either in full or in extracts, may be made only in accordance with the regulations of the University Library. Details of these regulations may be obtained from the Librarian. This page must form part of any such copies made.

III. The ownership of any patents, designs, trademarks and any and all other intellectual property rights except for the Copyright (the "Intellectual Property Rights") and any reproductions of copyright works, for example graphs and tables ("Reproductions"), which may be described in this thesis, may not be owned by the author and may be owned by third parties. Such Intellectual Property Rights and Reproductions cannot and must not be made available for use without the prior written permission of the owner(s) of the relevant Intellectual Property Rights and/or Reproductions

ACKNOWLEDGEMENTS

Initially, I would like to express my profound gratitude and appreciation to my dear supervisor Prof. Grant Campbell for his valuable guidance, persistent presence, continuous support, his patience, time and effort. It was a great pleasure to work under your direct supervision, you always motivated and supported me, I gained a massive lifetime knowledge. Words are not enough, but thank you for everything.

A warm thanks goes to Hayley Markham and all the technician team for their help and advice.

I would like to thank my fellow labmates and colleagues, Mariam, Umair, Gabrial… for the stimulating discussions, cooperation, and for all the fun we have had in the last year.

I am indebted to my friends, especially Malaz, words cannot describe my appreciation.

Last but not the least, I would like to thank CARA (the Council for At-Risk Academics), for giving me the valuable opportunity for pursuing my aims and chasing my ambitions. Your supports are truly “a lifeline to academics at risk”.

DEDICATIONS

"The patriot's blood is the seed of freedom's tree" – Poet Thomas Campbell

To those who are planting the seeds of the Syrian people freedom and integrity…

Extraction of soluble fibre food ingredients within integrated biorefineries

Abstract

Integrated biorefineries, in which several co-products are produced in ways that exploit interaction opportunities for enhancing efficiency and reviving economics, are necessary in order to make bioethanol commercially viable, while offering the opportunity to create new product markets. Arabinoxylans (AX) are potentially a novel class of food ingredients that could be extracted from cereal bran; production of AX involves precipitation with ethanol, hence the extraction could be integrated within bioethanol biorefineries. Having established this as a viable proposition in principle, now greater understanding of the functional properties of arabinoxylans is required, as these properties vary according to feedstock and extraction techniques. In order to investigate functional properties in food systems, significant quantities of AX extracts are necessary. Thus, the objective of the current research is to screen a range of pre-treatments approaches and extraction conditions for the purpose of facilitating the scale-up of AX from two biomass sources, wheat bran and sugarcane bagasse.

AX was extracted at lab-scale from wheat bran (WB) and sugar cane bagasse (SCB) via several extraction methods: alkaline extraction (pH 11.5, 60°C, 4 Hours), alkaline oxidative extraction (pH 11.5, H_2O_2 2%, 60°C for 4 hours) and enzymatic extraction utilizing three xylanases (β-xylanase, 1,4-β-D-xylanase, and Endo-1,4-β-xylanase) and feruloyl esterase. Several pre-treatment techniques were examined including cellulase treatment for 24 hours, milling and autoclaving. Purification of the extracts was investigated via two methods: firstly, washing the bran (water, 60°C for 20 minutes) prior to the extraction; and secondly, applying amylase and protease during the extraction. The samples were concentrated by ultrafiltration using a 10 kDa membrane, prior to ethanol precipitation, to reduce the ethanol requirement. Crude yields and total protein content were measured for all extracts.

Preliminary results demonstrated that the enzymatic extraction yield was the lowest, ranging between 4.6-9.3% for WB and 7.0-8.2% for SCB, while the alkaline extraction yielded 17.6 and 13.8% for WB and SCB, respectively, and the alkaline oxidative extraction yielded 33.7% for WB and 16.3% for SCB. Autoclaving wasn't successful in improving the yields, while milling increased the yield from WB and SCB to 39.79% and 18.2%, respectively. Higher yields were obtained by using cellulase pretreatment, reaching 40.12% and 23.8% for WB and SCB, respectively. The protein content in SCB samples was relatively low (0.6-0.8%) and much higher in WB samples (12.7-14.2%). Protease treatment reduced the protein content in the WB extracts to 8.2%. Preliminary monosaccharide analysis utilising HPEAC-PAD was undertaken. Unfortunately, these analyses were not sufficiently reliable, perhaps due to incomplete hydrolysis of samples. Nevertheless, significant progress was made towards understanding the basis for enhancing AX extraction yields from these feedstocks.

Table of Contents

List of Figures

List of Tables

Abbreviations

DDGS	distiller's dried grain with solubles
AX	Arabinoxylans
A/X	Arabinose/ Xylose ratio
AXOX	Arabinoxylan oligosaccharides
WEAX	Water Extractable Arabinoxylans
WUAX	Water Un-Extractable Arabinoxylans
Ds	Degree of Substitution
Mw	Molecular weight
CEL91	Cellulase - nsibc-91
XYL301	1,4-β-D-Xylanase PRO-E0301
XYL51	β-Xylanase PRO-E0051
XYL62	Endo-1,4-β-Xylanase PRO-E0062
FER356	Feruloyl esterase PRO-E0356
ALKOXI	Alkaline Oxidative
ALK	Alkaline
WB	Wheat Bran
SCB	Sugarcane Bagasse
AOAC	Association of Analytical Chemists
HPAEC	High Pressure Anion Exchange Chromatography
PAD	Pulsed Amperometric Detector
GS-MS	Gas Chromatography-Mass Spectroscopy
CE	Capillary Electrophoresis
TFA	Trifluoroacetic acid

1. Arabinoxylans, a potential co-product of integrated biorefineries

1.1. Biorefineries in the recent UK and global context

The finite food and fuel natural resources imposes unprecedented global challenges due to the rise in the human population, expected to reach 9 billion by 2050 (Alexandratos, 2006). Beddington, the UK's chief scientific advisor, described a "perfect storm" scenario with the world's demand of food, potable water and energy increasing by 50%, 30% and 50%, respectively (Beddington, 2009). This report indicates that failure to fulfil these needs is likely to lead to tragic consequences including public turmoil, cross-border conflicts and mass migrations.

Providing sufficient energy is an essential requirement for modern civilisation and its major challenge in the face of the progressive shortage of fossil fuel and increasingly urgent environmental issues. Renewable energy sources are constantly being considered as replacements for fossil fuel, including wind, tidal, wave, solar and biofuels (Valentine *et al*., 2012). Among these, biofuels are the sole option to supply energy as a liquid transport fuel form, in contrast to the other renewable energy sources. Furthermore, biofuels are not intermittent and do not require energy storage systems or employing other fuel technology as a backup, as is the situation with the wind, wave and tidal. The United Kingdom currently has a total biofuel production capacity of over 1,500 million litres per year. Figure 1-1 shows the increase in cumulative production capacity of commercial scale bioethanol and biodiesel plants in the UK (operational and planned), from which it is clear the biodiesel originally dominated UK biofuel production, while bioethanol has come to dominate in recent years.

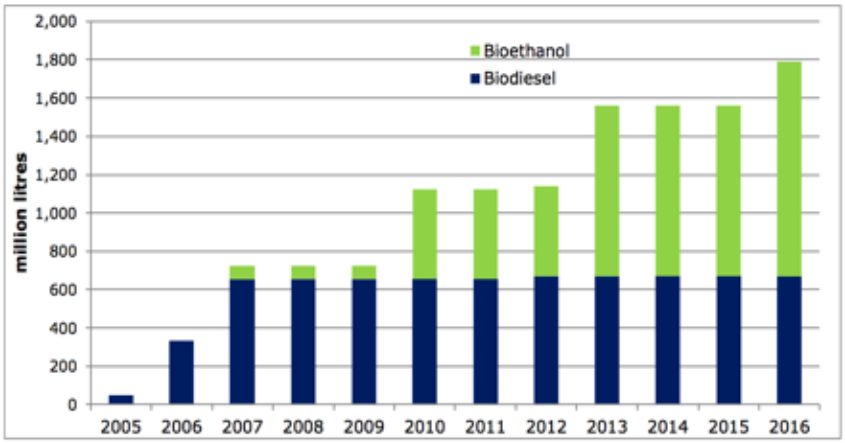

Figure 1-1 cumulative production capacity of commercial scale bioethanol and biodiesel plants in the UK. Source: (Alberici & Toop, 2013)

About 95% of the world's bioethanol production is in the USA, Brazil, China and Europe, utilising starch as the starting material (Stein, 2007). The production is chiefly from maize and soybeans in USA, sugar cane in Brazil, maize in China, and sugar beet, wheat and barley in Europe. In spite of the installed capacity to produce biofuel, the production of biofuel has been limited by many reasons such as the fluctuations and rise in food commodity prices (Alberici & Toop, 2013). Meanwhile, fossil fuels remain economically more attractive than bioethanol. Following substantial investment in bioethanol capacity, since 2009 UK bioethanol output has been remarkably lower than the available capacity, particularly in 2011 and 2012 when production was limited to only 6% and 17% of capacity, respectively, as Figure 1-2 displays (Alberici & Toop, 2013).

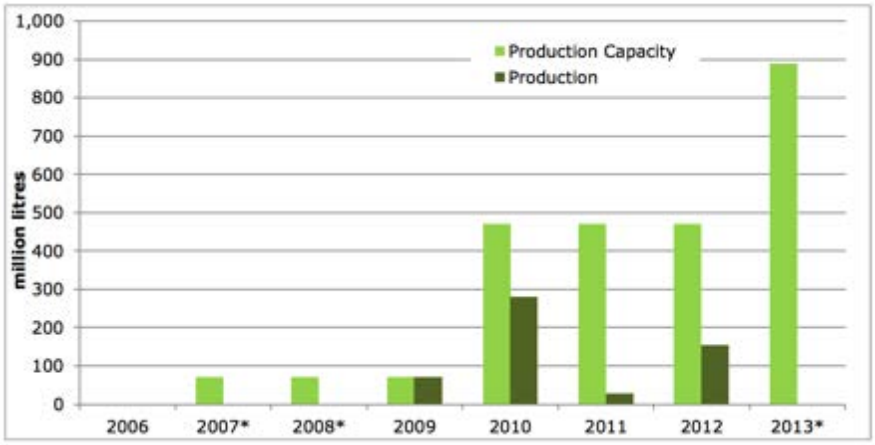

Figure 1-2 The Actual bioethanol production in UK. (Alberici & Toop, 2013)
**insufficient data*

Economically-viable production of biofuels is a challenge in the face of the low cost of oil-derived fuels. Looking at a crude oil refinery, it is apparent that the number of commodities produced in these refineries contributes to their economic performance (Asche *et al*, 2003). Production of a portfolio of products, such that "not a drop of oil is wasted", not only confers as much added value as possible to the whole feedstock, it also gives scope for highly integrated and hence efficient and economic processes. The same model, of integrated production of chemicals, materials, food and feed, is required for the feasible and competitive operation of biorefineries. Integrated biorefineries produce several bio-based chemicals beside fuel grade ethanol, for example, non-food starch, cellulose fibres and cellulose derivatives, oils, fatty acids and fermentation products such as ethanol and citric acid (de Jong *et al*., 2012).

Although a portfolio of co-produced products is key for biorefinery competitiveness, both for generating a range of revenue streams and for providing integration opportunities as exploited within oil refineries, not everyone working in biorefineries is as alert to the integration aspect as they are to the revenue stream aspect. Thus, for example, the definition developed by the International Energy Agency Bioenergy Task 42 Biorefinery reads:

> *"Biorefinery is the sustainable processing of biomass into a spectrum of marketable products (food, feed, materials, chemicals) and energy (fuels, power, heat)."*

This definition emphasises the spectrum of marketable products, but makes no reference to integrated processes that allow economic co-production of these products. In this respect a better definition is that given by the USA's National Renewable Energy Laboratory (www.nrel.gov/biomass/biorefinery.html):

> *"A biorefinery is a facility that integrates biomass conversion processes and equipment to produce fuels, power and chemicals from biomass. The biorefinery concept is analogous to today's petroleum refineries, which produce multiple fuels and products from petroleum."*

1.2. Arabinoxylans as a co-product of bioethanol production / context of the research

In order to be economically competitive and commercially viable, UK biorefineries must produce several co-products, with ethanol as one of them, and must seek to exploit integration opportunities arising from co-production. The major co-product of bioethanol production in cereal biorefineries is the distiller's dried grain with solubles (DDGS), a relatively low-value product that goes to animal feed. DDGS come from the non-starch fraction of the cereals, mainly the bran, along with leftover protein and yeast biomass. Cereal bran is the problematic fraction of cereals for biorefineries as it comprises a large fraction of the raw material and is underexploited economically. Thus, extracting components of the bran appears to be the most feasible route towards producing added-value products.

Recently, studies have focused on arabinoxylans production, a component of lignocellulosic biomasses, and a potential co-product of biorefineries that could provide a novel class of food ingredients and non-food products. One process for producing arabinoxylans involves precipitating with ethanol, giving opportunities for integration with bioethanol production within biorefineries. Previous work from our group has established that co-production of AX integrated with bioethanol could be economically feasible (Misailidis *et al*., 2009) and has established extraction protocols to produce AX from maize, wheat, and sugarcane bagasse. However, the properties of extracts as functional food ingredients, as affected by source and extraction conditions, have not been completely studied. Greater understanding of arabinoxylans' functional properties is required, as these properties vary according to feedstock and extraction conditions. Early studies on arabinoxylan extracts sometimes gave conflicting results due to differences in composition and purity of extracts (Biliaderis *et al*., 1995).

For the purpose of undertaking extensive functional property investigations in food systems, AX extracts need to be available in relatively large (kg) quantities. There is currently no commercial source of AX at this scale. Hence, to progress this line of research, there is a need to establish facilities for routine production of relatively pure AX in kg quantities.

The main objective of the current research is to enhance the extraction of arabinoxylans, in terms of increasing the resultant yields and rising the AX extract purity level, to facilitate further pilot scale extraction work and subsequent functionality studies. The studies reported in the current thesis follow on from an earlier proof-of-concept project, funded in 2015 by the

Lignocellulosic Biorefinery Network (LBNet), which investigated enzyme-assisted AX extraction from wheat bran and sugarcane bagasse. Substantially, the current study was an extension of this previous project, looking at a wider range of sample pre-treatments and extraction techniques.

1.3. Thesis Scope

Arabinoxylans are potentially a class of healthy soluble fibre food ingredients with a range of functional properties including viscosity enhancement, foaming, gel formation and prebiotic activity. One of the processes for producing arabinoxylans uses ethanol for precipitation; co-production of AX with bioethanol production therefore gives scope for integrated processing and economic production of a portfolio of AX and AX oligosaccharide (AXOS) products.

The evolving Arabinoxylans story in the context of UK cereal biorefineries is presented in more detail in Chapter 2 along with the description of arabinoxylan structure and classification.

Chapter 3 describes the physico-chemical properties of arabinoxylans and the relation of these properties with the structure of the polysaccharide, AX solubility, viscosity, water holding capacity, gel formation and foam stabilising effects.

Chapter 4 describes the materials and procedures used in the current work and the investigations undertaken using these materials and procedures. Chapter 5 presents the results of these investigations and discusses their significance in relation to the evolving knowledge base for commercial arabinoxylans production and use.

Chapter 6 concludes the thesis by summarising the main findings from the current work and makes recommendations about how extraction of AX could be progressed in a research based capacity for industrial application.

2. Arabinoxylans: Structure and Physicochemical Properties

2.1. Introduction

Arabinoxylans are novel polysaccharides that can be produced from biomasses, as they comprise a major portion of hemicellulose. AX is a non-starch polysaccharide that consists of a backbone of xylose with arabinose side chains. This chapter reviews AX structure, classification and occurrence along with physicochemical properties and their relation to the structure.

2.2. Brief background of arabinoxylans

Early in the 1920s, Hoffmann and Gortner (1927) extracted a gummy non-starch polysaccharide composed mainly of xylose and arabinose from wheat flour, and named the new gum as 'pentosans'. Better understanding of these pentosans was developed after describing the nature of araban-xylan association in cereals (rye, wheat and barley) as a simple type of molecule (Preece & Hobkirk, 1953). Subsequently, more reports stated the presence of AX in the outer-layers of cereal grains such as wheat, corn, rice, barley, oat, rye and sorghum (Vinkx & Delcour, 1996). AXs have evolved as test subjects in the field of cereal science and bioresource technology in the last few decades. According to bibliographic research, using the keyword "Arabinoxylan" in ScienceDirect (August 2016), the number of publications which include the keyword in their title, abstract or keyword has been significantly elevated in the last twenty years (Figures 2-1).

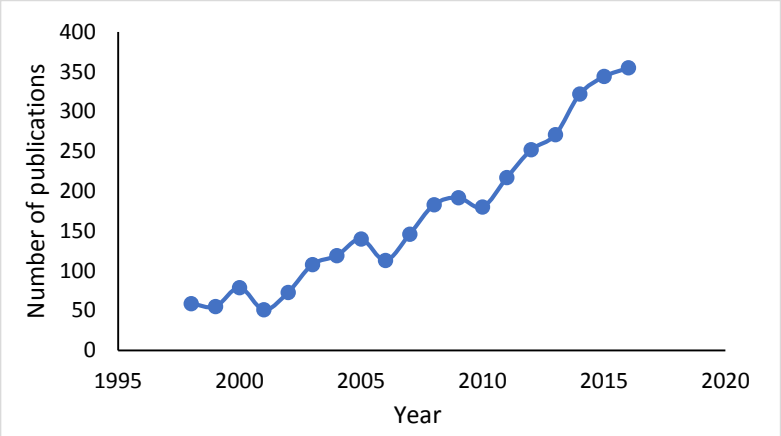

Figure 2-1 Number of publications about Arabinoxylans. Source: ScienceDirect (August 2016).

It is notable that the greater portion of the publications about AX are located in the same countries involved in biofuel and renewable energy studies as Figure 2-2 demonstrates (sample of top 180 relevant articles were counted).

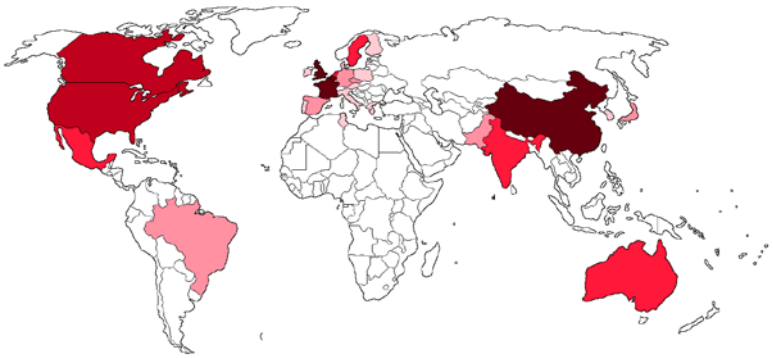

Figure 2-2 the geographic distribution of AX related researches.

Some of these studies describe methods for the extraction and isolation of AX at lab-scale. Scaling up the extraction is hampered chiefly by two factors: firstly, the cost of the extraction as it requires the use of ethanol in large quantities; and secondly, the absence of a clear market to commercialise it. This has maintained AX as a theoretical material that might have a promising future.

In order to stimulate markets for AX, an important number of studies have focused on the benefits of AX in the fields of human health and prebiotic properties, bread making and food industry, excipient in pharmaceutical industry and food packaging. However, the lack of commercial scale production of AX is a main problem, as sufficient amounts of AX are not available to demonstrate the uses of AX and promote their uptake in industry. In addition, differences in extraction methods and raw materials have led to conflicting results. Recently, with the evolving of integrated biorefinery concept, AX has appeared to be a promising co-product in biorefineries. Misailidis (2009) demonstrates that co-production of AX with ethanol within wheat biorefineries could be economically viable.

2.3. Structure of Arabinoxylans

Arabinoxylans constitute the main group of hemicelluloses, and exists mainly in the outer layer and endosperm cell walls of cereals associated with cellulose and lignin (Izydorczyk & Biliaderis, 1995). AX consist of a linear chain of *β*-D-xylopyranosyl linked via (1-4) glycosidic linkages. Izydorczyk (2008) stated that monomeric *α*-L-arabinofuranoside residues are attached to some of the xylopyranosyl chains at O-3, O-4 and/or both O-2,3 positions. The same study reported that, depending on arabinofuranoside position and number of substitution, four main structural units can be observed: monosubstituted xylopyranosyl at O-2 or O-3, disubstituted xylopyranosyl at both O-3,4 and unsubstituted xylopyranosyl (Izydorczyk & Dexter, 2008). Figure 2-3 illustrates the structure of AX.

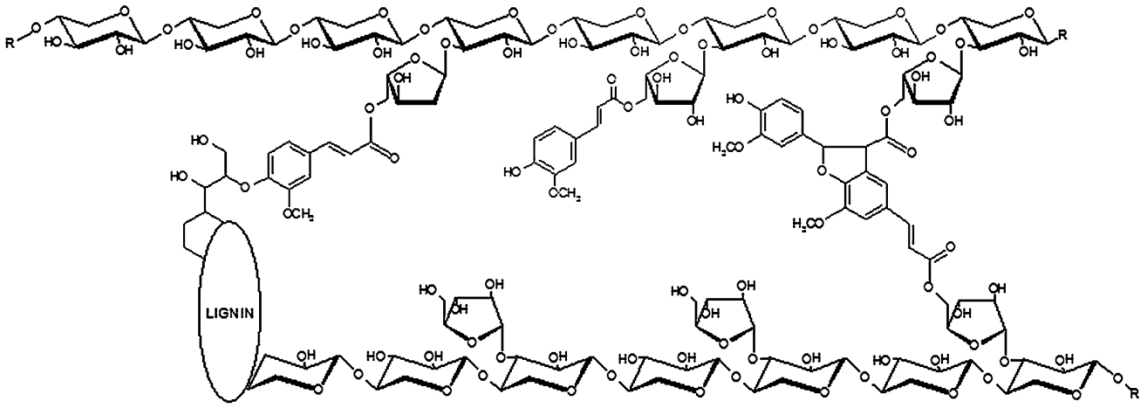

Figure 2-3 Structure of AX (Döring, Jekle, & Becker, 2015)

The sequence of the distribution of these four structural units varies depending chiefly on the source of arabinoxylans (Gruppen *et al*., 1993). Izydorczyk (1995) reported that the greater population of arabinofuranoside residues in AX is present as monomeric substituents, and minor proportion consisting of two or more residues linked via 1-2, 1-3, and 1-5 linkages can be seen (Izydorczyk & Biliaderis, 1995). The amount of AX embranchment can be estimated by the Arabinose/Xylose (A/X) ratio, which varies between sources and between different parts of the same plant, for instance, A/X of wheat endosperm AX was reported 0.7 (Rattan *et al*., 1994), whereas wheat bran has a higher ratio of 1.07 (Shiiba & Nagao, 1993).

Hydroxycinnamic, ferulic and *p*-coumaric acids were reported esterified to O-5 of arabino-furanoside residues in cell wall polysaccharides (Smith & Hartley, 1983). Ferulic acid esters

can form dimers of dehydrodiferulate esters, as seen in Figure 2-4, which are responsible for covalent cross-linking between AX chains and between AX and other cell wall components (e.g., lignin, proteins, etc.) (Santiago *et al*., 2006).

