Arabinoxylan as partial flour replacer: The effect on bread properties and economics of bread making

by

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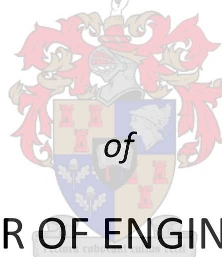
Thesis presented in partial fulfilment
of the requirements for the Degree



of

MASTER OF ENGINEERING
(CHEMICAL ENGINEERING)

in the Faculty of Engineering
at Stellenbosch University

Supervisor
Dr. A.F.A Chimphango

March 2016

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ABSTRACT

Wheat bran, used for animal feed, is a good candidate for production of higher value products such as arabinoxylan (AX). Extracted AX holds potential as a partial flour replacer in the bread making industry. The aim of this study was to maximise flour removal while using the minimum AX addition possible while maintaining physical bread properties.

The extraction of AX from wheat bran was accomplished using alkaline conditions. The purity of AX extracted at lab scale (275 ml) was 44.3% at the optimum extraction conditions (0.5 M NaOH, 240 min, 80°C). Large scale extraction (27 l) resulted in an extract with 49.3% purity, with addition of purification steps including ultrafiltration, anion exchange chromatography and ethanol precipitation. The two extracts obtained on small scale (E1) and large scale (E2) both had high average molecular weights (620 000 and 470 000 Da, respectively) and arabinose to xylose (A/X) ratios of 0.7 and 0.6. With inclusion of the additional purification steps at large scale, the whiteness index of the final extract was increased from 33 to 93. For the application purpose, the lighter extract colour will have a less prominent effect on bread colour and is therefore advantageous.

The high water binding capacity of AX allows for increased dough water absorption resulting in an altered final bread weight and volume. However, at optimal AX addition and flour removal levels, these product properties can be maintained. This was achieved with inclusion of 0.8% crude AX extract and 2.5% flour removal, while increasing water absorption by nearly 2%. The only physical difference between the AX containing loaves and the control was in colour, due to the darker colour of the extract. However, a discolouration step included in the extraction of E2 resulted in a significantly lighter final product compared to loaves containing E1. Comparison of E1 and E2 to highly pure AX resulted in similar final product properties indicating that the extracts' performance was not affected by the purity. Furthermore, inclusion of an oxidative enzyme, laccase, resulted in a softer final product as determined using a texture analyser.

AX production cost was estimated at R110/ kg resulting in higher production costs for AX containing loaves compared to commercial white bread. In order to maintain profit margins the selling price of AX containing loaves have to be increased by 9.6%.

In conclusion, crude AX extracted from the animal feed co-product, wheat bran, is a feasible candidate for application in the bread making process as a partial flour replacer.

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OPSOMMING

Graan semels, wat gebruik word vir dierevoer, is ‘n goeie kandidaat vir die produksie van hoër waarde produkte soos arabinoxylan (AX). Geëkstraeerde AX het die potensiaal om as gedeeltelike meel vervanger toegepas te word in die brood maak bedryf. Die doel van die studie was om meel verwydering te maksimeer en terselfdetyd die minimum hoeveelheid AX toe te voeg om sodoende die fisiese eienskappe van brood te behou.

Die ekstraksie van AX uit graan semels was uitgevoer onder alkaliese kondisies. Die suiwerheid van die AX geëkstraeer op laboratorium skaal (275 ml) was 44.3% by die optimum ekstraksie kondisies (0.5 M NaOH, 240 min, 80°C). Grootskaalse ekstraksie (27 l) het gelei tot ‘n ekstrak met 49.3% suiwerheid, deur middel van addisionele suiweringstappe insluitend, ultrafiltrasie, anioon uitruil kroomatografie en etanol presipitasie. Die twee ekstrakte wat verkry is vanaf klein skaal (E1) en groot skaal (E2) het beide hoë gemiddelde molekulêre massas (620 000 and 470 000 Da, onderskeidelik) en arabinose tot xylose (A/X) verhoudings van 0.7 en 0.6. Met die toevoeging van addisionele suiweringstappe op groot skaal, het die witheid indeks van die finale ekstrak toegeneem vanaf 33 na 93. Die ligter ekstrak kleur is voordelig vir toepassing in die bakproses.