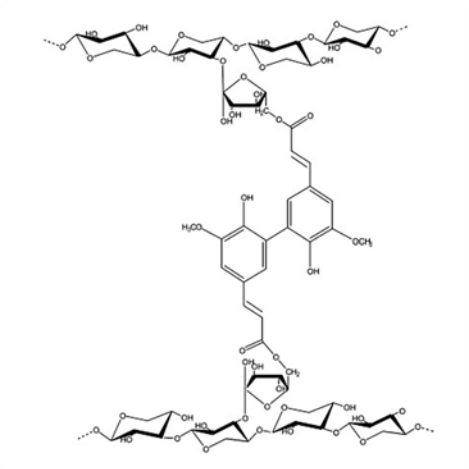

Figure 2-4 Dehydrodiferulate esters (Ferulic acid dimer) source: (Santiago & Malvar, 2010)

The unsubstituted xylan chains tend to form a three-fold, left-handed helix with three xylose residues per turn, which appears like an extended twisted ribbon (Courtin & Delcour, 2002; Fincher & Stone, 1986). This relatively flexible conformation is formed by hydrogen bonds between adjacent xylose residues (Izydorczyk & Biliaderis, 1995). Courtin (2002) suggested that substitutions on the xylan chain distort this structure, as arabinose side units sterically hinder this formation and a random coil conformation is suggested. Moreover, the presence of diferulic acid bridges between adjacent AX chains might form high molecular weight molecules with changed conformational properties (Courtin & Delcour, 2002).

2.4. Classification of arabinoxylans

Arabinoxylans are categorised according to the structural configuration of the sample used (Vinkx & Delcour, 1996). Thus, AX could be either water-extractable AX (WEAX) or water-unextractable AX (WUAX).

WEAX are so called because of the cell wall of these AX are loosely attached, causing easy migration of aqueous solution around the surface. This can be explained by an incomplete cross-linking with other components, small structural differences or initial enzymatic degradation (Izydorczyk & Biliaderis, 1995).

WUAX are not easily extractable with water as they are held in the cell wall matrix by non-covalent (e.g. hydrogen bonds) and covalent interactions (e.g. ester and ether bonds, diferulic acid bridges) with other AX molecules or with other cell wall constituents (Fengler & Marquardt, 1988; Izydorczyk & Biliaderis, 1995).

2.5. Occurrence of arabinoxylans

Arabinoxylans are the major non-starch non-cellulosic polysaccharides in cereal grains (the most known and studied source of AX). They were reported in the outer layers of cereal grains such as wheat, corn, rice, barley, oat, rye, and sorghum (Fincher & Stone, 1986; Saeed *et al.*, 2011; Vinkx & Delcour, 1996; Zhang *et al.*, 2014). Also, AX were reported in tissues of other plants such as bamboo (Ishii *et al*., 1990), ryegrass (Hartley & Jones, 1976), sugarcane bagasse (Sun *et al*., 2004), grape pomace (Minjares-Fuentes *et al*., 2016), garlic straws (Kallel *et al*., 2015), mucilage of tomatoes (Nascimento *et al*., 2016) and the bark of cinnamon (Pape-Gowda *et al*., 1980). Arabinoxylans form a major part of hemicellulose which occurs in a wide variety of plants and plays a key role in strengthening the cell wall by tethering the components of the cell wall (Scheller & Ulvskov, 2010).

2.6. Physicochemical Properties of Arabinoxylans

Arabinoxylans physicochemical behaviour is primarily attributed to its structural features, *i.e.*, the length of xylan chain, the degree of substitution (DS) (or the A/X ratio) and the oxidative linking of ferulic acid residues (Courtin & Delcour, 2002; Izydorczyk & Biliaderis, 1995). The main physicochemical properties of interest are water solubility, viscosity, water holding capacity and oxidative gelation, which depend on the structure of AX.

2.6.1. Water Solubility

The solubility of AX in water, in general, depends on structural factors such as chain length, degree of substitution, and substitution pattern (Saulnier *et al*. 2007). Degree of substitution is the major factor affecting AX solubility, as arabinose residues hinder the intermolecular aggregation of unsubstituted xylose residues (Andrewartha *et al*., 1979). This was demonstrated clearly by the significant decrease in solubility after removing the arabinose residues enzymatically (Andrewartha *et al*., 1979). In general, AX with higher DS are expected to exhibit more water solubility. However, AX molecular weight (MW) also affects the solubility, as decreasing MW increases the solubility (Mares and Stone 1973). Bian *et al*. (2010) who confirmed these observations, precipitated AX with graduated concentrations of ethanol (10, 20, 30, 45, 60 and 80%). It was found that the high MW AX with fewer branches were precipitated with lower concentrations of ethanol (less water soluble) while the lower MW AX with more branches precipitated in lower concentrations of ethanol (more water soluble) (Bian *et al*., 2010).

2.6.2. Viscosity

Owing to the relatively high molecular weight, stiff and semi-flexible random coil conformation, AX present the ability to form viscous solutions exhibiting a pseudoplastic behaviour (Girhammar & Nair, 1992). The viscosity of wheat flour AX is higher than the viscosity of other polysaccharides such as dextran and arabinan (Fincher & Stone, 1986). MW, pH, AX concentration and ferulic acid content have been observed to affect viscosity of AX solution in varying levels. Girhammar and Nair (1992) stated that the main factor affecting AX viscosity is its higher MW. In addition, pH affects the viscosity of AX solutions, with the highest viscosity obtained at neutral pH values (Girhammar & Nair, 1992). Of course, the

concentration of AX has the greater effect on the solution viscosity (Saulnier *et al*., 2007). Ferulic acid presence significantly increase the viscosity as it forms cross-links, which in turn increases the MW. Thus, the higher FA content results in more viscous solutions (Dervilly-Pinel *et al*. 2001; Saulnier *et al*., 2007)

2.6.3. **Water Holding Capacity**

Courtin and Delcour (2002) reported that AX are capable of absorbing water and swelling, WUAXs are capable of holding 7-10 times their weight in water, whereas WEAXs present slightly lower water-binding capacity at 4-6 times their weight. These findings were obtained by adding AX to bread dough while monitoring the Farinograph water absorption changes. Using Farinograph absorption method for the determination of water holding capacity is a questionable method as the increase in water absorption might be a result of the interaction of AX with other components in the dough, not due to the AX on its own (Courtin & Delcour, 2002). Thus, further investigations are required to determine AX water holding properties.

2.6.4. **Oxidative Gelation and Gel-Forming Capacity**

Izydorczyk *et al*. (1990) demonstrated that AX oxidative gelation occurs in the presence of oxidising agent (e.g. hydrogen peroxide or peroxidase), which catalyses the formation of covalent cross-linkages by dimerisation of ferulic acid residues (Izydorczyk *et al*., 1990), Figure 2-5 shows the oxidative coupling of ferulic acid.

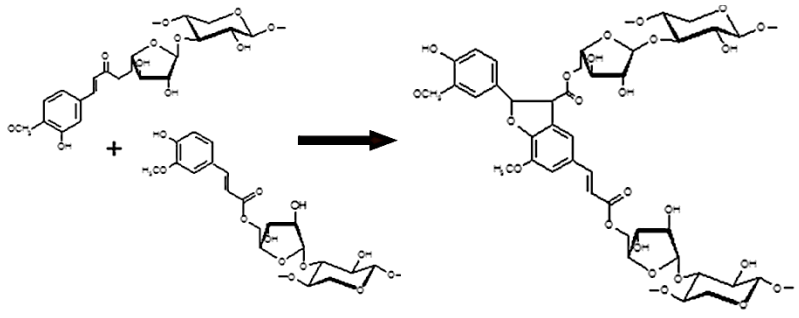

Figure 2-5 Oxidative coupling of ferulic acid (Döring *et al*., 2015)

These cross-linkages give rise to an augmentation in viscosity and eventually the formation of a gel (Dervilly-Pinel *et al*., 2001a; Vinkx *et al*., 1991). In general, high MW, high content of ferulate residues and relatively unsubstituted xylan chain (low A/X) are precursors of extensive

cross-linking (Izydorczyk & Biliaderis, 1995). However, the rigidity of the gel seems to be determined chiefly by the intrinsic viscosity, rather than the content of ferulate residues (Dervilly-Pinel *et al*., 2001a). Other linkages contribute in gel strengthening such as hydrogen bonding and van der Waals interactions (Saulnier *et al*., 2007).

2.6.5. **Foam Stabilising**

Arabinoxylans exhibit the ability of stabilising protein films against thermal disruption by increasing the viscosity of the interlamellar liquid, altering the drainage properties of foam films and mediating interactions between proteins in the absorbed layer (Izydorczyk *et al*., 1991; Sarker *et al*., 1998).

2.7. Summary

AX is a non-starch polysaccharide that consists of a back chain of xylose with side branches of arabinose; these side chains can be attached to ferulic acid via ester bonds. The molecular properties such as the length of the xylose chain, the number of arabinose substitutions and the content of ferulic acid are dependent on the source of AX. These molecular properties determine the physicochemical properties of AX. The higher the molecular weight of AX, the lower the water solubility with increase in viscosity and water holding capacity. In addition, more arabinose-substituted molecules exhibit greater water solubility than the less substituted ones. Moreover, the high ferulic acid content can promote more oxidative gelation.

In general, AX properties are subject to the molecular properties that in turn are subject to the extraction method. Since the extraction of AX depends on the solubilisation of the chains and breaking down the bonds between AX and other cell wall components, it follows that the extraction method will determine the properties of the extracted AX. The next chapter discusses the extraction methods of arabinoxylans and potential uses in industry, leading to the objectives for the current research.

3. Arabinoxylans extraction, analysis and potential use

3.1. Introduction

The fractionation of biomasses in order to produce valuable materials has attracted a great deal of attention recently. Insolating AX has been investigated utilising enzymatic and chemical methods. This chapter reviews the efforts addressed at extracting AX, discusses the effect of the extraction method on the final product and addresses the main potential uses of AX.

3.2. Arabinoxylans extraction methods

The liberation of the arabinoxylans from the cell wall matrix is restricted by the intricate and massive structure tying cell wall components together (Courtin & Delcour, 2001). The presence of ester and/or ether lignin-polysaccharide complexes, and hydrogen bonds between polysaccharides chains, limits the release of AX (Ebringerová & Heinze, 2000). Generally, rigorous treatment is needed to separate AX from other cell wall constituents (Courtin & Delcour, 2001). Extraction of AX typically consists of three major treatments: solubilising, mechanical-assisted hydrolysing, and purification.

3.2.1. Hydrolysing and solubilising of AX

3.2.1.1. Water extraction

It is suggested that water extraction can insulate high MW arabinoxylans and reserve the native structure of the molecule, but in low yields (Ebringerová & Heinze, 2000). Lower yields can be explained by the strong bonds to lignin or cellulose through ferulate links and hydrogen bonds between xylose chains and the cellulose (Maes & Delcour, 2002; Vinkx & Delcour, 1996). The non-covalent bonds are relatively weak chemical bonds, but when present excessively they can prevent the solubility under moderate extraction conditions (Biliaderis & Izydorczyk, 2006).

Higher temperature and pressure can be applied in water extraction in order to get higher yields. However, applying higher temperatures was reported to produce higher yield, lower purity, and lower degree of polymerisation (Schooneveld-Bergmans *et al*., 1999).

3.2.1.2.Alkaline Extractions

Alkaline solutions possess the ability of inducing modifications of the hydrogen and covalent bonds through breaking down the ester linkages and loosening the tethering between AX and other cell wall components (Cyran *et al*., 2004; Fincher & Stone, 1986). The extraction temperature exerts great influence on the yields; in a study by Bergmans *et al*. (1996), yield increased from 29% at 20°C to 50% at 90°C. By contrast, elevating the alkaline (NaOH) concentration from 0.25 to 0.5 M has no effect on the results (Ayala-Soto *et al*., 2016).

The nature of the alkali is suggested to affect the extraction yield (Chanliaud *et al*., 1995). Bergmans *et al*. (1996) reported that substitution of barium hydroxide with sodium hydroxide reduced the yield. Among various alkalis tested, only $Ba(OH)_2$ conferred a relatively pure arabinoxylans extract with a high yield (Bergmans *et al*., 1996; Gruppen *et al*., 1991). The mechanism behind this selectivity is not clear, but it was presumed that the presence of Ba^{2+} prevents the solubilisation of β-glucans (Gruppen *et al*., 1991).

Alkaline extraction can be associated with lignin removal using dilute concentrations of hydrogen peroxide (Maes & Delcour, 2001). Vinkx and Delcour (1996) argued that the oxidation might solubilise recalcitrant materials. Also, AX may undergo non-enzymatic oxidative gelation, the mechanism of this oxidative gelation is explained by the oxidative coupling of ferulate residues, forming cross-linking of AX molecules (Vinkx & Delcour, 1996). Oxidative gelation significantly increased the yields of alkaline extraction of AX; a concentration of 2% H_2O_2 at a pH of 11.5 for 4 h at 60°C recovered successfully 77% of the total arabinose and 65% of the total xylose (Maes & Delcour, 2001). Thus, alkaline oxidative extraction of AX is considered one of the most reliable methods of AX extraction.

3.2.1.3.Acids Extractions

The conversion of pentosans in acidic conditions into furan products such as 5-hydroxy methyl-furfural and 2-furfural aldehyde hindered development of this route of extraction (Almeida *et al*., 2007; Pedersen *et al*., 2010). Especially under severe conditions, *e.g*. high temperature, these furfural products could be converted into unpleasant compounds such as formic and levulinic acids (Larsson *et al*., 1999). However, it was reported that a formula of formic acid,

acetic acid, H_2O in a ratio of 30:60:10 was effective to yield 76.5% of the total hemicelluloses of wheat straws (Xu *et al*., 2006).

3.2.1.4. Enzyme extraction

Some enzymes, *viz.*, xylanases, are capable of hydrolysing the AX main backbone into a lower molecular weight or shorter chain or even into its constructional monosaccharides (Dekker, 1985). Xylanases have been categorized into two main groups according to their mechanism: Exo-xylanases, which attack the terminal unit of the polysaccharide; and Endo-xylanases, which attack the polysaccharide's backbone in an arbitrary manner resulting in a general decrease in degree of polymerisation (Araki & Kitamikado, 1982; McCleary *et al*., 1982). Xylanases were found in wheat grain (Kulp, 1968; Preece & MacDougall, 1958), wheat bran (Bergmans *et al*., 1996), wheat flour (Cleemput *et al*., 1997), barley (Taiz & Honigman, 1976) and in germinated barley (Slade *et al*., 1989). Also, they were isolated from bacteria (Gilbert & Hazlewood, 1993; Paice *et al*, 1986), fungi (Haltrich *et al*., 1996; Polizeli *et al*., 2005), marine algae, protozoans, crustaceans, insects, snails and seeds of land plants (Sunna & Antranikian, 1997).

Endo-β-(1,4)-xylanases (EC 3.2.1.8) are the most common among the xylanases group (Escarnot *et al*., 2012), which is from microbial origin and belonging to glycanase families GH10 and GH11 (Biely *et al*., 1997). Generally, xylanases are classified according to the similarities in their amino acid sequence into two main families, GH10 and GH11 (Henrissat, 1991). Xylanases are used in AX extraction as they can attack the polysaccharide main backbone and release (solubilise) portions of WUAX (Courtin & Delcour, 2001). GH11 xylanases were reported to be more effective than GH10 in extracting AX from destarched wheat bran, with yields of 41% and 18% respectively (Maes *et al*., 2004). GH11 xylanases were assumed to be more selective to insoluble AX (Maes *et al*., 2004) or to have greater ability to penetrate the cell wall matrix (Beaugrand *et al*., 2004).

Other enzymes have been exploited in the extraction of AX in combination with xylanases, including feruloyl esterase (Faulds *et al*., 2003; Kroon *et al*., 1999). Feruloyl esterases cooperate with xylanases to release AX by breaking down the ester linkage between AX and ferulic acid (Faulds *et al*., 2002; Faulds & Williamson, 1995). Furthermore, cellulase in combination with endoxylanases resulted in greater yields of AX extracted from spelt bran

(Escarnot, Aguedo, & Paquot, 2011). Also, α-L- arabinofuranosidases shave the arabinose side branches, thereby longer regions of unsubstituted xyloses will be formed, which are more degradable by xylanases, resulting in more solubilised AX (Figueroa-Espinoza & Rouau, 1998). In contrast, the combination of different xylanases together did not enhance the yields (Beaugrand *et al*., 2004; Escarnot *et al*., 2012).

Enzymatically extracted AX showed a lower A/X ratio as xylanases prefer attacking low substituted AX (Beaugrand al., 2004), and much lower yields compared to alkaline hydrogen peroxide method (Zhou *et al*., 2010).

3.2.2. **Mechanically assisted extraction**

With respect to the increase of the effectiveness of the AX extraction process, many mechanical approaches have been investigated in favour of solubilising AX or loosening the cell wall to allow the release of AX.

3.2.2.1. Ultrasound treatment

Ultrasound technique was applied in the process of AX extraction in combination of both enzymatic and alkaline routes. With both methods, ultrasound increased the yields of the extraction (Ebringerova *et al*., 2002; Minjares-Fuentes *et al*., 2016; Wang *et al*., 2014). Changes in the functional properties of the extracted AX were observed such as weak "gel properties" and lower intrinsic viscosity; this could be a result of ultrasound degradation and decreasing AX MW (Ebringerova & Hromadkova, 1997; Sun *et al*., 2002).

3.2.2.2. Steam explosion

In this method, raw material is exposed to high pressure steam, then the pressure is released rapidly leading to an explosive depolymerisation (Cara *et al*., 2006). It is considered environmentally friendly and an effective procedure to break down the cell wall matrix (Avellar & Glasser, 1998). It is suggested that steam explosion breaks down the interpolymeric phenolic cross-links which loosens the cell wall structure (Merali *et al*., 2015). However, steam explosion notably depolymerised AX into its oligosaccharide and monosaccharide constituents (Sun *et al*., 2005). This degradation is the main disadvantage of steam explosion, as it affects the functional properties of the extracted AX.

3.2.2.3. Extrusion

Twin-screw extrusion was investigated for AX extraction, yields were relatively low with significantly lower purity (Jacquemin *et al*., 2015). However, extrusion exhibited many advantages such as short treatment time, lower water consumption and an environmentally friendly process (Jacquemin *et al*., 2012; Zeitoun *et al*., 2010).

3.2.3. Purification of AX

After the extraction, purification of crude AX extracts is usually necessary. Typically, the purification involves the inactivation of the enzymes used in the extractions, and the employment of hydrolytic enzymes to hydrolyse starch and protein in the crude extracts, followed by concentration and precipitation of the final AX.

3.2.3.1. Amylase and protease purification

Dervilly *et al.* (2000) applied α-amylase and amylo-glucosidase to degrade starch, leading to partial elimination of the starch from the extracts. In the same manner, protease was used to reduce the protein content and the extracts contained slightly less protein after the treatment (Dervilly *et al.*, 2000).

3.2.3.2. Concentrating methods

Dialysis membranes method is one of the first concentration processes used at a laboratory scale, and the most commonly used method (Preece & Hobkirk, 1953). Ultrafiltration is a promising concentration method as it can be used in pilot and industrial scales (Krawczyk *et al*., 2008). In previous unpublished work in the University of Huddersfield, ultrafiltration successfully decreased the volume of the extracts (prior to the ethanol precipitation) to one-fifth the original volume, thus significantly reducing the amount of ethanol required for precipitation and hence the cost and safety hazards.

3.2.3.3. AX precipitating

Regardless of the extraction method, precipitating AX is required. Ethanol has successfully been used to separate AX from the suspension. Several studies showed that the concentration of ethanol used to precipitate AX affects the A/X ratio; the increase in ethanol concentrations

results in higher ratio of A/X in the extracts (Gruppen *et al*., 1992; Viëtor *et al*, 1992). Bian *et al*., (2010) studied the effect of ethanol concentration on AX precipitating process by applying gradual concentrations of ethanol (10, 20, 30, 45, 60 and 80%). Differences in the branching distribution was observed according to the ethanol concentration, *i.e.*, the higher MW AX and lower branching AX were precipitated by lower concentrations of ethanol; and by increasing the ethanol concentration, the lower MW AX with higher branches precipitated (Bian *et al*., 2010).

Ammonium sulphate can be used for AX precipitation. Izydorczyk *et al*. (1992) demonstrated the effect of ammonium sulphate concentration on AX by applying gradual concentrations of the salt in the precipitation process (60, 70, 80, and 95%). the results showed that increasing the concentration of ammonium sulphate increased the A/X ratio of the resultant AX and precipitated lower MW AX (Izydorczyk & Biliaderis 1992).

3.3. Characterisation of Arabinoxylan

AX consists of a linear chain of β-D-xylopyranosyl and branches of α-L-arabinofuranoside, which are attached to the xylan chain. The most fundamental element of the structural characterisation of AX polysaccharides is the determination of the monosaccharide compositions, based on two essential terms, the identification of monomers and the relative A/X ratio.

3.3.1. Monomer Analysis

There are numerous analytical methods used for the analysis of saccharides such as high performance liquid chromatography with refractive index detector, and colorimetric methods (Henshall, 2001). However, the most frequently used methods for both quantitative and qualitative analysis of monosaccharides are chromatographic methods viz. gas chromatography (GC) and high pressure anion exchange chromatography (HPEAC) (Albersheim *et al.*, 1967; Blake & Richards, 1970; Gerwig *et al.* 1978).

Prior to the sample analysis process, polysaccharide depolymerisation is required, usually by acid hydrolysis. Applying gas chromatographic analysis necessitates the implementation of a

derivatization step in order to convert the monosaccharide compounds into volatile equivalents (Henshall, 2001).

High pressure anion exchange chromatography with pulsed amperometric detection (HPEAC-PAD) has been widely used for polysaccharide analysis. The HPEAC-PAD analytical method is superior to GC as it does not requires the derivatization step (Rocklin & Pohl, 1983)

3.3.1.1. HPEAC-PAD:

HPEAC-PAD is a relatively recently developed technique for the identification and quantification of monosaccharides. Principally, HPEAC-PAD is a high performance anion exchange chromatographic separative method coupled with pulsed amperometric detector (Gruter *et al.* 1992, 1993; Lemoine *et al*., 1997; Levander *et al*., 2001). In fact, the concept of this technique relies on the variable degree of ionization of monosaccharides corresponding to the change in pH of ambient medium; monosaccharides tend to ionise under high pH conditions, thereby allowing for the ease of separation in an ion exchange column (Corradini *et al.*, 2004; Speight, 2005).

Predominantly, in anion exchange chromatography, strong anions are retained towards the oppositely positive charged resin (the stationary phase) due to ion-pair formation (Corradini *et al.*, 2004). The extent of retention of the saccharide compounds depends on the degree of ionization in the mobile phase. Generally, the inverse relationship between the pKa value of the hydroxyl ion and the ionisation degree aids on the high retention of the targeted analyte through the chromatographic system (Rocklin & Pohl, 1983).

Subsequently, after the completion of separation step through the chromatography column, the detection is achieved with the pulsed amperometric detector utilization, which functions as an electric current changes monitoring through a gold or platinum electrode. When a monosaccharide reaches the surface of the electrode an oxidization process occurs as a result of potential variations of the detector.; this generates a current that can be measured (Rosselló-Mora, 2003).

The absence of derivatization and the relatively rapid separation make HPEAC-PAD an advantageous selection to perform the intended analytical separation. Moreover, this technique

possesses valuable features including the ability of detecting low quantities of the saccharide compounds (in the pico-mole ranges) through the PAD detector; furthermore, the eluents are cost-effective and safe to be handled (Henshall, 2001; Speight, 2005).

3.3.1.2. Gas Chromatography-Mass Spectrometry

GC and GC–MS are considered as highly appropriate techniques for the analysis of carbohydrates; notwithstanding, the preparation of adequate derivatives is a prime requirement. The main feature of GC separation is the greater separation capacity. Indeed, various monosaccharides can be separated through this technique, the retention times are relatively short with high resolution.

Specifically, monosaccharides, classified as a polar and relatively non-volatile compounds, require to be derivatized to a higher volatility state prior to the gas chromatographic analysis (Gómez-González *et al.*, 2010; Medeiros & Simoneit, 2007; Ruiz-Matute *et al.*, 2011).

Many published related derivatization methods are mostly addressing the substitution of the polar groups of the sugar entity through their stipulated methodological approaches. Particularly, the popular derivatives of carbohydrates are the methyl ethers, acetates, trifluoroacetates and trimethylsilyl ethers (Albersheim *et al.*, 1967).

3.3.1.3. Capillary Electrophoresis (CE)

The use of capillary electrophoresis (CE) has been described extensively as a vital separation method for the analysis of sugar compounds. In fact, CE has emerged recently as a highly promising technique for the analysis of mono- and oligosaccharides. The presence of the negative charge in alkaline solutions of monosaccharides can permit their ease of separation through the tubular capillary under a fixed electric field. The detection can be feasibly performed utilizing a pulsed amperometric detector or mass spectroscopy (Henshall, 2001; Q. Wang & Fang, 2004).

3.4. Potential usage of AX

The functional and structural properties of arabinoxylans promote potential uses in the food industry, particularly in the bread making industry. Due to the unique properties of AX such

as water holding capacity and viscosity, AX can have a prominent impact on the process of bread making and the quality of the finished bread product *i.e.*, loaf volume, crumb texture, and firming characteristics (Biliaderis *et al*., 1995; Saulnier *et al*., 2007). AX can also inhibit the ice formation during freezing of dough, which improves the quality of frozen dough (Wang *et al*., 2016).

In addition, the literature reported several healthy effects associated with AX such as decreasing the levels of blood cholesterol and low-density lipoprotein (Anderson & Hanna, 1999), improving glycaemic control as it reduces postprandial blood glucose and insulin levels (Slavin, 2005), in addition to the prebiotic effect that AX and AXOS can play (Grootaert *et al*., 2007; Hughes *et al*., 2007).

Furthermore, AX properties have promoted its use as a natural pharmaceutical excipient as it showed a promising results a binder and dispenser excipient (Erum *et al*., 2015). Also, AX presents a pH-independent sustained release coating material (Iqbal *et al*., 2011).

In the increasing demands for deploying films from renewable sources in food packaging, AX appears to form a promising packaging films (Stepan *et al*., 2012; Stepan *et al*., 2014; Stevanic *et al*., 2011; Velkova *et al*., 2015).

3.1. Research objectives

The larger scale production of AX extracts is an absolute requirement permitting the optimum utilization of this entity into variable sectors.

Substantially, the fundamental step in arabinoxylan production sequence is the precipitation phase through ethanol utilization. Thus, integrating bioethanol production with AX production can promote the commercial viability and the economical competitiveness of biorefineries through the effective investment of resources and raw materials.

Accordingly, several extraction methods have been evaluated including acid, alkaline and enzymatic extraction. Further potential extraction approaches need to be investigated

thoroughly, aiming for enhancing the extraction output in terms of extraction effectiveness aspects and elevating the purity of the yielded extracts.

Therefore, the objective of the current research was to screen a range of pre-treatments approaches and extraction conditions for the purpose of obtaining AX from two biomass sources, wheat bran and sugarcane bagasse. The experimental studies included the appraisal of xylanases capability of releasing arabinoxylan, in comparison with the chemical routes. A further objective was to compare the merits of enzymatic (viz. cellulase) and mechanical pre-treatments (viz. milling and thermal treatment). Finally, the value of enzymatic purification (via protease and amylase) of AX extracts was investigated.Summary

A great deal of attention has been paid in the research literature to find a suitable extracting method for AX. This chapter has reviewed the main extraction methods in the literature *viz.*, water, acids and alkaline extraction. Water extraction appears to be the only method that preserves the original structure of AX molecules, but the low yields hinder the transition of water extraction to larger scales. Enzymatic extraction show promising potential as it offers low cost and an environmental friendly process, but the xylanases alter the molecular shape of AX and produce relatively lower MW AX. This will affect the functional properties of AX products, as the MW is a key factor in AX functionality. Among all the reviewed methods, alkaline extraction is the most convenient method in term of yields. The changes in molecular properties are less in the chemical extraction, maintaining the main functional properties such as viscosity and water holding and gel formation, which are essential for introducing AX to the food industry. Mechanically-assisted extraction utilising ultrasound and steam explosion increases the yields and affects the properties of AX, as both tend to hydrolyse AX to its oligosaccharides. Purification of the extracted AX is needed as the impurities such as protein, cellulose and lignin can alter and change the characteristics of the extracts. Enzymatic purification along with ultrafiltration seems to be a realistic method to purify the extracts. Ethanol precipitation is the main method used for recovering the extracted AX, though it adds cost and brings in safety issues in handling the ethanol. The objective of the current work was to investigate a range of pretreatment and extraction conditions, along with post-treatmetn with enzymes, to enhance the yield and purity of AX extracts from wheat bran and bagasse.

4. Materials & Methods

The current and the following chapter describe the investigations of arabinoxylans extraction from two biomass sources, wheat bran and sugarcane bagasse, through exploiting chemical and enzymatic methods. The influence of applying pre-treatment strategies on the extraction, the effectiveness of protease and amylase for extracts purifications, and crude extract characterisation are addressed. The current chapter presents the materials and methods utilized to accomplish these investigations; the subsequent chapter expounds and discusses the results.

4.1. AX Extraction

Initially, arabinoxylans were extracted from wheat bran (WB) and sugarcane bagasse (SCB) raw materials through the employment of three methodologies, alkaline, alkaline oxidative and enzymatic methods. Then, Samples were pre-treated using milling, autoclaving, and cellulase pre-treatment. Enzymatic purifications of extracts using protease and amylase were extensively investigated.

4.1.1. Materials used

Wheat bran and sugarcane bagasse were provided by AB Sugar (Peterborough, UK).

4.1.1.1. Chemicals, Enzymes and Instruments

4.1.1.1.1. Chemicals

Sulphuric acid, Hydrogen peroxide (>30%w/v), Methylated industrial spirit (MSI) and citric acid were purchased from (Fisher Scientific UK Limited, analytical grade). Sodium hydroxide disodium phosphate from (Sigma-Aldrich, UK). The antifoaming agent used is Dimeticon SILFAR® SE 4 (Wacker Chemie AG, Germany).

4.1.1.1.2. Enzymes

β-Xylanase (*C.mixtus*, PRO-E0051), Endo-1,4-β-Xylanase (*N.Patriciarum*, PRO-E0062), 1,4-β-D-Xylanase (*O.terroe*, PRO-E0301) and Feruloyl esterase (*A.cellulolyticus*, PRO-E0356) from Prozomix UK. Cellulase (nsibc-91) from Novozymes UK. Protease (P-4860) and amylase (alpha-amylase 9001-19-8) from Sigma-Aldrich, UK.

4.1.1.1.3. Instruments

During the extraction process samples were centrifuged utilising Beckman centrifuge model GS-6S (Beckman instruments, Inc., Palo Alto, California). Samples were concentrated utilising Sartorius™, Stedim Vivaflow™200 Ultrafiltration System equipped with a membrane of 10 kDa molecular weight cut-off, the filtration runs on (2.5-3) bar pressure (Sartorius Stedim Biotech, Goettingen, Germany). The drying was performed employing Christ Freeze Dryer Alpha 1-4 ld plus, Germany. For milling the raw material and the extracts a domestic coffee grinder was used (Andrew James, Co. Durham, UK).

4.1.2. **Methodologies**

4.1.2.1.AX Extraction from Wheat Bran

Figure 4-1 demonstrates the general extraction process of AX from wheat bran. The extraction was screened in terms of three levels: the efficiency of the main hydrolysis and extraction method; the importance of pre-treatment; and the possibility of producing extracts featuring with higher purity through utilizing the enzymatic treatment.

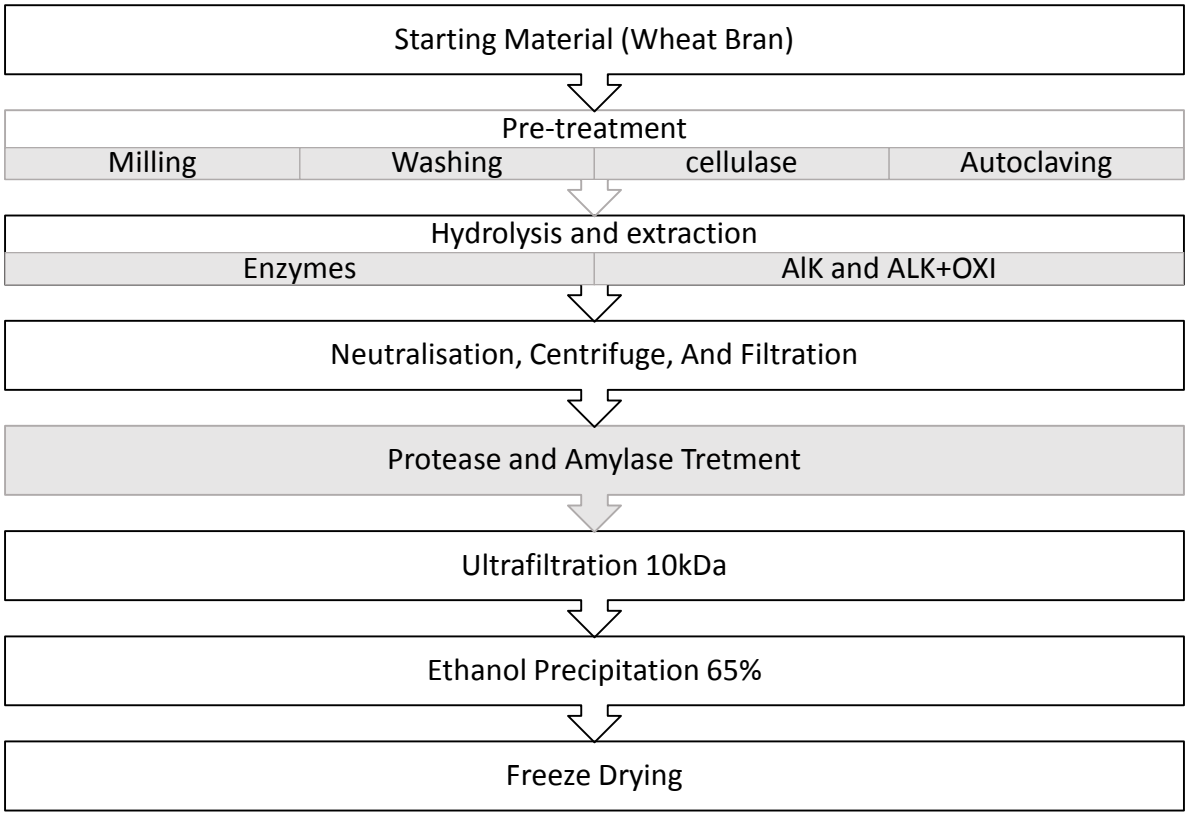

*Figure 4-1 the extraction process from WB, *coloured steps are studied*

4.1.2.1.1. Enzymatic and alkaline oxidative extraction

Both Alkaline oxidative (ALKOXI) and enzymatic methods were executed punctually as following:

Alkaline oxidative method: 30 g of WB was transferred carefully into 1 litre Duran bottle, then, and 270 ml water was added, followed by a gradual addition of 15 ml of 5M NaOH under continuous vigorous stirring. Then, 10 drops of anti-foaming agent and 20 ml of H_2O_2 30% were both added in small proportions (2 ml at a time) while the stirring was persisted. Observably, after H_2O_2 addition, foaming starts to grow as Figure 4-2 illustrates, consequently, extra few drops of anti-foaming agent, under continuous stirring, were added to control the foaming.

Figure 4-2 Foaming occurs with ALKOXI method

The resultant bottles containing the dispersions were placed in a water bath pre-heated to 60°C for 4 hours, the pH values were monitored and adjusted accordingly through the extraction process. The total measured volume of the slurry was approximately 350 ml with solid/liquid ratio of 1:11 for WB. Thereafter, the bottles were reserved under ambient conditions to equilibrate to room temperature (~23°C), then, neutralization with 4 M sulphuric acid to pH 7±0.2 was performed. Subsequently, the resultant sludge was transferred to 750 ml centrifuge bottles, then, centrifugation was implemented (3500 rpm for 15 min). Filtration process were performed in order to obtain the desired supernatant, which moved to a clean Duran bottle, two washing steps for the remaining solid were executed with 300 ml water under constant stirring for 10 minutes then centrifuged. The produced filtrates were pooled to the former resultant one. The total volume of the repetitive filtrates washing steps was approximately 900 ml of AX-rich solution. Figure 4-3 shows the water bath, the centrifuge instrument, and the filtration step of the tested samples.

.

Figure 4-3 Water bath, Beckman Centrifuge, and filtering

The solution was concentrated using Stedim Vivaflow™200 ultrafiltration system in order to reduce the volume to 200 ml. Figure 4-4 shows the ultrafiltration process.

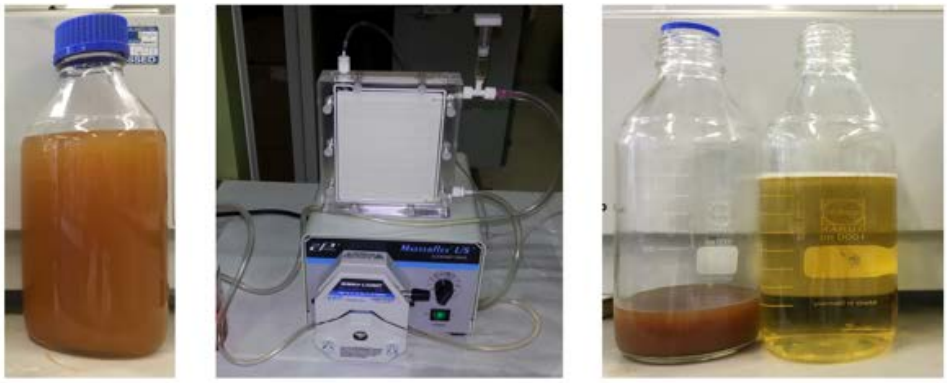

Figure 4-4 Viv flow 200 Ultrafiltration system

AX was precipitated from the 200 ml retentate by the addition of 400 ml of 99% methylated industrial spirit (MIS), aiming to achieve ~65% concentration of ethanol. The precipitation occurs immediately after the addition of MIS as Figure 4-5 shows. In order to assure the maximum precipitation of AX, the bottles were kept overnight under fixed temperature (4°C).

Figure 4-5 AX precipitation with 65% ethanol

Subsequently, the produced suspensions were centrifuged, supernatants, which contains the ethanol were discarded, and AX-rich extract was recovered quantitatively. The samples were transferred into suitable petri-dishes prior to freeze drying. Figure 4-6 illustrates the wet AX extract sample.

Figure 4-6 the wet AX extract

Despite the implementation of the freezing step under a very low temperature on the resultant sample (approximately –14°C), unfortunately, some samples exhibited incomplete freezing which could be due to the authentic cryostabilising properties of AX.

Consequently, the prepared petri-dishes were frozen in a styropor box with the presence of a dry ice (–78°C) for 1 hour in order to assist the complete freezing of the extracts. Thereafter, extracts were placed in the freeze dryer manifold chamber, as displayed in Figure 4-7. The pressure was adjusted to 0.02 mbar allowing water sublimation.

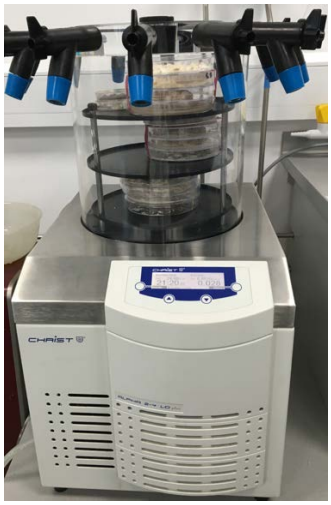

Figure 4-7 Freeze Dryer

The dried extracts were recovered, weighed for the purpose of yield calculation, milled using a coffee miller in order to reach the form of fine powder formation, and reserved for further

analysis. The sample was coded as **WB1**. Experiments of all samples were performed in triplicate.

Enzymatic extraction: 30 g of WB was measured accurately into Duran bottles, and 300 ml of phosphate buffer (pH 6.5) was added (107.4 ml of 0.2 M disodium phosphate, 42.6 of 0.1 M citric acid and 150 ml of water). Using a micropipette, 30 µl of β-Xylanase E0051 (XYL51) and 30 µl of feruloyl esterase E0356 (FER356) were added to the intended bottles. The resultant solutions were stirred for 2 hours in a 40°C water bath. Thereafter, the same steps of the chemical method were applied (*i.e.*, cooling to room temperature, centrifuging, washing, ultrafiltration, alcohol precipitation and freeze-drying). Sample was coded as **WB2**.

4.1.2.1.2. Cellulase in enzymatic method

WB samples were treated by integrating a combination of 30 µl XYL51, 30 µl of FER356 and 250 µl of cellulase nsibs-91 (CEL91) for 2 hours following the extraction procedure steps described above. Sample was coded **WB3**.

4.1.2.1.3. Enzymes treatment time

Sample WB3 conditions were repeated, but with extended incubating times of 4 and 6 hours (samples **WB4** and **WB5**). In addition, three prepared reference samples containing only the buffer were incubated for 2, 4, and 6 hours (CON1, CON2 and CON3).

4.1.2.1.4. Applying different xylanases

1,4-β-D-xylanase E0301 (XYL301) and Endo-1,4-β-xylanase E0062 (XYL62) were tested under the previous conditions (*i.e.*, 30 µl xylanase with 30 µl FER356 and 250 µl CEL91 for 4 hours' treatment). Samples were coded **WB6** and **WB7** for XYL301 and XYL62, respectively.

4.1.2.1.5. Cellulase pre-treatment

Exploiting cellulase as a pre-treatment method in the alkaline oxidative extraction was investigated in two approaches:

WB8 was treated with 250 µl CEL91 for 24 hours followed by chemical extraction, which was performed for 4 hours.

WB9 was incubated with 250 µl CEL91 for only 4 hours followed by chemical extraction for 2 hours.

4.1.2.1.6. Combining cellulase pre-treatment with alkaline extraction

Alkaline extraction was carried out with and without the cellulase pre-treatment steps as follows:

WB10 was implemented using 0.1 M sodium hydroxide only for 4 hours.

WB11 was pre-treated with CEL91 for 24 hours. Thereafter, extraction with sodium hydroxide utilisation for only 4 hours.

4.1.2.1.7. Milling the bran

WB was milled using a coffee miller (details). Then, 30 g were extracted using ALKOXI method, sample was coded (**WB14**).

4.1.2.1.8. Auto clave pre-treatment

30 g of WB were soaked in phosphate buffer at pH 7 and autoclaved for 1 hour at 121°C followed by ALKOXI extraction.

4.1.2.1.9. Bran washing

30 g of raw bran were placed into 1 litre Duran bottle containing 800 ml distilled water, the bottle was heated to 60°C for 20 minutes using the water bath applying vigorous stirring. Subsequently, the bottle was removed from the water bath, and left under ambient condition to permit precipitation of the solids materials. The separated liquid phase was poured into a clean container, then the precipitated remaining solids were re-extracted with ALKOXI (**WB12**). The liquid phase was concentrated utilising the rotary evaporator, freeze dried and reserved for further analysis and quantifications.

4.1.2.1.10. Starch and protein removal

30 g of WB were washed following the exact procedure (as in sample WB14), followed by CEL91 treatment (250 µl) for 24 hours and chemical extraction. Prior to the ultrafiltration step,

the solution was treated with amylase protease and cellulose for 4 hours at 60°C. Sample was coded as WB13.

Table 4-1 lists all the prepared samples on wheat bran.

Table 4-1 Wheat Bran AX extraction methodologies

Sample	Procedure	Time
WB1	ALKOXI	4 H
WB2	XYL51+ FER356	2 H
CON1	Buffer	2 H
WB3	XYL51 + FER356 + CEL91	2 H
CON2	Buffer	2 H
WB4	XYL51 + FER356 + CEL91	4 H
CON3	Buffer	4 H
WB5	XYL51 + FER356 + CEL91	6 H
CON4	Buffer	6 H
WB6	XYL301+ FER356 + CEL91	4 H
WB7	XYL62 + FER356 + CEL91	4 H
WB8	CEL91 + ALKOXI	24 H + 4H
WB9	CEL91 + ALKOXI	4 H + 2 H
WB10	ALK ONLY	4H
WB11	CEL91 + ALK ONLY	24 H + 4H
WB12	Washing + ALKOXI	4H
WB13	Washing + CEL91 + ALKOXI+ PRT + AML	24 H + 4H + 6H
WB14	Milling + ALKOXI	4H
WB15	Autoclave + ALKOXI	1 H + 4 H

4.1.2.1.11. Yield Calculations

The extraction yields were calculated as the percentage of the weight of the freeze-dried crude extract to the weight of the starting raw material:

$$Yield\ \% = \frac{\text{weight of the freeze\_dried crude extract}}{\text{weight of the original sample}} \times 100$$

4.1.2.2.AX Extraction from Sugarcane Bagasse

Figure 4-8 illustrates the AX extraction process variations investigated for sugarcane bagasse.

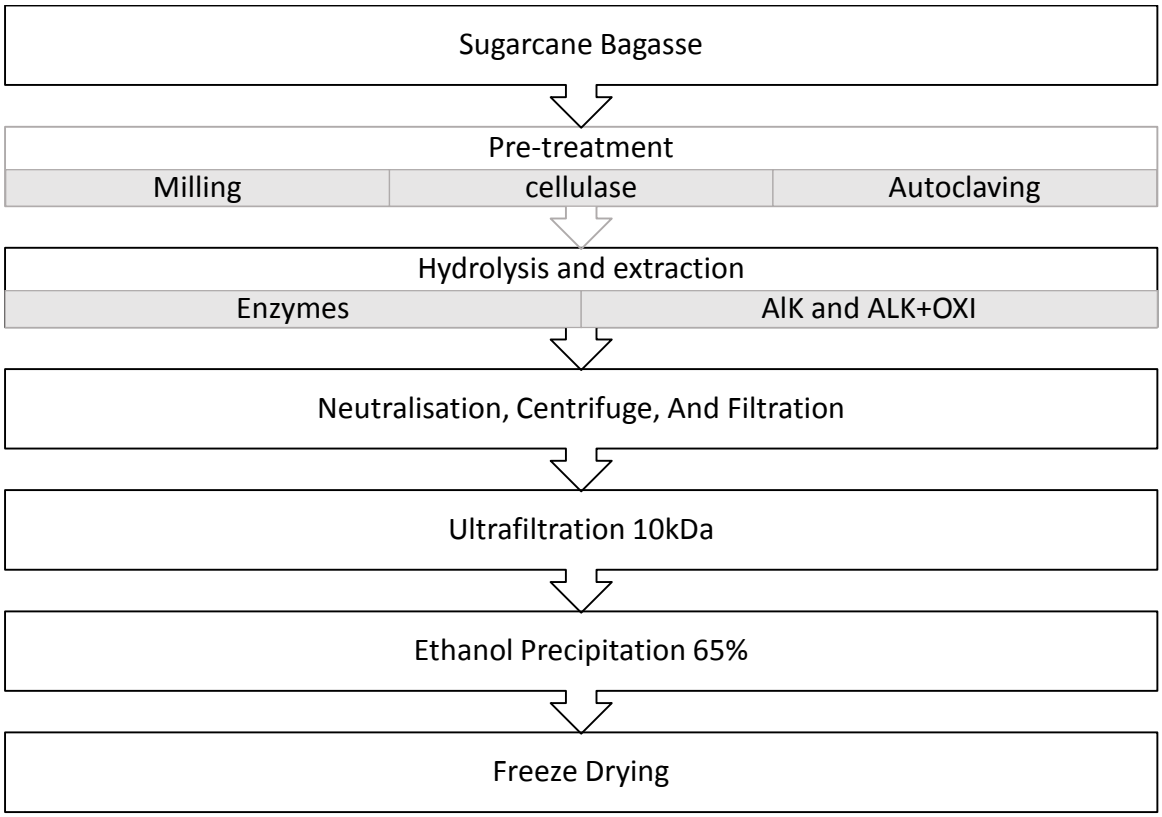

*Figure 4-8 the extraction process from SCB, *coloured steps are studied*

4.1.2.2.1. Alkaline oxidative extraction

Starting with only 10 g of sugarcane bagasse (SGB), as it forms a very thick slurry when mixed with water, the extraction was carried out in the same steps as in case of wheat bran with two significant differences:

- The solid/liquid ratio of the extraction was 1:33
- The amount of anti-foaming agent needed was less as the oxidation produced less foaming with the bagasse.

These resultant samples were coded as **SCB1**. All samples were executed in triplicate.

4.1.2.2.2. Enzymatic extraction

Two xylanase enzymes were utilised in this trial: XYL301 and XYL62 in combination with FER356 and CEL91 for 4 hours. The resultant samples were coded as **SCB2** and **SCB3** for XYL301 and XYL62, respectively.

4.1.2.2.3. Cellulase pre-treatment

The efficiency of cellulase pre-treatment was tested following the same approach of WB pre-treatment procedure:

SCB4 was treated with CEL91 for 24 hours followed by alkaline oxidative extraction process performed for 4 hours.

SCB5 was incubated with CEL91 for only 4 hours, then alkaline oxidative extraction was implemented for 2 hours.

4.1.2.2.4. Combining cellulase pre-treatment with alkaline extraction

SCB6 was carried out using sodium hydroxide for 4 hours only.

SCB7 was pre-treated with CEL91 for 24 hours. Thereafter extraction with sodium hydroxide was performed for 4 hours.

4.1.2.2.5. Auto clave pre-treatment

10 g of SCB was soaked in phosphate buffer at pH 7. The produced sample was subjected to autoclaving for 1 hour at 121°C. Thereafter, ALKOXI extraction was performed for 4 hours, sample was coded as **SCB8.**

4.1.2.2.6. Milling the bagasse

In order to convert SCB into a fine powder form, milling was executed with the utilisation of a coffee grinder. Then, 10 g of the sample was utilised in AX extraction by ALKOXI method for 4 hours. The resultant sample was coded as **SCB9.**

Table 4-2 lists the investigations performed on SCB samples.

Table 4-2 Sugarcane Bagasse AX extraction methodologies

Sample	Procedure	Time
SCB1	ALKOXI	4 H
CON5	Buffer	4 H
SCB2	XYL301+ FER356 + CEL91	4 H
SCB3	XYL62 + FER356 + CEL91	4 H
SCB4	CEL91 + ALKOXI	24 H + 4H
SCB5	CEL91 + ALKOXI	4 H + 2 H
SCB6	ALK ONLY	4H
SCB7	CEL91 + ALK ONLY	24 H + 4H
SCB8	Autoclave + ALKOXI	4 H
SCB9	Milling + ALKOXI	4 H

4.2. Protein Content Determination

4.2.1. Materials and apparatus used

4.2.1.1. Chemicals

Sulphuric acid, methyl red (pH indicator) and hydrochloric acid were purchased from Fisher scientific (Loughborough, UK). Kjeltabs® digestion catalyser, sodium hydroxide and boric acid were supplied from SIGMA-ALDRICH (Poole, United Kingdom)

4.2.1.2. Apparatus

Kjeldahl digestion system utilised in this analysis is Turbotherm Rapid Digestion Systems, Gerhardt®, Model TT125 supplied from Gerhardt (Bonn, Germany). Steam Distillation System Gerhardt Kjeldahl VAPODEST®, model Vapodest-10 provided from Gerhardt (Bonn, Germany). Auto titrator Schott TITRONIC® basic titration burette obtained from Schott Instruments (GmbH, Germany).

4.2.2. Methodology

The Kjeldahl approach for total nitrogen determination method was followed aiming for acquiring the protein contents of both starting materials (*i.e.* WB and SCB) and the AX-rich extracts. Kjeldahl method consisted of three stages: digestion, distillation and titration.

During the first digestion stage: 1 g of the tested subject was placed into digestion tubes along with 2 Kjeltabs , anhydrous sodium sulphate and a catalyst .Then , 20 ml concentrated sulphuric acid was added along with few drops of anti-bumping agents for prevention of bubbling. Consequently, digestion process converted the nitrogen into ammonia (NH_4SO_4), and other organic matter to CO_2 and H_2O.

Accordingly, a control sample was prepared, then digested for 1 hour and 20 minutes period following the former digestion procedure. Thence, at the end stage of the digestion, a greenish blue colour was developed as an indication for the digestion process completion. Basically, increasing the digestion time was needed in order to obtain the intended green colour as recommended by the manufacturer manual.

At the termination of the digestion process, digestion tubes were left to equilibrate with the ambient room temperature.

The Gerhardt Kjeldahl Vapodest distillation unit was used aiming for the conversion of the ammonium sulphate into ammonia gas by the addition of sodium hydroxide. The formed ammonia was distilled to a receiving flask containing 25 ml of 3% boric acid and 2 drops of methyl red (pH indicator). Boric acid was utilised aiding for the conversion of the ammonia into ammonium borate.

Lastly, the estimation of the nitrogen content was executed through titration procedure of the formed ammonium borate along with 0.1 M hydrochloric acid, the average titration volumes were obtained and the blank titre value were used for calculating the nitrogen percentage presented in the samples.

$$\%N = \frac{[Sample\ titration\ volume\ (ml) - Blank\ titration\ volume(ml)] \times Molarity\ of\ HCl \times 14 \times 100}{Weight\ of\ sample\ (mg)}$$

After the determination of the total nitrogen content, the resultant nitrogen was converted to a protein percentage context by the protein conversion factor multiplication (F = 5.7) for wheat products (AOAC 979.09).

$$\%\ Protein\ content = \%N \times F$$

4.3. Monomer Analysis

Aiming for evaluating the degree of purity for the most promising resultant extracts, monosaccharide analysis was executed utilising HPAEC-PAD. An hydrolysis step was implemented before introducing the sample to the chromatographic instrument.

4.3.1. Standards preparation

The monosaccharides standards were purchased from SIGMA-ALDRICH (Poole, United Kingdom). Preliminarily, a stock standard solution containing 1000 ppm of each of the three sugar compounds, arabinose, glucose and xylose, was prepared. Subsequently, three standard

solutions were prepared at concentrations of 20, 40 and 60 ppm by diluting the stock solution as illustrated in Table 4-3.

Table 4-3 The preparation of standards solution

	St 20 ppm	St 40 ppm	St 60 ppm
ml of stock St	0.1	0.2	0.3
ml of ultra-purified water	4.9	4.8	4.7

4.3.2. **Sample preparation/Hydrolysis**

The prime aim of the hydrolysis phase is to induce chemical bonds cleavage through water addition, which can facilitate the conversion of arabinoxylans into its sugar moieties sub-components (Manns *et al.*, 2014). For attaining this purpose, 4.0 mg of AX extract was transferred carefully into a pressurized tube. 2 ml of 2M trifluoroacetic acid (Fisher Scientific, UK) was added to the former tube. Tubes were placed in a hot oil-bath 120°C for 2 hours, then removed from the oil-bath and left to equilibrate to the ambient room temperature. A constant stream of nitrogen gas at 60°C was exploited in order to stimulate the complete sample dryness upon evaporation.

Subsequently, the dried residues were dissolved in 4 ml of ultra-purified water. The hydrolysis phase yielded the intended monomeric solution which was ready to proceed through further analytical procedure utilizing HPAEC-PAD. The selected most promising extracts were assigned as WB1, WB8, WB13, SCB1, SCB4, SCB5 and SCB7.

4.3.3. **High Performance Anion Exchange Chromatography Pulsed Amperometry Detection (HPAEC-PAD)**

The HPAEC Instrumentation used in this research was a Dionex ICS–3000 Ion Chromatography System (Dionex Corporation, CA, USA), which comprises the Dionex Auto Sampler, Dionex ICS 3000 Eluent Organiser, Dionex ICS 3000 Detector Chromatography, Dionex ICS 3000 Dual Pump System and Dionex ICS 3000 Eluent Generator Reagent-Free Ion Chromatography. The column used was a CarboPac® PA20 analytical column, 3×150 mm,

6.0 µm particle size. The chosen mobile Phase was 10mM NaOH with adapted flow rate of 0.45 ml/min and injection volume of 25.0 µl. The pressure was adjusted to 2815 psi. Chromeleon® Xpress software was utilised for data processing.

4.4. Ultrafiltration maintenance

Evidently, arabinoxylans are responsible, for a specific extent, in affecting the filtration performance in beer production (Krawczyk *et al*., 2008). AX residual caused the fouling of the membrane during the filtration process. Unfortunately, the filtration time was extended due to this issue, and the efficiency of the filtration process was decreased.

Owing to the fact that sodium hydroxide can solubilise partially both AX and lignin; it can be used for the removal of the fouling. The ultrafiltration membrane made of polyethersulfone can withstand the high alkalinity of the 1M sodium hydroxide based on the manufacturer recommendations. Washing the ultrafiltration membrane with 0.1M NaOH appeared to be advantageous in cleaning the membrane and boost the filtration efficiency. Elution of the ultrafiltration cassettes with sodium hydroxide was performed after three experimental runs for at least 6 hours.

Moreover, in order to control the effectiveness and the quality of the ultrafiltration method, the samples from the filtrates were collected periodically, and preserved for further molecular weight distribution analysis.

5. Results and Discussion

5.1. Introduction

After extracting AX from both sugarcane bagasse and wheat bran, the resultant extraction products were calculated and total nitrogen content analysis was performed. The results are presented and discussed in this chapter.

5.2. Wheat Bran AX Extracts

5.2.1. Wheat Bran AX Extract Physical state and appearance:

Wheat bran AX-rich extract colours varied according to the extraction methods; enzymatically and alkaline extracted AX gave a yellowish darker off-white colour, whereas the alkaline oxidative produced extracts were more whitish. This could be as a result of lignin oxidation bleaching.

In general, all the extracts were odourless and have a non-crystalline starchy appearance (after milling). However, the physical appearance of the extracts could be subject to the high content of impurities (*i.e.*, lignin, protein, starch). Nevertheless, further investigation of the composition of the extracts needs to be conducted.

5.2.2. Wheat Bran AX Extracts Yields

Figure 5-1 illustrates the enzymatic method of WB2, utilising the mixture of both XYL51 and FER356 without releasing AX as expected in comparison to the control buffer (CON1), whereas the alkaline oxidative method (WB1) gained higher crude yield of AX-rich extract.

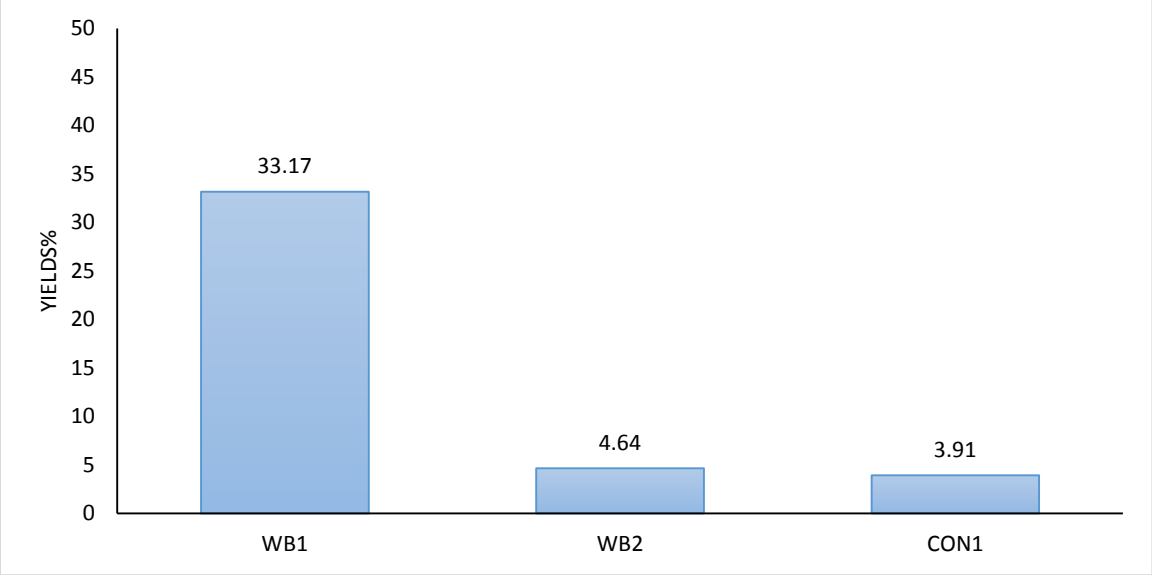

Figure 5-1 Extraction yields of samples WB1, WB2 and the control Buffer

Figure 5-2 shows that utilising cellulase in the enzymatic extraction (WB3) slightly increased the yield of the extraction. In addition, increasing the enzymes treatment time (WB4, WB5) slightly decreased the yield compared to the buffer.

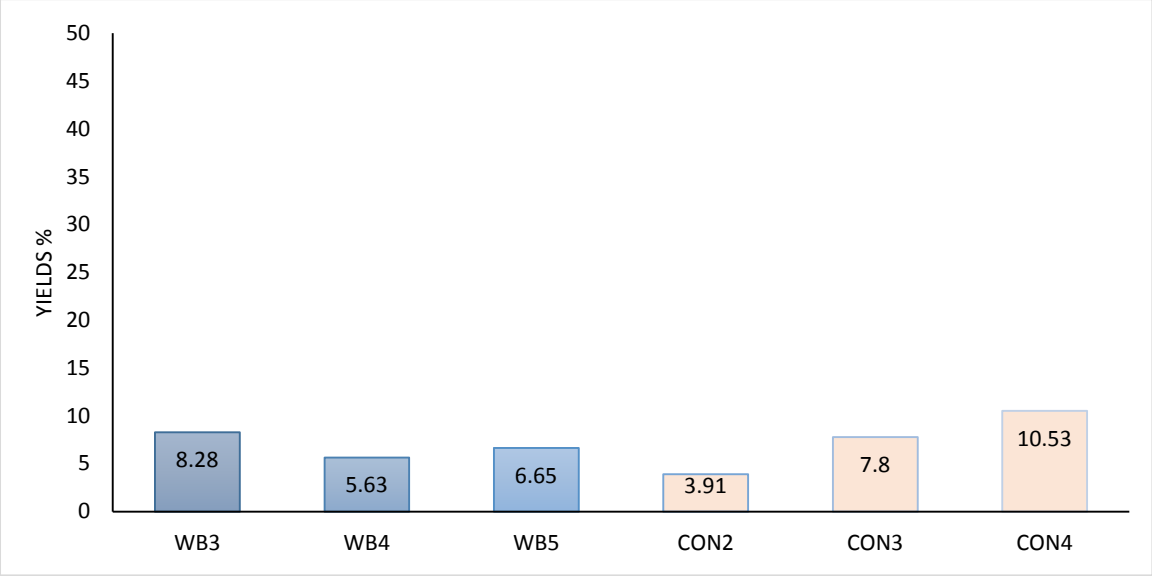

Figure 5-2 Extraction yields of XYL51 after 2, 4, and 6 H Treatment

Irrespective of the xylanases used, higher yields were not observed in the samples WB6, WB7. As Figure 5-2 demonstrates, the yields obtained by the three enzymes are close to the control buffer and ranged between 5.6-9.3%.

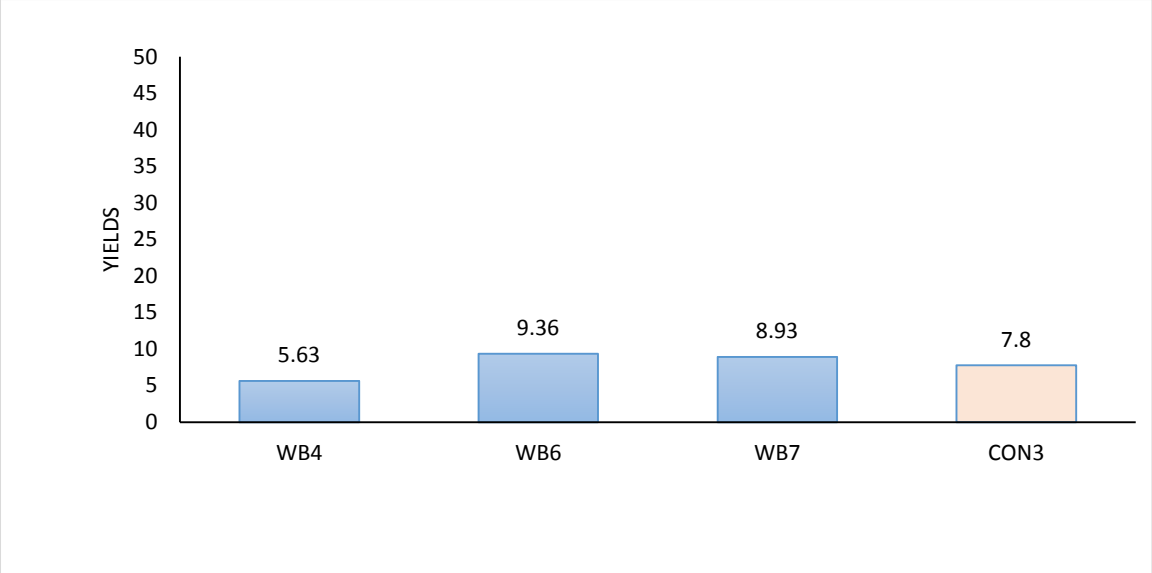

Figure 5-3 Extraction yields for XYL51, XYL62, and XYL301

Figure 5-4 illustrates that cellulase pre-treatment with alkaline oxidative extraction (24 h treatment) (WB8) gave the highest yield (40.12%) among all the tried approaches, whereas 4 hours pre-treatment followed by only 2 hours chemical extraction gave almost the same yield as 4 hours chemical extraction without pre-treatment.

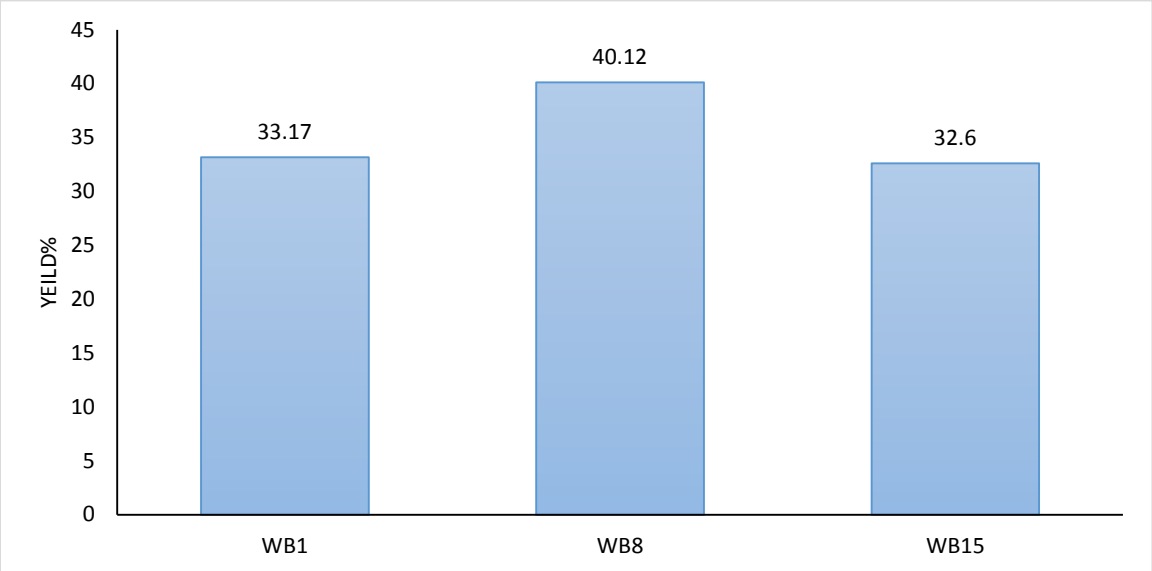

Figure 5-4 Cellulase pre-treatment yields

Alkaline extraction (WB10) yielded only 17.6%, which is almost half the yield of the alkaline-oxidative method. Unexpectedly, cellulase pre-treatment (WB11) did not enhance the extraction and the yield was 17.4%. Figure 5-5 compares the obtained results for alkaline extraction and alkaline oxidative extraction.

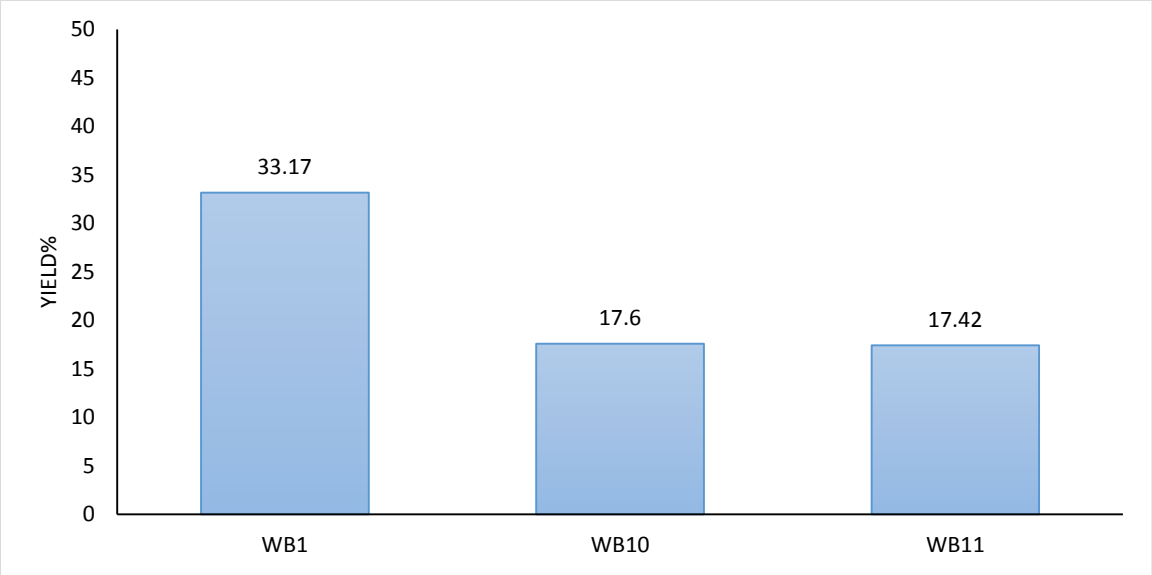

Figure 5-5Alkaline extraction yields

Washing the bran (WB12) with water at 60°C for 20 minutes slightly reduced the yield (31.54%) of ALKOXI extraction. In addition, enzymatic removal of starch and protein (WB13) significantly reduced the extraction yield to 28.2%.

Figure 5-6 shows that autoclaving (WB15) as a thermal pre-treatment did not increase the yields. In contrast, milling the bran (WB14) significantly increased the yields of the extraction.

Figure 5-6 illustrates the effectiveness of autoclaving, milling, and cellulase pre-treatment.

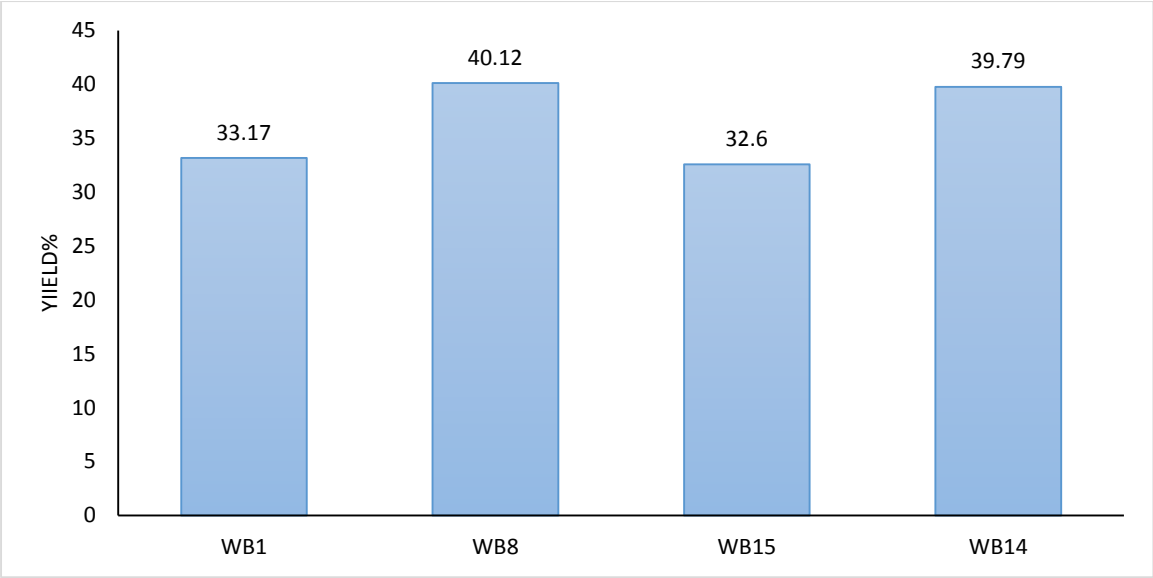

Figure 5-6 ALKOXI extraction without pre-treatment, cellulase, autoclaving, and milling pre-treatment.

Table 5-1 and Figure 5-7 summarise the results of extracting AX from wheat bran.

Table 5-1 AX extraction from WB methodologies and yields

Sample	Procedure	Time (Hour)	Yield %
WB1	ALKOXI	4	33.17
WB2	XYL51 + FER356	2	4.64
CON1	Buffer	2	3.91
WB3	XYL51 + FER356 + CEL91	2	8.28
WB4	XYL51 + FER356 + CEL91	4	5.63
CON2	Buffer	4	7.8
WB5	XYL51 + FER356 + CEL91	6	6.65
CON3	Buffer	6	10.53
WB6	XYL301+ FER356 + CEL91	4	9.36
WB7	XYL62 + FER356 + CEL91	4	8.93
WB8	CEL91 + ALKOXI	24 + 4	40.12
WB9	CEL91 + ALKOXI	4 + 2	33.5
WB10	ALK ONLY	4	17.6
WB11	CEL91 + ALK ONLY	24 + 4	17.42
WB12	Washing + ALKOXI	4	31.54
WB13	CEL91 + ALKOXI + PRT + AML	24 + 4 + 6	28.2
WB14	Milling + ALKOXI	4	39.79
WB15	Autoclave + ALKOXI	4	32.6

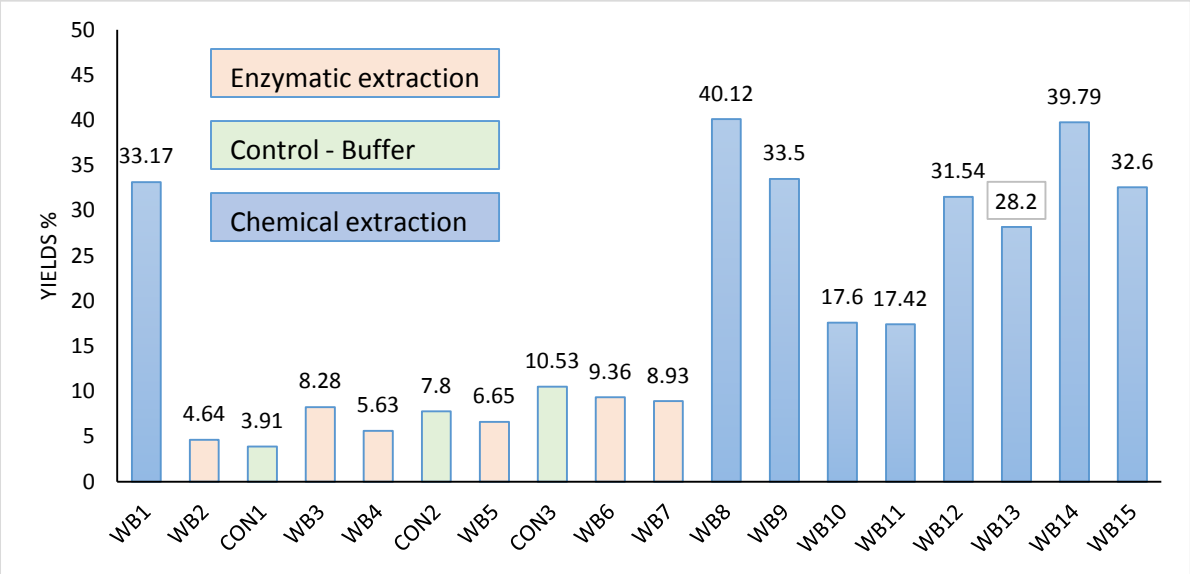

Figure 5-7 AX extraction yields from WB

5.3. Sugarcane Bagasse AX Extracts

5.3.1. SCB AX Extract Physical state and appearance:

The colour of SCB AX-rich extracts, just like WB extracts, varied according to the extraction method, however the extracts were much darker than WB extracts and had a brownish colour as Figure 5-8 illustrates.

Figure 5-8 WB-AX and SCB-AX Crude extracts

All the extracts were odourless and had a non-crystalline appearance. The physical appearance of the extracts could be subject to the content of impurities, especially lignin.

5.3.2. SCB AX Extracts Yields

The extraction yields were calculated with respect to the methods detailed in Section 5.2.2.

5.3.2.1. Enzymatic and Alkaline Oxidative Extraction

Figure 5-9 compares the alkaline oxidative extraction (**SCB1)** and the enzymatic extraction (**SCB2**, **SCB3**). The results show that the chemical method gave much higher yield.

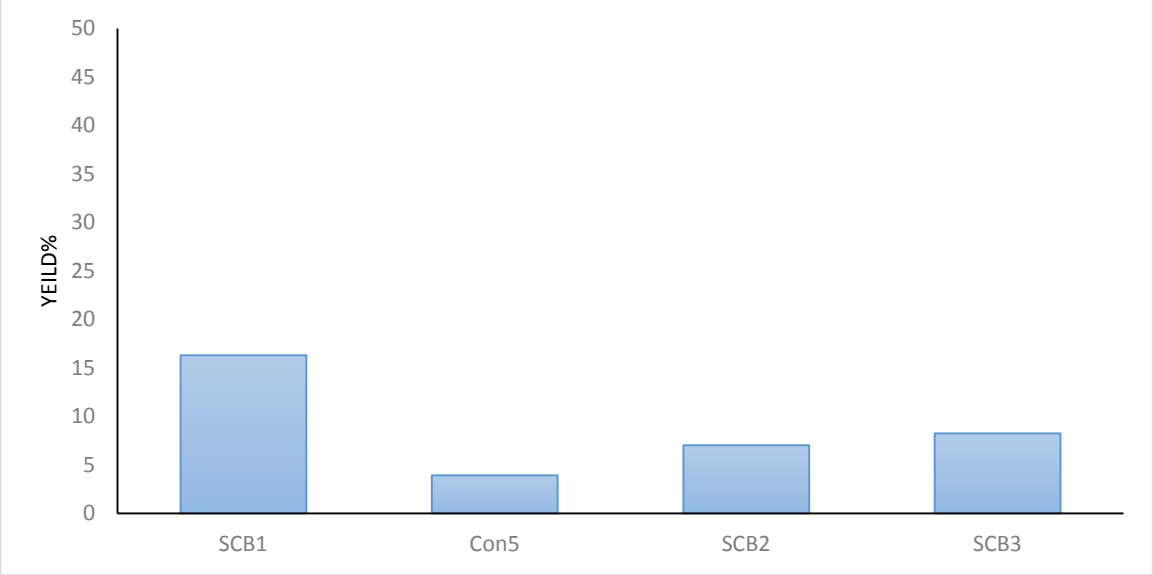

Figure 5-9 Extraction yields of SCB-AX in ALKOXI and enzymatic methods

5.3.2.2. Cellulase pre-treatment

Figure 5-10 shows cellulase treatment for 24 hours (**SCB4**) yielded 21.77% and treatment for 4 hours followed by 2 hours' chemical extraction (**SCB5**) yielded 23.8%.

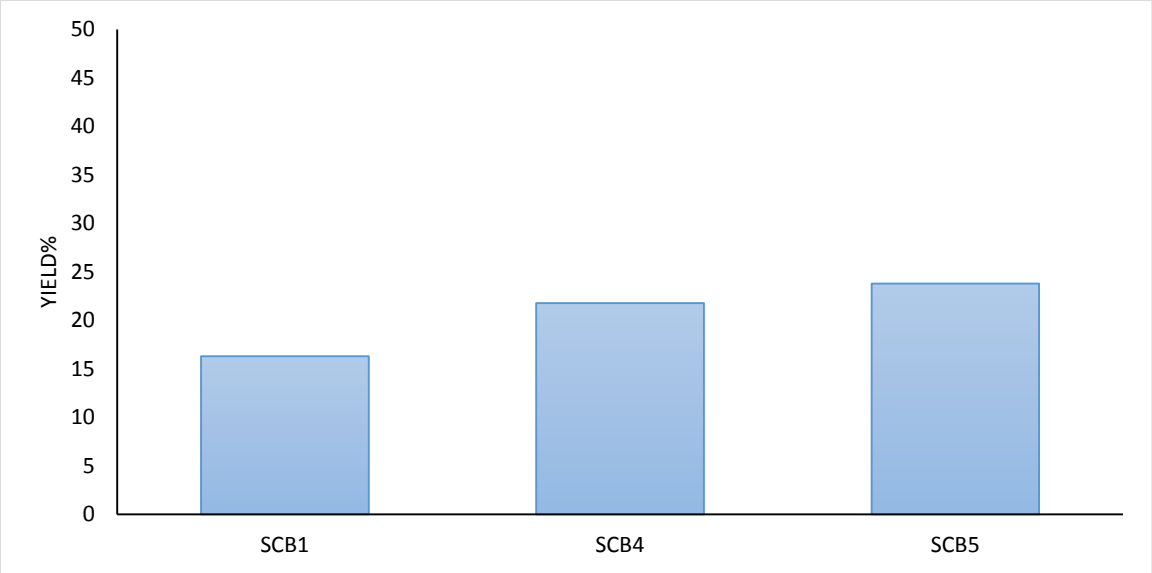

Figure 5-10 Cellulase pre-treatment prior to ALKOXI extraction

5.3.2.3. Alkaline extraction

Extracting AX from SCB with alkaline solution only yielded 13.8% AX from **SCB6**, whereas, the cellulase pre-treatment (**SCB7**) notably increased the yield to 18.7%. Figure 5-11 compares the alkaline method to the alkaline oxidative method (with/without pre-treatment).

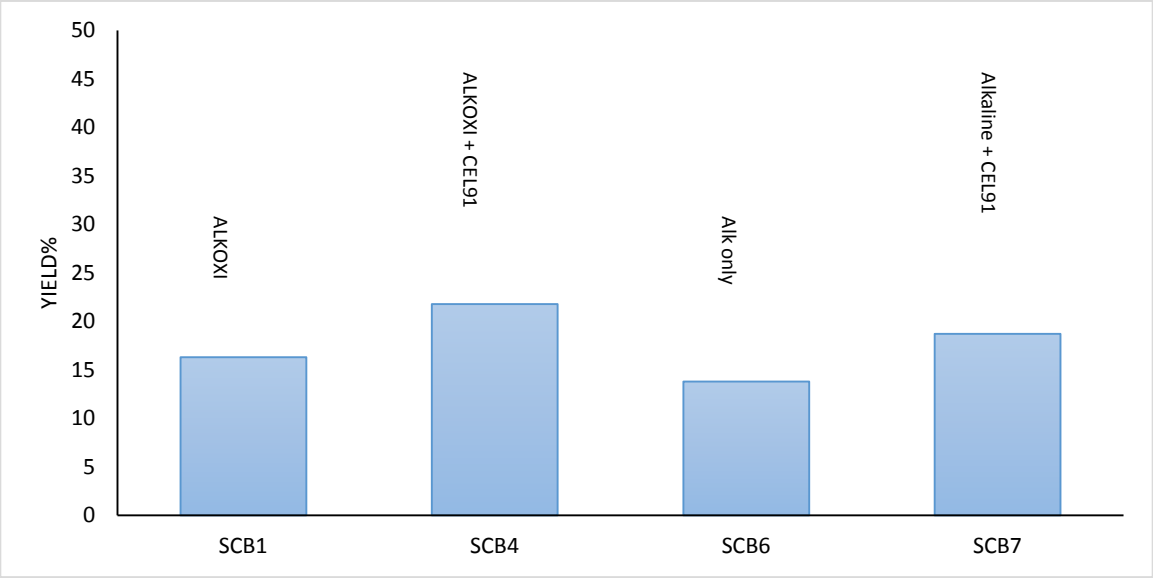

Figure 5-11 ALKOXI and alkaline extraction with an without cellulase pre-treatment

5.3.2.4. Autoclaving and milling

Figure 5-12 demonstrates that treating the bagasse with autoclave did not offer any improvement to the extraction yield as the yields did not change after this treatment (**SCB8**). Milling the bagasse **SCB9** increased the extraction yields to some extent.

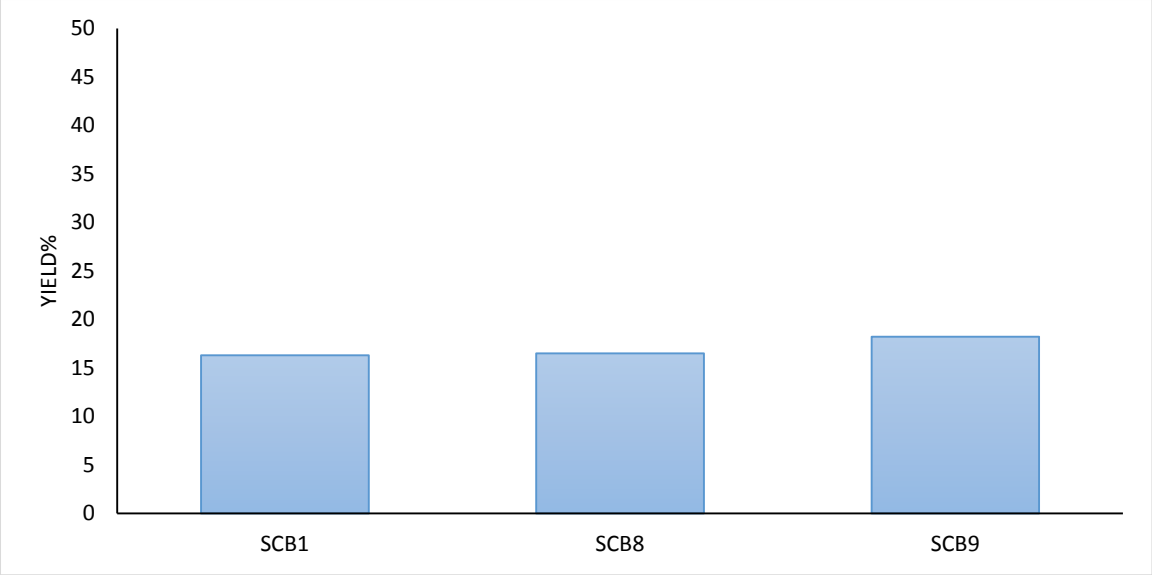

Figure 5-12 SCB-AX with Autoclave and milling pre-treatment

To sum up, enzymatic extraction did not result in acceptable yields (the yields ranged between 8.23 and 7.02%). Chemical extraction seems a more effective way as the yields reached 23.8% with cellulase pre-treatment. Figure 5-13 and Table 5-2 summarise the extraction of sugar cane bagasse arabinoxylans.

Table 5-2 AX extraction from SCB methodologies and yields

Sample	Procedure	Time (Hour)	Yield %
SCB1	ALKOXI	4	16.3
CON5	Buffer	2	6.2
SCB2	XYL301+ FER356 + CEL91	4	7.03
SCB3	XYL62 + FER356 + CEL91	4	8.23
SCB4	CEL91 + ALKOXI	24 + 4	21.77
SCB5	CEL91 + ALKOXI	4 + 2	23.8
SCB6	ALK ONLY	4	13.8
SCB7	CEL91 + ALK ONLY	24 + 4	18.7
SCB8	Autoclave + ALKOXI	4	16.5
SCB9	Milling + ALKOXI	4	18.2

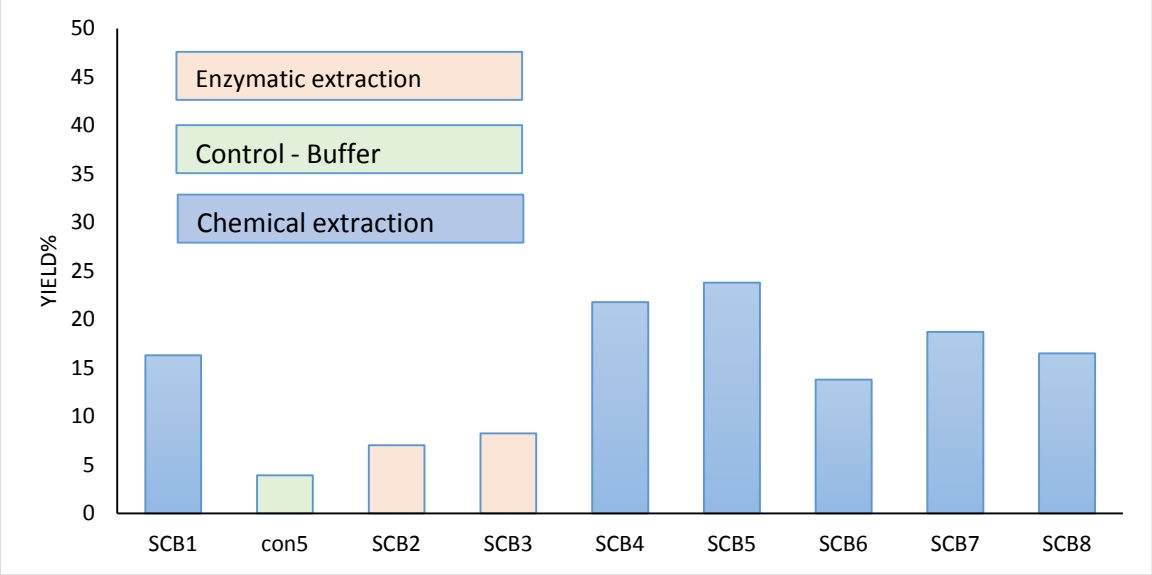

Figure 5-13 AX extraction yields from SCB

5.4. Protein Content Determination

Table 5-3 presents the titres obtained for each sample, the calculated average titre, the % nitrogen, and the % protein content.

Table 5-3 AX-rich extracts total protein determination

Sample	Titre 1	Titre 2	Titre 3	Average Titre	Average Blank	N %	Protein %
Bran	23.9	23.7	24.4	24	1.4	3.16	**18**
WB1	19.2	19.1	19.2	19.17	1.4	2.49	**14.2**
WB6	17.7	17.6	17.4	17.57	1.4	2.26	**12.9**
WB8	18.9	18.8	19.1	18.9	1.4	2.45	**14**
WB9	19	19.3	19.2	19.17	1.4	2.49	**14.2**
WB10	17.1	17.2	17.4	17.2	1.4	2.22	**12.6**
WB11	17.5	17.3	17.1	17.3	1.4	2.23	**12.7**
WB12	19	19	19.5	19.17	1.4	2.49	**14.2**
WB13	11.8	12	11.3	11.7	1.4	1.5	**8.2**
WB14	19.3	19.1	19	19.13	1.4	2.48	**14.2**
WB15	18.3	18.1	18.5	18.3	1.4	2.37	**13.5**
Bagasse	3.1	3	2.7	2.9	1.4	0.21	**1.2**
SCB1	2.4	2.3	2	2.23	1.4	0.12	**0.7**
SCB4	2.2	2.1	2.2	2.17	1.4	0.11	**0.6**
SCB6	2.5	2.2	2.4	2.37	1.4	0.14	**0.8**
SCB7	2.1	2.4	2	2.17	1.4	0.11	**0.6**
SCB8	2.3	2.3	2.1	2.23	1.4	0.12	**0.7**
SCB9	2.3	2.1	2.2	2.2	1.4	0.11	**0.6**

5.4.1. Wheat Bran AX protein content

The protein content in the raw bran was 18%, the protein content in AX crude extracts ranged between 12.6 and 14.2%. The protease treatment reduced the protein content from 14.2 to 8.2%. Furthermore, washing the bran (WB12) had no effect on the protein content in the extract; this indicates that stronger protein removal approaches are required.

Figure 5-14 presents the protein content in chemically extracted AX from wheat bran.

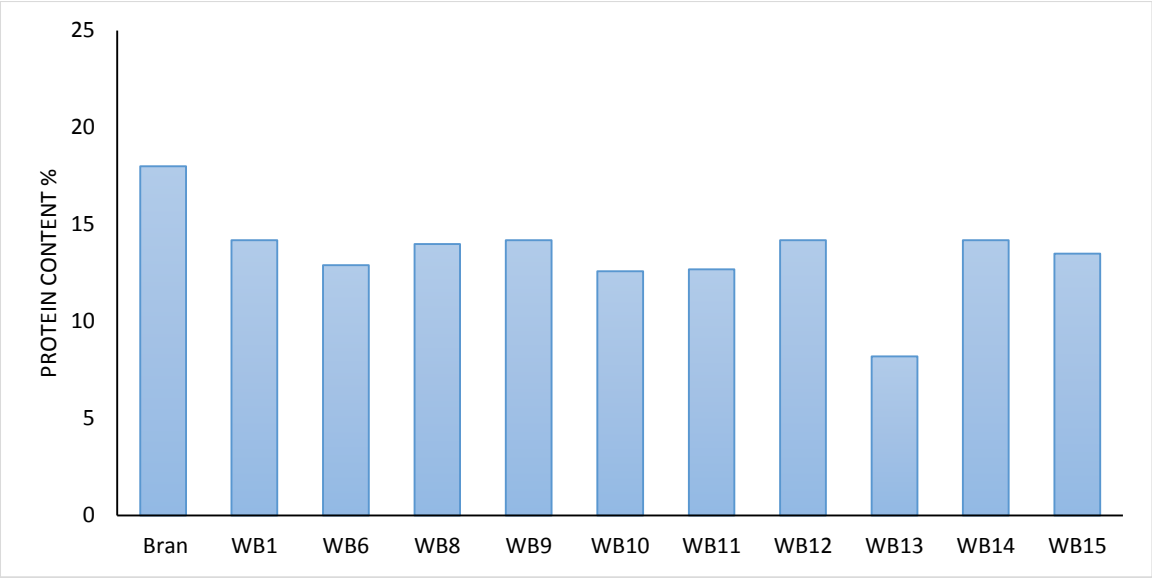

Figure 5-14 WB-AX extracts Protein Content

5.4.2. Sugarcane Bagasse AX protein content

The raw bagasse protein content was 1.2% and the protein content in the crude extracts ranged between 0.6 and 0.8 %. Figure 5-15 presents the protein content in chemically extracted AX from sugarcane bagasse.

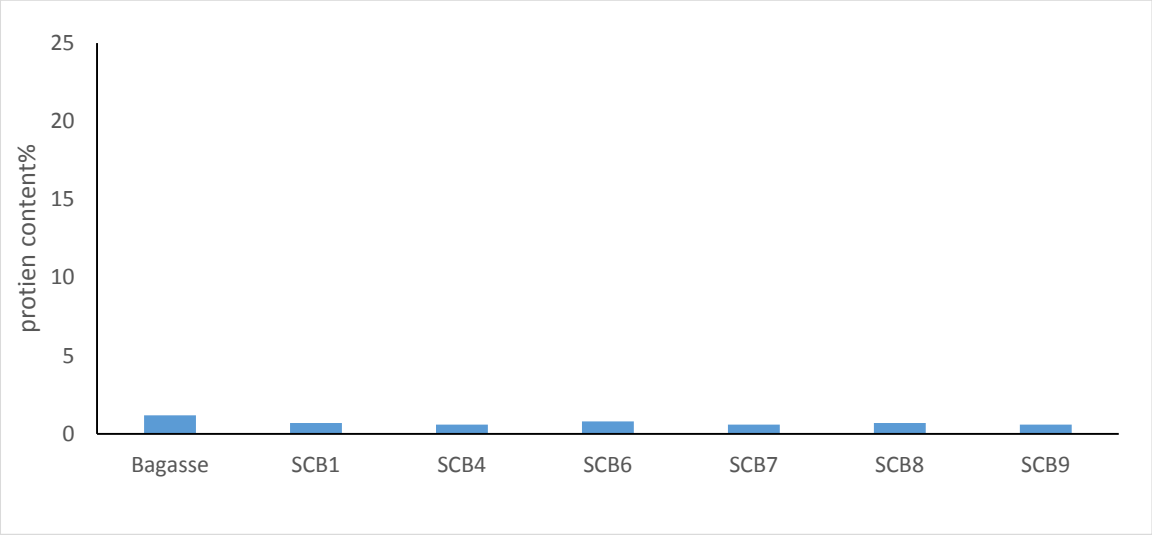

Figure 5-15 SCB-AX extracts protein Content

5.5. Monomer Analysis

Both samples and standards were analysed via HPEAC-PAD as previously described in the materials and methods section (4.3). Calibration curves were constructed for the three consecutive standards. Thereafter, the samples sugar contents were determined.

5.5.1. Calibration Curves Construction

The mixed standard solutions were injected carefully into the instrument, then, the area under the curve (AUC) for each peak was calculated. The peaks were identified by injecting three different solutions each contains a single sugar. Then, a comparison between sugar retention times (RT) was performed. Figure 5-16 illustrates the standards mixture chromatogram.

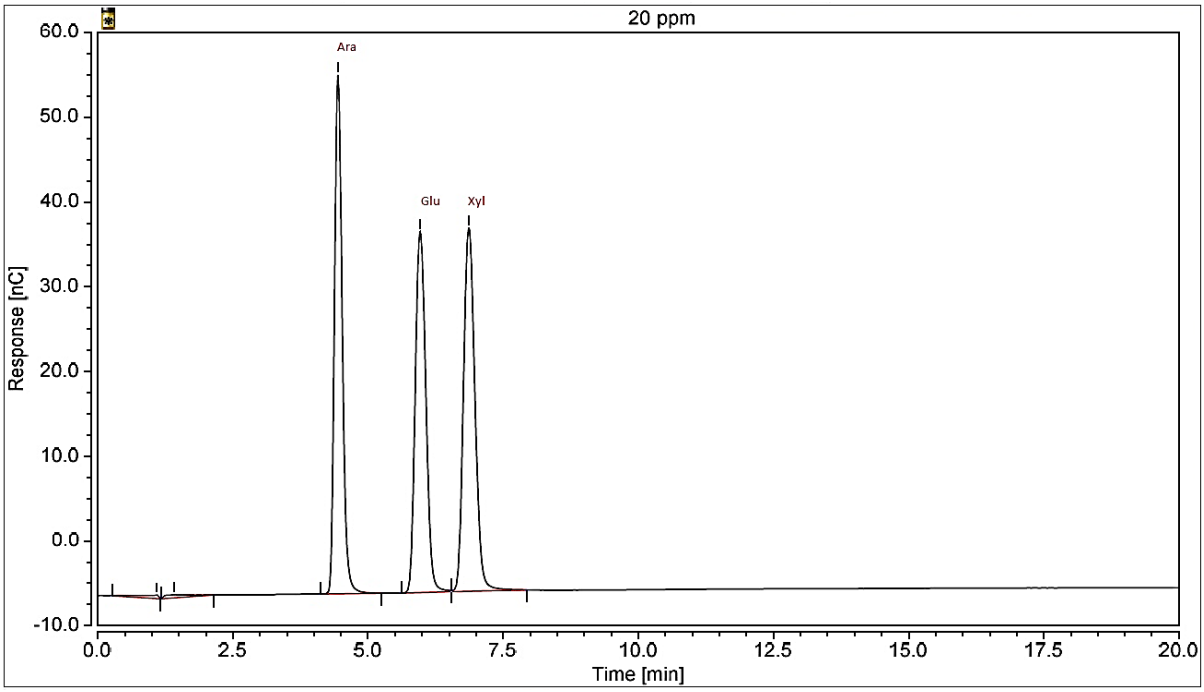

Figure 5-16 Standards separation chromatogram

Retention times were recorded as a robust tool to provide a valid comparative background between the injected analysed samples. Table 5-4 demonstrates the calculated retention times corresponded to the three saccharides compounds: arabinose, glucose and xylose.

Table 5-4 Retention times of the standard monosacchrides

Sugar	RT (min)
Arabinose	4.38 ± 0.06
Glucose	5.82 ± 0.07
Xylose	6.71 ± 0.03

To construct calibration curves, AUC values were calculated via the chromatographic software Chromeleon®. Tables 5-5 displays the AUC of the standards peaks.

Table 5-5 AUC of the standards peaks

Standard Concentration (ppm)		**AUC**
Arabinose (ppm)	**20**	10.24 ± 0.12
	40	18.50 ± 0.25
	60	24.53 ± 0.24
Glucose (ppm)	**20**	9.785 ± 0.08
	40	17.01 ± 0.27
	60	22.35 ± 0.20
Glucose (ppm)	**20**	10.61 ± 0.12
	40	18.93 ± 0.28
	60	25.28± 0.12

Calibration curves were plotted in order to provide a representation about the relationship between the AUC belongs to each peak against the standard concentration. Figures 5-17, 5-18 and 5-19 show the resultant curves.

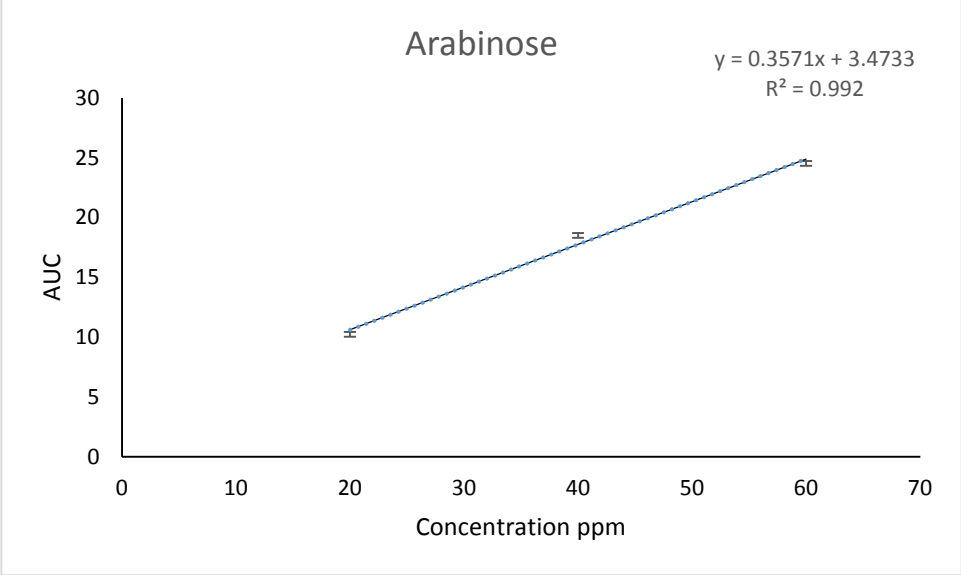

Figure 5-17 Arabinose calibration curve

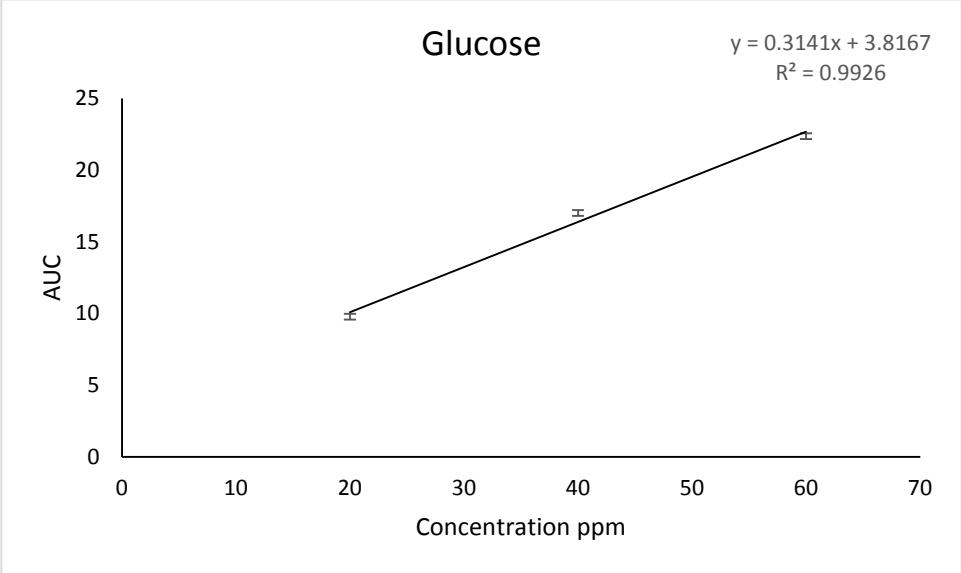

Figure 5-18 Glucose calibration curve

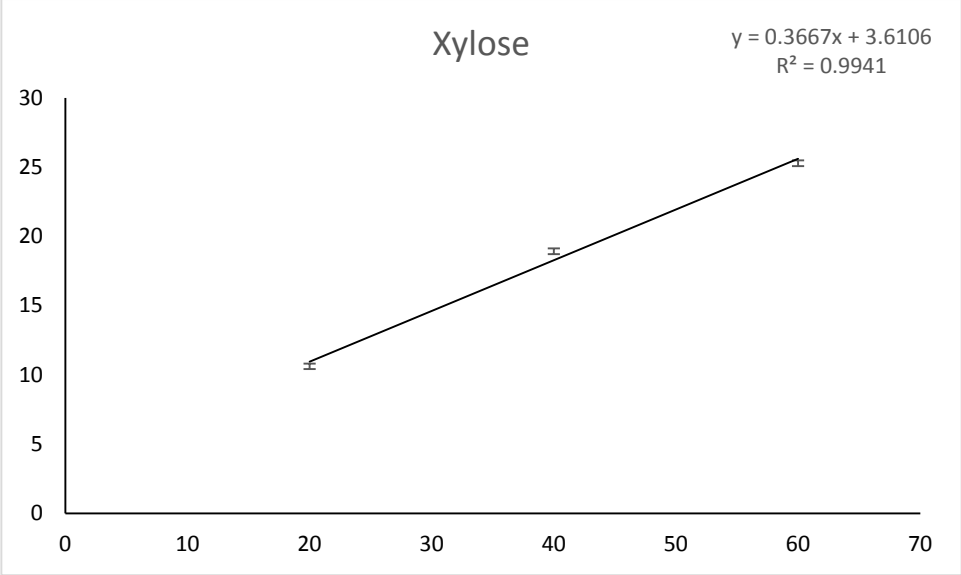

Figure 5-19 Xylose calibration curve

5.5.2. **Extract's Monosaccharides Content**

After hydrolysing each extract, the yielded hydrolysed solutions were introduced to the HPEAC instrumentation. Comparisons were implemented between the resultant peaks and the standards aiming for identification and quantification purposes. Figures 5-20 and 5-21 illustrate the chromatographic diagrams of wheat bran and sugarcane bagasse sugar content analysis.

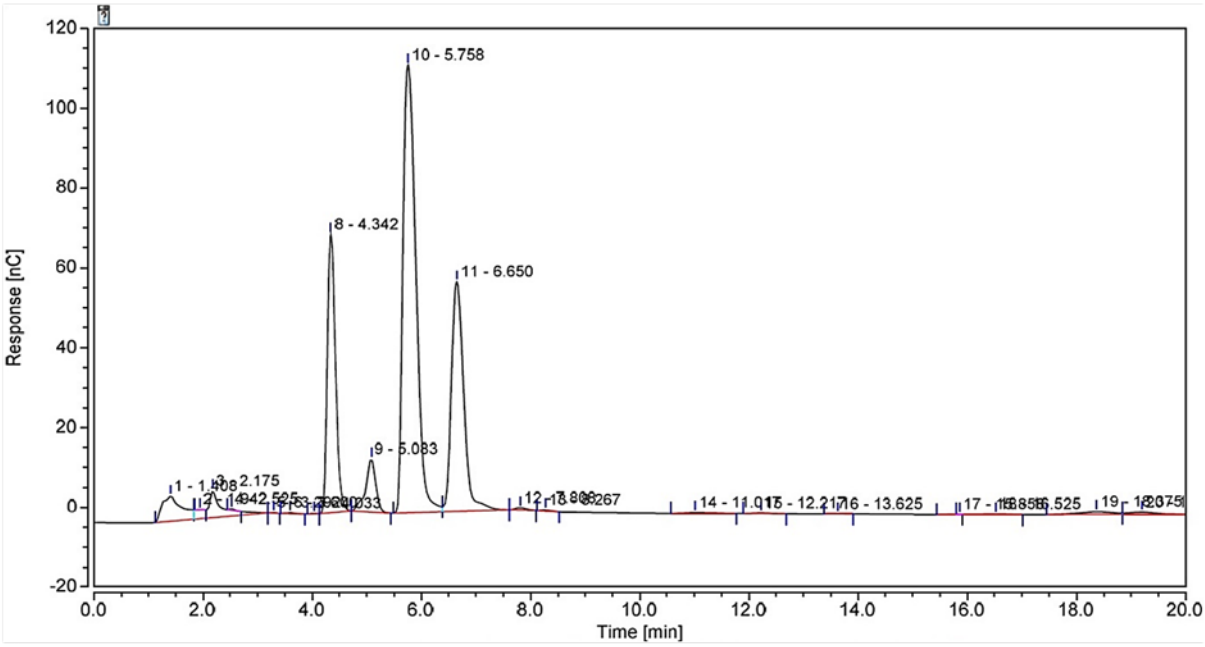

Figure 5-20 Chromatographic diagrams of wheat bran saccharides

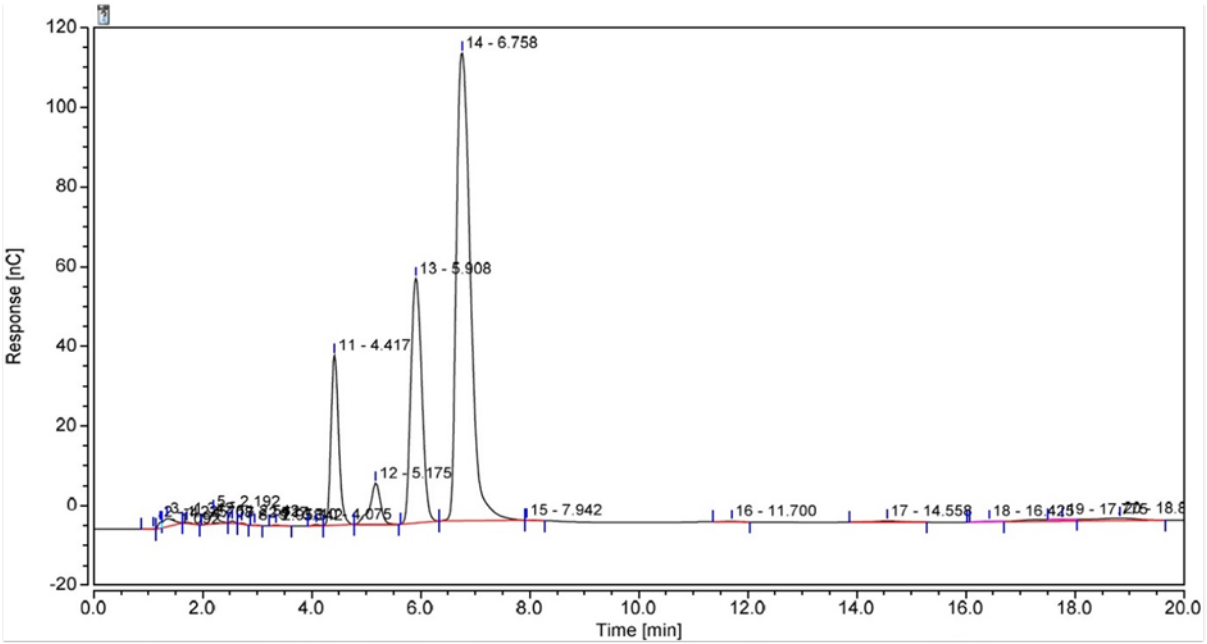

Figure 5-21 Chromatographic diagrams of sugarcane bagasse saccharides

The calculated concentrations of sugars in the extracts were determined according to the plotted calibration curves. Table 5-6 and Figure 5-22 summarise the extracts' sugar contents.

Table 5-6 The percentage of monosaccharides in the studied samples

%	WB	WB1	WB8	WB13	SBC	SBC1	SBC4	SBC5	SBC7
Arabinose	2.6	3.7	4.1	6.45	0.9	2.23	1.14	0.57	2.56
Glucose	8.44	6.82	8.44	8.69	3.3	2.51	1.72	0.96	3.4
Xylose	3.1	3.27	3.36	6.15	8.51	9.5	6.86	4.69	2.9

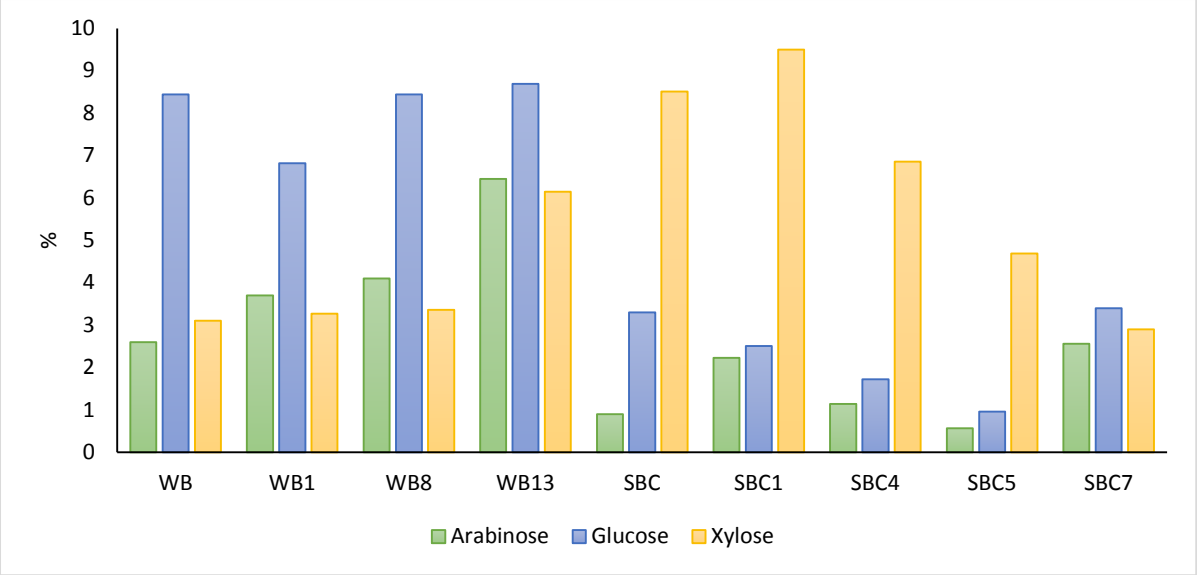

Figure 5-22 The percentage of monosaccharides in the studied samples

Arabinoxylans content in the yielded extracts was calculated as [(arabinose + xylose) × 0.88]. The (0.88) factor accounts for the addition of a water molecule during the hydrolysis. Table 5-7 and figure 5-23 show the AX contents in the studied samples.

Table 5-7 AX content in the studied samples

	WB	WB1	WB8	WB13	SBC	SBC1	SBC4	SBC5	SBC7
AX %	5.02	6.13	6.56	11.1	8.28	10.32	7.04	4.63	4.81

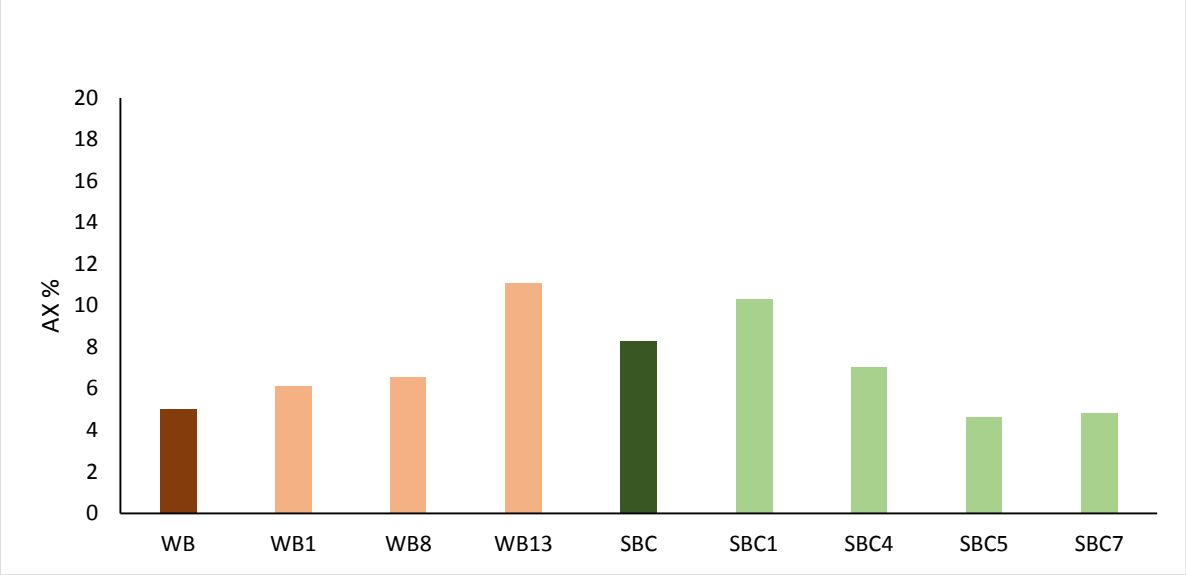

Figure 5-23 AX content in the studied samples

The branching ratio was calculated as the arabinose to xylose ratio, table 5-8 and figure 5-24 illustrate the A/X for the analysed samples.

Table 5-8 A/X ratio in the analysed samples

	WB	WB1	WB8	WB13	SBC	SBC1	SBC4	SBC5	SBC7
A/X	0.84	1.13	1.22	1.05	0.11	0.23	0.17	0.12	0.88

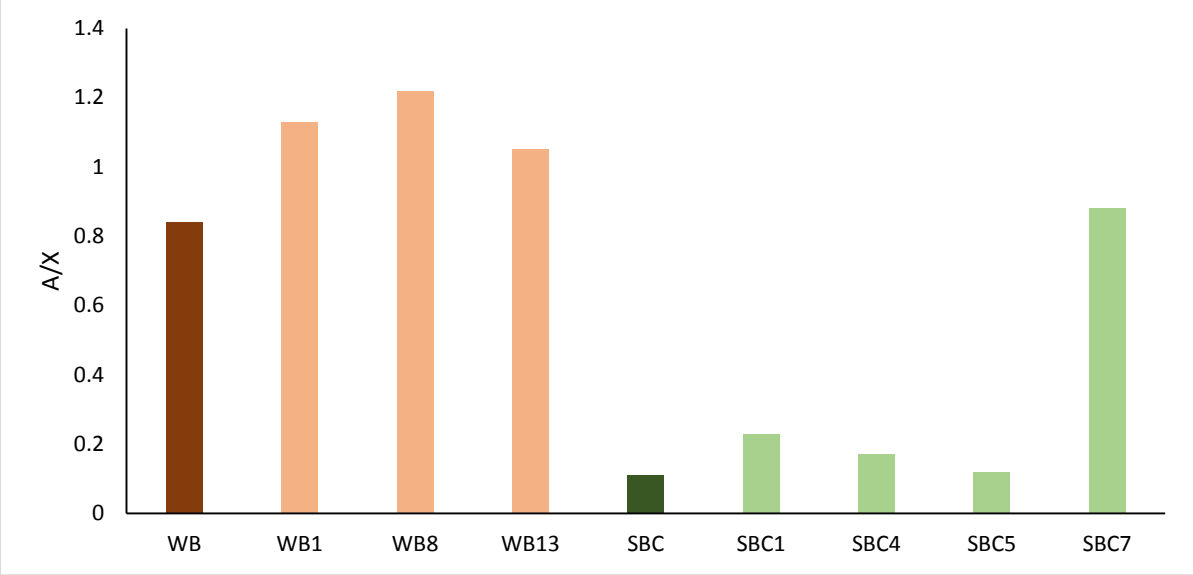

Figure 5-24 A/X ratio in the analysed samples

5.6. Discussion

5.6.1. Extraction yields

It is important to clarify that the calculated yields are for crude extracts. These crude extracts contain a significant amount of impurities such as starch, protein and lignin; the protein content analysis showed that WB AX-rich extracts contained about 14% protein. It has not yet been possible to undertake monosaccharide analysis of these samples; this forms part of the future work plan. For the moment, the crude yields give an indication of the extraction effectiveness.

5.6.2. Enzymatic AX extraction

The xylanase enzymes used in this work were not able to release AX from the cell wall matrix significantly compared to chemical routes. There was an insignificant variation in the effectiveness of the three xylanases, but in general, the yields were much lower than from the chemical method. The use of ultrafiltration might be a key factor affecting the yields of enzymatic extraction, as xylanases tend to produce small molecular weight AX. This is supported by the lower yield gained by increasing the xylanase treatment time. Measurement of AX in the filtrate is required to confirm this hypothesis.

5.6.3. Chemical extraction

Alkaline oxidative extraction method was the most effective of the methods investigated. Foaming is the main disadvantage of this method, requiring the use of anti-foaming agent. Alkaline extraction with sodium hydroxide seemed to be less effective in the case of wheat bran, but for sugarcane bagasse, it might be effective if coupled with an effective pre-treatment method.

5.6.4. Cellulase pre-treatment

Pre-treating wheat bran and sugar cane bagasse with cellulase offers a promising pre-treatment method for extracting arabinoxylans; it distinctly helped to break open the tenacious cell wall matrix, allowing more AX to be solubilised. However, further analysis is required to identify the composition of the extracts and to determine the increased component after the cellulase treatment.

5.6.5. Autoclaving pre-treatment

Autoclaving the bran and the bagasse showed no improvement in the extraction yields. Apparently, the temperature and pressure in the autoclave was not adequate to loosen the coherent cell wall structure.

5.6.6. Bran Washing

Washing the bran with water did not add any obvious improvement to the extraction of AX, the yield decreased 2 g after washing the bran. This decrease might be a result of partial removal of the starch associated with the bran and a lower starch content in the AX-rich extract. Washing the bran had no effect on protein content.

5.6.7. Protease and Amylase purification

Treating the extracts with protease and amylase enzymes, prior to the ultrafiltration step, in order to reduce protein and starch content significantly reduced the weight of the extract. The total protein analysis clarifies the effectiveness of protease treatment as the protein content decreased from 24.2 to 8.2%. The results suggest that more purification can be achieved by increasing the protease dose or treatment time. Monosaccharide analysis should confirm that the reduction in protein increased the purity of the extracts.

Following amylase treatment, the decrease in the extraction yield from 33.2 to 29.2% indicates that alpha amylase usage reduced the starch content in the extracts.

5.6.8. Monomers Analysis

The chromatographic separation conditions conducted throughout this research were capable of producing a good resolution between the monosaccharides peaks. The use of calibration curves constructed based on the obtained sugars standards AUC is considered as a valid representative tool intended for illustrating the relationship between the calculated concentrations and their corresponding measured responses.

Nevertheless, notwithstanding the successful separation with high resolution between the analysed compounds peaks, unexpectedly, the starting material produced results that were significantly lower than anticipated. The calculated total content of AX in the wheat bran was

5.02% db, which is much lower than reported AX contents from wheat bran sources of typically around 25-28% db (Hollmann & Lindhauer, 2005). Similarly for the sugarcane bagasse, the calculated content of AX was 8.28% db, much lower than reported AX contents of up to 27% of the dry weight.

The rationale behind conducting the hydrolysis phase is to induce a full liberation of the monosaccharides moieties to be readily ionised and detected. Therefore, it seems the hydrolytic procedure was inadequate to achieve full chain breakage between AX chemical bonds, hence, low monosaccharides yields were released and detected, leading to the low contents of AX calculated.

However, the results advocate and support the efficiency of the enzymatic purification, proved by the high AX percentage quantified in the purified sample (WB13, 11.1%) among wheat bran extracts.

The bagasse samples showed, as expected, a noticeably lower A/X ratio in comparison with the wheat bran AX. The relatively elevated A/X ratio in wheat bran samples suggests that the hydrolysis of arabinose branches is more achievable than the hydrolysis of the main xylose backbone.

Overall, the results suggest the effectiveness of the alkaline oxidative approach upon yielding the highest purity of AX among the rest of oxidative strategies.

5.6.9. **Ultrafiltration**

The use of ultrafiltration in the AX extraction gives the disadvantage of discarding the lower MW water extractable arabinoxylans. On the other hand, ultrafiltration offers the extraction process two major benefits: firstly, reducing the amount of ethanol used for the precipitation of AX up to one fifth the original volume; and secondly, with enzyme purification, ultrafiltration can be beneficial in isolating the broken starch and proteins. Sacrificing WEAX in order to achieve lower extraction cost and higher purity may be a sensible approach.

5.7. Summary

Based on the calculated extraction yields and the total protein contents obtained from the preliminary results of the AX extraction investigations, the alkaline oxidative method generated the greater yields (33.7% for WB and 16.3% for SCB). In contrast, the enzymatic extraction yields were the lowest, ranging between 4.6-9.3% for WB and 7.0-8.2% for SCB.

In regards to the pretreatment methods, cellulase pretreatment conferred higher yields, reaching up to 40.12% and 23.8% for WB and SCB, respectively. Autoclaving did not offer any enhancement to the extraction process. The total protein content analysis results demonstrated that SCB extracts had a relatively low protein content (0.6-0.8%), while WB extracts contained a greater amount of protein (12.7-14.2%). Enzymatic purification with amylase and protease appeared to be effective in reducing the protein and starch contents in the extracts.

The work presented in this chapter has given insights into approaches for extracting and characterising arabinoxylans and has pointed towards further studies. Chapter 6 summarises the conclusions from the work so far and recommends future investigations.

6. Conclusions and Recommendations

6.1. Progress made in the current work

In the context of exploring strategies for enhancing the economic income from biorefineries, the literature advocates the integrated biorefinery approach as an effective basis for exploiting interaction opportunities arising from the co-production of several products. Biorefineries are currently derived principally from ethanol production, and ethanol can serve as a precipitating agent for arabinoxylans, which makes arabinoxylans potentially a natural co-product of bioethanol production, and raising the opportunity for bringing a new class of functional food ingredients to the market.

Arising from this literature review, the primary objective of the current work was identified earlier as screening a range of pre-treatments approaches and extraction conditions in the purpose of obtaining AX from two biomass sources, wheat bran and sugarcane bagasse. Additional objectives were to investigate mechanical and enzymatic pretreatments and along with post-extraction enzymatic purification of AX extracts. The purpose of these preliminary studies was to provide information relevant to future plans to scale up AX extraction to pilot scale, in order to produce kg quantities of AX for functionality studies in food and other systems.

The current study has addressed the prime objective in order to provide a background for understanding the arabinoxylan extraction phases and purification methods and to establish methodological characterisation and additional requirements needed for adequate characterisation. The investigation of AX extraction techniques included enzymatic approaches (*i.e.* xylanases and feruloyl esterase) and chemical approaches (*i.e.* alkaline and alkaline oxidative). Several pretreatment methods, autoclaving, milling and cellulase incubation, were investigated.

The results demonstrated that the alkaline oxidative extraction method appears to be the most efficient technique for the production of AX. However, cellulase pretreatment may offer an effective option for enhancing the extraction process in terms of yield. Enzymatic purification utilising amylase and protease, coupled with a functional ultrafiltration system, can elevate the opportunities of producing more pure AX extracts in a cost effective manner.

The chromatographic analysis resulted data were not adequately reliable to complement and support these conclusive points; in future, efforts need to be dedicated towards optimising the hydrolysis process of the studied biomasses.

6.2. Recommendations for future work

Investigations of the arabinoxylan extraction from biomasses within biorefineries offers a valuable opportunity to produce commercial products with higher value and to increase the profitability of biorefineries.

The current project focused on the possibility of improving AX extraction process utilising enzymes and other pretreatment techniques. The investigation presented in this work suggests the viability of implementing enzymatic pretreatment process for enhancing the outcomes of the extraction process.
Aiming for optimisation, further investigations exploiting different enzymatic strategies for pretreatment process, such as feruloyl esterase and laccase, need to be conducted and screened. Moreover, exploring the utilisation of gluco-amylase alongside alpha amylase and protease within the purification approach is required, as current extracts appear to contain substantial residual starch.

Overall, a greater understanding of the characteristics of arabinoxylan and the effect of the extraction process on these characteristics is necessary. Routine production of kg quantities of AX from different feedstocks and under different processing conditions is an essential next step for taking arabinoxylan studies further towards commercialisation.

Arabinoxylans are a promising food ingredient that can be co-produced within biorefineries from the under-valorised by-products. This approach has the potential to increase the commercial viability of biorefineries and thus to enhance the benefits of bioethanol production, while bringing additional benefits in the form of new functional food ingredients.

References

Alberici, S., & Toop, G. (2013). UK biofuel industry overview, 2. Retrieved from https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/266090/ecofys-uk-biofuel-industry-overview-v1.5.pdf

Albersheim, P., Nevins, D. J., English, P. D., & Karr, A. (1967). A method for the analysis of sugars in plant cell-wall polysaccharides by gas-liquid chromatography. *Carbohydrate Research*, *5*(3), 340–345.

Alexandratos, N. (2006). The Mediterranean diet in a world context. *Public Health Nutrition*, *9*(1a), 111–117. http://doi.org/10.1079/PHN2005932

Almeida, J. R. M., Modig, T., Petersson, A., Hähn-Hägerdal, B., Lidén, G., & Gorwa-Grauslund, M. F. (2007). Increased tolerance and conversion of inhibitors in lignocellulosic hydrolysates by Saccharomyces cerevisiae. *Journal of Chemical Technology and Biotechnology*, *82*(4), 340–349.

Anderson, J. W., & Hanna, T. J. (1999). Impact of nondigestible carbohydrates on serum lipoproteins and risk for cardiovascular disease. *The Journal of Nutrition*, *129*(7), 1457S–1466s.

Andrewartha, K. A., Phillips, D. R., & Stone, B. A. (1979). Solution properties of wheat-flour arabinoxylans and enzymically modified arabinoxylans. *Carbohydrate Research*, *77*(1), 191–204. http://doi.org/10.1016/S0008-6215(00)83805-7

Araki, T., & Kitamikado, M. (1982). Purification and characterization of a novel exo-β-mannanase from Aeromonas sp. F-25. *Journal of Biochemistry*, *91*(4), 1181–1186.

Asche, F., Gjølberg, O., & Völker, T. (2003). Price relationships in the petroleum market: An analysis of crude oil and refined product prices. *Energy Economics*, *25*(3), 289–301. http://doi.org/10.1016/S0140-9883(02)00110-X

Avellar, B. K., & Glasser, W. G. (1998). Steam-assisted biomass fractionation. I. Process considerations and economic evaluation. *Biomass and Bioenergy*, *14*(3), 205–218. http://doi.org/10.1016/S0961-9534(97)10043-5

Ayala-Soto, F. E., Serna-Saldívar, S. O., & Welti-Chanes, J. (2016). Effect of processing time, temperature and alkali concentration on yield extraction, structure and gelling properties of corn fiber arabinoxylans. *Food Hydrocolloids*, *60*, 21–28. http://doi.org/10.1016/j.foodhyd.2016.03.014

Beaugrand, J., Chambat, G., Wong, V. W. K., Goubet, F., Rémond, C., Paës, G., … Chabbert, B. (2004). Impact and efficiency of GH10 and GH11 thermostable endoxylanases on wheat bran and alkali-extractable arabinoxylans. *Carbohydrate Research*, *339*(15), 2529–40. http://doi.org/10.1016/j.carres.2004.08.012

Beddington, J. R. (2009). Food, Energy, Water and the Climate: a Perfect Storm of Global Events. In *Lecture to Sustainable Development UK 09 Conference* (Vol. 19, pp. 1–9). Retrieved from http://www.bis.gov.uk/assets/goscience/docs/p/perfect-storm-paper.pdf

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*(3), 235–245. http://doi.org/10.1006/jcrs.1996.0024

Bian, J., Peng, F., Peng, P., Xu, F., & Sun, R. C. (2010). Isolation and fractionation of hemicelluloses by graded ethanol precipitation from Caragana korshinskii. *Carbohydrate Research*, *345*(6), 802–809. http://doi.org/10.1016/j.carres.2010.01.014

Biely, P., Vršanská, M., Tenkanen, M., & Kluepfel, D. (1997). Endo-β-1, 4-xylanase families: differences in catalytic properties. *Journal of Biotechnology*, *57*(1), 151–166.

Biliaderis, C. G., & Izydorczyk, M. S. (2006). *Functional food carbohydrates*. CRC Press.

Biliaderis, Izydorczyk, & Rattan. (1995). Effect of arabinoxylans on bread-making quality of wheat flours. *Food Chemistry*, *53*(2), 165–171.

Blake, J. D., & Richards, G. N. (1970). A critical re-examination of problems inherent in compositional analysis of hemicelluloses by gas-liquid chromatography. *Carbohydrate Research*, *14*(3), 375–387. http://doi.org/10.1016/S0008-6215(00)80011-7

Brillouet, J.-M., & Joseleau, J.-P. (1987). Investigation of the structure of a heteroxylan from the outer pericarp (beeswing bran) of wheat kernel. *Carbohydrate Research*, *159*(1), 109–126. http://doi.org/10.1016/S0008-6215(00)90009-0

Cara, C., Ruiz, E., Ballesteros, I., Negro, M. J., & Castro, E. (2006). Enhanced enzymatic hydrolysis of olive tree wood by steam explosion and alkaline peroxide delignification. *Process Biochemistry*, *41*(2), 423–429. http://doi.org/10.1016/j.procbio.2005.07.007

Chanliaud, E., Saulnier, L., & Thibault, J. F. (1995). Alkaline extraction and characterisation of heteroxylans from maize bran. *Journal of Cereal Science*, *21*(2), 195–203. http://doi.org/10.1016/0733-5210(95)90035-7

Cleemput, G., Hessing, M., van Oort, M., Deconynck, M., & Delcour, J. A. (1997). Purification and characterization of a [beta]-D-xylosidase and an endo-xylanase from wheat flour. *Plant Physiology*, *113*(2), 377–386.

Corradini, C., Bianchi, F., Matteuzzi, D., Amoretti, A., Rossi, M., & Zanoni, S. (2004). High-performance anion-exchange chromatography coupled with pulsed amperometric detection and capillary zone electrophoresis with indirect ultra violet detection as powerful tools to evaluate prebiotic properties of fructooligosaccharides and inulin. *Journal of Chromatography A*, *1054*(1), 165–173. http://doi.org/10.1016/j.chroma.2004.07.109

Courtin, C. M. M., & Delcour, J. A. A. (2001). Relative activity of endoxylanases towards water-extractable and water-unextractable arabinoxylan. *Journal of Cereal Science*, *33*(3), 301–312. http://doi.org/10.1006/jcrs.2000.0354

Courtin, C. M. M., & Delcour, J. A. Al. (2002). Arabinoxylans and endoxylanases in wheat flour bread-making. *Journal of Cereal Science*, *35*(3), 225–243. http://doi.org/10.1006/jcrs.2001.0433

Cyran, M., Courtin, C. M., & Delcour, J. A. (2004). Heterogeneity in the Fine Structure of Alkali-Extractable Arabinoxylans Isolated from Two Rye Flours with High and Low Breadmaking Quality and Their Coexistence with Other Cell Wall Components. *Journal of Agricultural and Food Chemistry*, *52*(9), 2671–2680. http://doi.org/10.1021/jf030550r

de Jong, E., Higson, A., Walsh, P., & Wellisch, M. (2012). Bio-based chemicals value added products from biorefineries. *IEA Bioenergy, Task42 Biorefinery*.

Dekker, R. F. H. (1985). Biodegradation of the hemicelluloses. *Biosynthesis and Biodegradation of Wood Components*, 505–533.

Dervilly-Pinel, G., Rimsten, L., Saulnier, L., Andersson, R., & Åman, P. (2001). Water-extractable Arabinoxylan from Pearled Flours of Wheat, Barley, Rye and Triticale. Evidence for the Presence of Ferulic Acid Dimers and their Involvement in Gel Formation. *Journal of Cereal Science*, *34*(2), 207–214. http://doi.org/10.1006/jcrs.2001.0392

Dervilly, G., Saulnier, L., Roger, P., & Thibault, J.-F. (2000). Isolation of Homogeneous Fractions from Wheat Water-Soluble Arabinoxylans. Influence of the Structure on Their Macromolecular Characteristics. *Journal of Agricultural and Food Chemistry*, *48*(2),

270–278. http://doi.org/10.1021/jf990222k
Döring, C., Jekle, M., & Becker, T. (2015). Technological and Analytical Methods for Arabinoxylan Quantification from Cereals. *Critical Reviews in Food Science and Nutrition*, *56*(6), 999–1011. http://doi.org/10.1080/10408398.2012.749207
Ebringerová, A., & Heinze, T. (2000). Xylan and xylan derivatives - Biopolymers with valuable properties, 1: Naturally occurring xylans structures, isolation procedures and properties. *Macromolecular Rapid Communications*, *21*(9), 542–556. http://doi.org/10.1002/1521-3927(20000601)21:9<542::aid-marc542>3.3.co;2-z
Ebringerova, A., & Hromadkova, Z. (1997). The effect of ultrasound on the structure and properties of the water-soluble corn hull heteroxylan. *Ultrasonics Sonochemistry*, *4*(4), 305–309. http://doi.org/10.1016/S1350-4177(97)00037-0
Ebringerova, A., & Hromadkova, Z. (2002). Effect of ultrasound on the extractibility of corn bran hemicelluloses. *Ultrasonics Sonochemistry*, *9*(4), 225–229. http://doi.org/10.1016/S1350-4177(01)00124-9
Erum, A., Bashir, S., Saghir, S., & Sarfraz, R. M. (2015). Arabinoxylan from Plantago ovate (Husk) a novel binder and superdisintegrant. *Dhaka University Journal of Pharmaceutical Sciences*, *13*(2), 133–141. http://doi.org/10.3329/dujps.v13i2.21891
Escarnot, E., Aguedo, M., & Paquot, M. (2011). Characterization of hemicellulosic fractions from spelt hull extracted by different methods. *Carbohydrate Polymers*, *85*(2), 419–428. http://doi.org/10.1016/j.carbpol.2011.03.005
Escarnot, E., Aguedo, M., & Paquot, M. (2012). Enzymatic hydrolysis of arabinoxylans from spelt bran and hull. *Journal of Cereal Science*, *55*(2), 243–253. http://doi.org/10.1016/j.jcs.2011.12.009
Faulds, C. B., & Williamson, G. (1995). Release of ferulic acid from wheat bran by a ferulic acid esterase (FAE-III) from Aspergillus niger. *Applied Microbiology and Biotechnology*, *43*(6), 1082–1087.
Faulds, C. B., Zanichelli, D., Crepin, V. F., Connerton, I. F., Juge, N., Bhat, M. K., & Waldron, K. W. (2003). Specificity of feruloyl esterases for water-extractable and water-unextractable feruloylated polysaccharides: influence of xylanase. *Journal of Cereal Science*, *38*(3), 281–288. http://doi.org/10.1016/S0733-5210(03)00029-8
Faulds, C., Sancho, A., & Bartolomé, B. (2002). Mono-and dimeric ferulic acid release from brewer's spent grain by fungal feruloyl esterases. *Applied Microbiology and Biotechnology*, *60*(4), 489–494.
Fengler, A. I., & Marquardt, R. R. (1988). Water soluble pentosans from rye: II. Effects on rate of dialysis and on the retention of nutrients by the chick. *Cereal Chemistry*, *65*(4), 298–302.
Figueroa-Espinoza, M. C., & Rouau, X. (1998). Oxidative Cross-Linking of Pentosans by a Fungal Laccase and Horseradish Peroxidase: Mechanism of Linkage Between Feruloylated Arabinoxylans 1. *Cereal Chemistry*, *75*(2), 259–265.
Fincher, G. B., & Stone, B. A. (1986). Cell walls and their components in cereal grain technology. *Advances in Cereal Science and Technology (USA)*.
Gerwig, G. J., Kamerling, J. P., & Vliegenthart, J. F. G. (1978). Determination of the d and l configuration of neutral monosaccharides by high-resolution capillary g.l.c. *Carbohydrate Research*, *62*(2), 349–357. http://doi.org/10.1016/S0008-6215(00)80881-2
Gilbert, H. J., & Hazlewood, G. P. (1993). Bacterial cellulases and xylanases. *Microbiology*, *139*(2), 187–194.
Girhammar, U., & Nair, B. M. (1992). Certain Physical-Properties of Water-Soluble Nonstarch Polysaccharides From Wheat, Rye, Triticale, Barley and Oats. *Food Hydrocolloids*, *6*(4),

329–343.
Gómez-González, S., Ruiz-Jiménez, J., Priego-Capote, F., & Luque de Castro, M. D. (2010). Qualitative and Quantitative Sugar Profiling in Olive Fruits, Leaves, and Stems by Gas Chromatography− Tandem Mass Spectrometry (GC-MS/MS) after Ultrasound-Assisted Leaching. *Journal of Agricultural and Food Chemistry*, *58*(23), 12292–12299.
Grootaert, C., Verstraete, W., & Van de Wiele, T. (2007). Microbial metabolism and prebiotic potency of arabinoxylan oligosaccharides in the human intestine. *Trends in Food Science and Technology*, *18*(2), 64–71. http://doi.org/10.1016/j.tifs.2006.08.004
Gruppen, H., Hamer, R. J., & Voragen, A. G. J. (1991). Barium hydroxide as a tool to extract pure arabinoxylans from water-insoluble cell wall material of wheat flour. *Journal of Cereal Science*, *13*(3), 275–290. http://doi.org/10.1016/S0733-5210(09)80006-4
Gruppen, H., Hamer, R. J., & Voragen, A. G. J. (1992). Water-unextractable cell wall material from wheat flour. 1. Extraction of polymers with alkali. *Journal of Cereal Science*, *16*(1), 41–51. http://doi.org/10.1016/S0733-5210(09)80078-7
Gruppen, H., Kormelink, F. J. M., & Voragen, A. G. J. (1993). Water-unextractable Cell Wall Material from Wheat Flour. 3. A Structural Model for Arabinoxylans. *Journal of Cereal Science*, *18*(2), 111–128. http://doi.org/10.1006/jcrs.1993.1040
Gruter, M., Leeflang, B. R., Kuiper, J., Kamerling, J. P., & Vliegenthart, J. F. G. (1992). Structure of the exopolysaccharide produced by lactococcus lactis subspecies cremoris H414 grown in a defined medium or skimmed milk. *Carbohydrate Research*, *231*, 273–291. http://doi.org/10.1016/0008-6215(92)84025-N
Gruter, M., Leeflang, B. R., Kuiper, J., Kamerling, J. P., & Vliegenthart, J. F. G. (1993). Structural characterisation of the exopolysaccharide produced by Lactobacillus delbrückii subspecies bulgaricus rr grown in skimmed milk. *Carbohydrate Research*, *239*, 209–226. http://doi.org/10.1016/0008-6215(93)84216-S
Haltrich, D., Nidetzky, B., Kulbe, K. D., Steiner, W., & Župančič, S. (1996). Production of fungal xylanases. *Bioresource Technology*, *58*(2), 137–161.
Hartley, R. D., & Jones, E. C. (1976). Diferulic acid as a component of cell walls of Lolium multiflorum. *Phytochemistry*, *15*(7), 1157–1160.
Henrissat, B. (1991). A classification of glycosyl hydrolases based on amino acid sequence similarities. *Biochemical Journal*, *280*(2), 309–316. http://doi.org/10.1042/bj2800309
Henshall, A. (2001). High-performance liquid chromatography techniques used in dietary fiber and complex carbohydrates analysis. *FOOD SCIENCE AND TECHNOLOGY-NEW YORK-MARCEL DEKKER-*, 375–414.
Hoffmann, W. F., & Gortner, R. A. (1927). Wheat Flour with special reference to the globulin, albumin and proteose fractions. *Cereal Chemistry*, *4*, 221–229.
Hollmann, J., & Lindhauer, M. (2005). Pilot-scale isolation of glucuronoarabinoxylans from wheat bran. *Carbohydrate Polymers*, *59*(2), 225–230. http://doi.org/10.1016/j.carbpol.2004.09.015
Horwitz, W. (2000). Protein in grains. Method 979.09. *Official Method of Analysis of AOAC International*, 30–34.
Hughes, S. A., Shewry, P. R., Li, L., Gibson, G. R., Sanz, M. L., & Rastall, R. A. (2007). In Vitro Fermentation by Human Fecal Microflora of Wheat Arabinoxylans. *Journal of Agricultural and Food Chemistry*, *55*(11), 4589–4595. http://doi.org/10.1021/jf070293g
Iqbal, M. S., Akbar, J., Hussain, M. A., Saghir, S., & Sher, M. (2011). Evaluation of hot-water extracted arabinoxylans from ispaghula seeds as drug carriers. *Carbohydrate Polymers*, *83*(3), 1218–1225. http://doi.org/10.1016/j.carbpol.2010.09.024
Ishii, T., Hiroi, T., & Thomas, J. R. (1990). Feruloylated xyloglucan and p-coumaroyl

arabinoxylan oligosaccharides from bamboo shoot cell-walls. *Phytochemistry*, *29*(6), 1999–2003. http://doi.org/10.1016/0031-9422(90)85055-K

Izydorczyk, M., Biliaderis, C. G., & Bushuk, W. (1991). Comparison of the structure and composition of water-soluble pentosans from different wheat varieties. *Cereal Chemistry (USA)*.

Izydorczyk, M. S., & Biliaderis, C. G. (1992). Effect of molecular size on physical properties of wheat arabinoxylan. *Journal of Agricultural and Food Chemistry*, *40*(4), 561–568. http://doi.org/10.1021/jf00016a006

Izydorczyk, M. S., Biliaderis, C. G., & Bushuk, W. (1990). Oxidative gelation studies of water-soluble pentosans from wheat. *Journal of Cereal Science*, *11*(2), 153–169.

Izydorczyk, M. S., & Dexter, J. E. (2008). Barley ??-glucans and arabinoxylans: Molecular structure, physicochemical properties, and uses in food products-a Review. *Food Research International*, *41*(9), 850–868. http://doi.org/10.1016/j.foodres.2008.04.001

Izydorczyk, & Biliaderis. (1995). Cereal arabinoxylans: advances in structure and physicochemical properties. *Carbohydrate Polymers*, *28*(1), 33–48.

Jacquemin, L., Mogni, A., Zeitoun, R., Guinot, C., Sablayrolles, C., Saulnier, L., & Pontalier, P.-Y. (2015). Comparison of different twin-screw extraction conditions for the production of arabinoxylans. *Carbohydrate Polymers*, *116*, 86–94. http://doi.org/10.1016/j.carbpol.2014.06.071

Jacquemin, L., Zeitoun, R., Sablayrolles, C., Pontalier, P.-Y., & Rigal, L. (2012). Evaluation of the technical and environmental performances of extraction and purification processes of arabinoxylans from wheat straw and bran. *Process Biochemistry*, *47*(3), 373–380. http://doi.org/10.1016/j.procbio.2011.10.025

Kallel, F., Driss, D., Bouaziz, F., Neifer, M., Ghorbel, R., & Ellouz Chaabouni, S. (2015). Production of xylooligosaccharides from garlic straw xylan by purified xylanase from Bacillus mojavensis UEB-FK and their in vitro evaluation as prebiotics. *Food and Bioproducts Processing*, *94*, 536–546. http://doi.org/10.1016/j.fbp.2014.07.012

Krawczyk, H., Persson, T., Andersson, A., Jönsson, A.-S., Avellar, B. K., Glasser, W. G., … Holmbom, B. (2008). Isolation of hemicelluloses from barley husks. *Food and Bioproducts Processing*, *86*(1), 31–36. http://doi.org/10.1016/j.fbp.2007.10.018

Kroon, P. A., Garcia-Conesa, M. T., Fillingham, I. J., Hazlewood, G. P., & Williamson, G. (1999). Release of ferulic acid dehydrodimers from plant cell walls by feruloyl esterases. *Journal of the Science of Food and Agriculture*, *79*(3), 428–434.

Kulp, K. (1968). Enzymolysis of pentosans of wheat flour. *Cereal Chem*, *45*(339), I968.

Larsson, S., Palmqvist, E., Hahn-Hägerdal, B., Tengborg, C., Stenberg, K., Zacchi, G., & Nilvebrant, N.-O. (1999). The generation of fermentation inhibitors during dilute acid hydrolysis of softwood. *Enzyme and Microbial Technology*, *24*(3), 151–159.

Lemoine, J., Chirat, F., Wieruszeski, J. M., Strecker, G., Favre, N., & Neeser, J. R. (1997). Structural characterization of the exocellular polysaccharides produced by Streptococcus thermophilus SFi39 and SFi12. *Applied and Environmental Microbiology*, *63*(9), 3512–8. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9293002

Levander, F., Svensson, M., Rådström, P., Vuyst, L. De, Degeest, B., Looijesteijn, P., … Råström, P. (2001). Small-scale analysis of exopolysaccharides from Streptococcus thermophilus grown in a semi-defined medium. *BMC Microbiology*, *1*(1), 23. http://doi.org/10.1186/1471-2180-1-23

Maes, C., & Delcour, J. a. (2002). Structural Characterisation of Water-extractable and Water-unextractable Arabinoxylans in Wheat Bran. *Journal of Cereal Science*, *35*(3), 315–326. http://doi.org/10.1006/jcrs.2001.0439

Maes, C., & Delcour, J. A. (2001). Alkaline Hydrogen Peroxide Extraction of Wheat Bran Non-starch Polysaccharides. *Journal of Cereal Science*, *34*(1), 29–35. http://doi.org/10.1006/jcrs.2001.0377

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*(2), 181–186.

Manns, D., Deutschle, A. L., Saake, B., & Meyer, A. S. (2014). Methodology for quantitative determination of the carbohydrate composition of brown seaweeds (Laminariaceae). *RSC Adv.*, *4*(49), 25736–25746. http://doi.org/10.1039/C4RA03537B

McCleary, B. V, Travel, F. R., & Cheetham, N. W. H. (1982). Preparative-scale isolation and characterisation of 6 1-α-d-galactosyl-(1→ 4)-β-d-mannobiose and 6 2-α-d-galactosyl-(1→ 4)-β-d-mannobiose. *Carbohydrate Research*, *104*(2), 285–297.

Medeiros, P. M., & Simoneit, B. R. T. (2007). Analysis of sugars in environmental samples by gas chromatography–mass spectrometry. *Journal of Chromatography A*, *1141*(2), 271–278.

Merali, Z., Collins, S. R. A., Elliston, A., Wilson, D. R., Käsper, A., Waldron, K. W., … Zia-ul-Hussnain, S. (2015). Characterization of cell wall components of wheat bran following hydrothermal pretreatment and fractionation. *Biotechnology for Biofuels*, *8*(1), 23. http://doi.org/10.1186/s13068-015-0207-1

Minjares-Fuentes, R., Femenia, A., Garau, M. C., Candelas-Cadillo, M. G., Simal, S., & Rosselló, C. (2016). Ultrasound-assisted extraction of hemicelluloses from grape pomace using response surface methodology. *Carbohydrate Polymers*, *138*, 180–91. http://doi.org/10.1016/j.carbpol.2015.11.045

Misailidis, N., Campbell, G. M., Du, C., Sadhukhan, J., Mustafa, M., Mateos-Salvador, F., & Weightman, R. M. (2009). Evaluating the feasibility of commercial arabinoxylan production in the context of a wheat biorefinery principally producing ethanol: Part 2. Process simulation and economic analysis. *Chemical Engineering Research and Design*, *87*(9), 1239–1250.

Moore, A. M., Martinez-Munoz, I., & Hoseney, R. C. (1990). Factors affecting the oxidative gelation of wheat water-solubles. *Cereal Chem*, *67*(1), 81–84.

Nascimento, G. E. do, Baggio, C. H., Werner, M. F. de P., Iacomini, M., & Cordeiro, L. M. C. (2016). Arabinoxylan from Mucilage of Tomatoes (Solanum lycopersicum L.): Structure and Antinociceptive Effect in Mouse Models. *Journal of Agricultural and Food Chemistry*, *64*(6), 1239–1244. http://doi.org/10.1021/acs.jafc.5b05134

Paice, M. G., Bourbonnais, R., Desrochers, M., Jurasek, L., & Yaguchi, M. (1986). A xylanase gene from Bacillus subtilis: nucleotide sequence and comparison with B. pumilus gene. *Archives of Microbiology*, *144*(3), 201–206.

Pape Gowda, J., Channe Gowda, D., & Anjaneyalu, Y. V. (1980). Structure of a new arabinoxylan from the bark of Cinnamomum iners. *Carbohydrate Research*, *87*(2), 241–248. http://doi.org/10.1016/S0008-6215(00)85210-6

Pedersen, M., Viksø-Nielsen, A., & Meyer, A. S. (2010). Monosaccharide yields and lignin removal from wheat straw in response to catalyst type and pH during mild thermal pretreatment. *Process Biochemistry*, *45*(7), 1181–1186.

Polizeli, M., Rizzatti, A. C. S., Monti, R., Terenzi, H. F., Jorge, J. A., & Amorim, D. S. (2005). Xylanases from fungi: properties and industrial applications. *Applied Microbiology and Biotechnology*, *67*(5), 577–591.

Preece, I. A., & Hobkirk, R. (1953). NON-STARCHY POLYSACCHARIDES OF CEREAL GRAINS III. HIGHER MOLECULAR GUMS OF COMMON CEREALS, *59*.

http://doi.org/10.1002/j.2050-0416.1953.tb02733.x
Preece, I. A., & MacDougall, M. (1958). ENZYMIC DEGRADATION OF CEREAL HEMIGELLULOSES II. PATTERN OF PENTOSAN DEGRADATION. *Journal of the Institute of Brewing*, *64*(6), 489–500.
Rattan, O., Izydorczyk, M. S., & Biliaderis, C. G. (1994). Structure and Rheological Behaviour of Arabinoxylans from Canadian Bread Wheat Flours. *LWT - Food Science and Technology*, *27*(6), 550–555. http://doi.org/10.1006/fstl.1994.1108
Rocklin, R. D., & Pohl, C. A. (1983). Determination of Carbohydrates by Anion Exchange Chromatography with Pulsed Amperometric Detection. *Journal of Liquid Chromatography*, *6*(9), 1577–1590. http://doi.org/10.1080/01483918308064876
Rosselló-Mora, R. (2003). Opinion: The Species Problem, Can We Achieve a Universal Concept? *Systematic and Applied Microbiology*, *26*(3), 323–326. http://doi.org/10.1078/072320203322497347
Ruiz-Matute, A. I., Hernandez-Hernandez, O., Rodríguez-Sánchez, S., Sanz, M. L., & Martínez-Castro, I. (2011). Derivatization of carbohydrates for GC and GC–MS analyses. *Journal of Chromatography B*, *879*(17), 1226–1240.
Saeed, F., Pasha, I., Anjum, F. M., & Sultan, M. T. (2011). Arabinoxylans and arabinogalactans: a comprehensive treatise. *Critical Reviews in Food Science and Nutrition*, *51*(5), 467–476. http://doi.org/10.1080/10408391003681418
Santiago, R., Butrón, A., Reid, L. M., Arnason, J. T., Sandoya, G., Souto, X. C., & Malvar, R. A. (2006). Diferulate content of maize sheaths is associated with resistance to the Mediterranean corn borer Sesamia nonagrioides (Lepidoptera: Noctuidae). *Journal of Agricultural and Food Chemistry*, *54*(24), 9140–9144.
Santiago, R., & Malvar, R. A. (2010). Role of Dehydrodiferulates in Maize Resistance to Pests and Diseases. *International Journal of Molecular Sciences*, *11*(2), 691–703. http://doi.org/10.3390/ijms11020691
Sarker, D. K., Wilde, P. J., & Clark, D. C. (1998). Enhancement of Protein Foam Stability by Formation of Wheat Arabinoxylan-Protein Crosslinks, *75*(4).
Saulnier, L., Sado, P.-E., Branlard, G., Charmet, G., & Guillon, F. (2007). Wheat arabinoxylans: Exploiting variation in amount and composition to develop enhanced varieties. *Journal of Cereal Science*, *46*(3), 261–281. http://doi.org/10.1016/j.jcs.2007.06.014
Scheller, H. V., & Ulvskov, P. (2010). Hemicelluloses. *Annual Review of Plant Biology*, *61*(1), 263–289. http://doi.org/10.1146/annurev-arplant-042809-112315
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*(1), 63–75. http://doi.org/http://dx.doi.org/10.1006/jcrs.1998.0222
Shiiba, K., Yamada, H., Hara, H., Okada, K., & Nagao, S. (1993). Purification and characterization of two arabinoxylans from wheat bran. *Cereal Chemistry*, *70*(2), 209–214.
Slade, A. M., Hoj, P. B., Morrice, N. A., & Fincher, G. B. (1989). Purification and characterization of three (1→ 4)-β-d-xylan endohydrolases from germinated barley. *European Journal of Biochemistry*, *185*(3), 533–539.
Slavin, J. (2005). Dietary Fiber and Body Weight. *Nutrition*, *21*(3), 411–418.
Smith, M. M., & Hartley, R. D. (1983). Occurrence and nature of ferulic acid substitution of cell-wall polysaccharides in graminaceous plants. *Carbohydrate Research*, *118*, 65–80. Retrieved from http://www.sciencedirect.com/science/article/pii/0008621583880367

Speight, J. G. (2005). *Lange's handbook of chemistry* (Vol. 1). McGraw-Hill New York.
Stein, K. (2007). *Food vs Biofuel*. *Journal of the American Dietetic Association* (Vol. 107).
Stepan, A. M., Ansari, F., Berglund, L., & Gatenholm, P. (2014). Nanofibrillated cellulose reinforced acetylated arabinoxylan films. *Composites Science and Technology*, *98*, 72–78.
Stepan, A. M., Höije, A., Schols, H. A., de Waard, P., & Gatenholm, P. (2012). Arabinose content of arabinoxylans contributes to flexibility of acetylated arabinoxylan films. *Journal of Applied Polymer Science*, *125*(3), 2348–2355. http://doi.org/10.1002/app.36458
Stevanic, J. S., Joly, C., Mikkonen, K. S., Pirkkalainen, K., Serimaa, R., Rémond, C., … Salmén, L. (2011). Bacterial nanocellulose-reinforced arabinoxylan films. *Journal of Applied Polymer Science*, *122*(2), 1030–1039. http://doi.org/10.1002/app.34217
Sun, J. ., Sun, X. ., Sun, R. ., & Su, Y. . (2004). Fractional extraction and structural characterization of sugarcane bagasse hemicelluloses. *Carbohydrate Polymers*, *56*(2), 195–204. http://doi.org/10.1016/j.carbpol.2004.02.002
Sun, R. C., Sun, X. F., & Ma, X. H. (2002). Effect of ultrasound on the structural and physiochemical properties of organosolv soluble hemicelluloses from wheat straw. *Ultrasonics Sonochemistry*, *9*(2), 95–101. http://doi.org/10.1016/S1350-4177(01)00102-X
Sun, X. F., Xu, F., Sun, R. C., Geng, Z. C., Fowler, P., & Baird, M. S. (2005). Characteristics of degraded hemicellulosic polymers obtained from steam exploded wheat straw. *Carbohydrate Polymers*, *60*(1), 15–26. http://doi.org/10.1016/j.carbpol.2004.11.012
Sunna, A., & Antranikian, G. (1997). Xylanolytic enzymes from fungi and bacteria. *Critical Reviews in Biotechnology*, *17*(1), 39–67.
Taiz, L., & Honigman, W. A. (1976). Production of cell wall hydrolyzing enzymes by barley aleurone layers in response to gibberellic acid. *Plant Physiology*, *58*(3), 380–386. http://doi.org/10.1104/pp.58.3.380
Valentine, J., Clifton-Brown, J., Hastings, A., Robson, P., Allison, G., & Smith, P. (2012). Food vs. fuel: The use of land for lignocellulosic "next generation" energy crops that minimize competition with primary food production. *GCB Bioenergy*, *4*(1), 1–19. http://doi.org/10.1111/j.1757-1707.2011.01111.x
Velkova, N., Doliška, A., Fras Zemljič, L., Vesel, A., Saake, B., & Strnad, S. (2015). Influence of carboxymethylation on the surface physical-chemical properties of glucuronoxylan and arabinoxylan films. *Polymer Engineering & Science*, *55*(12), 2706–2713. http://doi.org/10.1002/pen.24059
Viëtor, R. J., Angelino, S., & Voragen, A. G. J. (1992). Structural features of arabinoxylans from barley and malt cell wall material. *Journal of Cereal Science*, *15*(3), 213–222.
Vinkx, C. J. A., & Delcour, J. A. (1996). Rye (Secale cerealeL.) Arabinoxylans: A Critical Review. *Journal of Cereal Science*, *24*(1), 1–14. http://doi.org/10.1006/jcrs.1996.0032
Vinkx, C. J. A., Van Nieuwenhove, C. G., & Delcour, J. (1991). Physicochemical and Functional-Properties of Rye Nonstarch Polysaccharides. 3. Oxidative Gelation of a Fraction Containing Water-Soluble Pentosans and Proteins. *Cereal Chemistry*, *68*(6), 617–622.
Wang, J., Sun, B., Liu, Y., & Zhang, H. (2014). Optimisation of ultrasound-assisted enzymatic extraction of arabinoxylan from wheat bran. *Food Chemistry*, *150*, 482–8. http://doi.org/10.1016/j.foodchem.2013.10.121
Wang, P., Tao, H., Jin, Z., & Xu, X. (2016). Impact of water extractable arabinoxylan from rye bran on the frozen steamed bread dough quality. *Food Chemistry*, *200*, 117–24.

http://doi.org/10.1016/j.foodchem.2016.01.027
Wang, Q., & Fang, Y. (2004). Analysis of sugars in traditional Chinese drugs. *Journal of Chromatography B*, *812*(1), 309–324.
Xu, F., Liu, C. F., Geng, Z. C., Sun, J. X., Sun, R. C., Hei, B. H., … Je, J. (2006). Characterisation of degraded organosolv hemicelluloses from wheat straw. *Polymer Degradation and Stability*, *91*(8), 1880–1886. http://doi.org/10.1016/j.polymdegradstab.2005.11.002
Yoo, C. G., Kim, H., Lu, F., Azarpira, A., Pan, X., Oh, K. K., … Kim, T. H. (2015). Understanding the Physicochemical Characteristics and the Improved Enzymatic Saccharification of Corn Stover Pretreated with Aqueous and Gaseous Ammonia. *BioEnergy Research*, *9*(1), 67–76. http://doi.org/10.1007/s12155-015-9662-6
Zeitoun, R., Pontalier, P. Y., Marechal, P., & Rigal, L. (2010). Twin-screw extrusion for hemicellulose recovery: influence on extract purity and purification performance. *Bioresource Technology*, *101*(23), 9348–54. http://doi.org/10.1016/j.biortech.2010.07.022
Zhang, Z., Smith, C., & Li, W. (2014). Extraction and modification technology of arabinoxylans from cereal by-products: A critical review. *Food Research International*, *65*, 423–436. http://doi.org/10.1016/j.foodres.2014.05.068
Zhou, S., Liu, X., Guo, Y., Wang, Q., Peng, D., & Cao, L. (2010). Comparison of the immunological activities of arabinoxylans from wheat bran with alkali and xylanase-aided extraction. *Carbohydrate Polymers*, *81*(4), 784–789. http://doi.org/10.1016/j.carbpol.2010.03.040