Die fisiese-chemiese eienskappe van AX beïnvloed hul funksionalitiet in die brood maak proses. Die hoë water bindingskapasiteit van AX laat toe vir toenemende deeg water absorpsie wat veranderinge in brood gewig en volume tot gevolg het. Alhoewel, by optimale AX toevoegingsvlakke en meel verwyderingsvlakke kan hierdie brood eienskappe behou word. Dit was moontlik deur toevoeging van 0.8% AX en meel verwydering van 2.5%, terwyl water absorpsie met bykans 2% toegeneem het. Die enigste opmerkbare verksil tussen brode met AX toevoeging en die kontrole was die kleur, as gevolg van die donker kleur van die ekstrak self. Die toevoeging van ‘n ontkleuringstap tydens die ekstraksie proses van E2 het ‘n aansienlike ligter finale produk tot gevolg gehad, in vergelyking met E1. Vergelyking van E1 en E2 met kommersiële AX het gelei tot finale produkte met ooreenstemmende eienskappe. Dit dui daarop dat die suiwerheid van die AX ekstrak nie sy prestasie beïnvloed het nie. Verder, toevoeging van die oksidatiewe ensiem, laccase, het ‘n finale produk met ‘n sagter tekstuur tot gevolg gehad.

Die produksie koste van AX was beraam as R110/ kg. Hierdie koste het gelei tot ‘n hoër produksie koste vir brode met AX toevoeging in vergelyking met kommersiële witbrood. Om wins marge te behou moet die verkoopprys van brode met AX toevoeging na beraming 9.2% meer wees.

Ten slotte, geëkstraeerde AX, vanaf graan semels, is ‘n realistiese kandidaat vir toepassing in die brood maak proses as ‘n gedeeltelike meel vervanger.

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ACKNOWLEDGEMENTS

I recognise and thank the following persons and institutions for their contribution to the successful completion of this thesis:

My heavenly Father, who blessed me with this opportunity and gave me the strength and perseverance to complete this project

Dr Annie Chimphango, my study leader, for guidance and support throughout my project

Kim O'Kennedy and Carien Roets (Pioneer Foods, Essential Grains, Research and Development) for their valued assistance and generosity

Pioneer Foods, Essential Grains, Research and Development department, who allowed me to use their facilities, equipment and laboratory staff

Prof Pierre-Yves Pontallier (ENSIACET, Toulouse) who allowed me to use his laboratory and offered valued assistance and advice

Vincent Oriez (ENCIACET, Toulouse) for training and assistance

Mr Henry Soloman (Wood Science) for technical assistance and moral support

Analytical staff, Jaco van Rooyen and Levine Simmers, for analyses and technical support

Technical staff for assistance (Department of Process Engineering)

Dr Eugene van Rensburg and Dr. Maria Garcia for their guidance and advice

Jane de Kock (Department of Microbiology) for assistance and use of equipment

Anchen Lombard and Prof. Marena Manley (Department of Food Science) for assistance and use of facilities

Sasko Milling and Baking, Malmesbury for their kind provision of wheat flour and bran

The National Research Foundation (NRF) and Department of Process Engineering for financial funding

My fellow post-graduate students for their encouragement, support and advice

My family, Jacques, Norma, Arzanne, and William for their continuous love, support and motivation

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ABBREVIATIONS

A	Arabinose
AACC	American Association of Cereal Chemistry
ACS	American Chemical Society
AGX/GAX	Arabinoglucurunoxylan/Glucuronuarabinoxylan
ANOVA	Analysis of variance
AOAC	Association of Official Analytical Chemists
Ara*f*	Arabinofuranosyl
ASAX	Alkaline soluble arabinoxylan
AX	Arabinoxylan
BU	Brabender units
DF	Dietary fibre
Di-FA	Dehydrodi-ferulic acid
Dw	Dry weight
FA	Ferulic acid
GI	Glycaemic index
HMW	High molecular weight
HPLC	High performance liquid chromatography
IR	Infrared
kDa	kilo Dalton
KOH	Potassium hydroxide
kWh	kilo Watt hour
LMW	Low molecular weight
MW	Molecular weight
MWCO	Molecular weight cut-off
NMR	Nuclear magnetic resonance
NREL	National Renewable Energy Laboratory

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NaOH	Sodium hydroxide
NSP	Non-starch polysaccharides
RH	Relative humidity
WPC	Wheat pentosan concentrate
SD	Standard deviation
WEAX	Water-extractable arabinoxylans
WI	Whiteness index
WSAX	Water-soluble arabinoxylans
WUAX	Water-unextractable arabinoxylans
X	Xylose
Xyl*p*	Xylopyranosyl

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1 INTRODUCTION

Current economic and environmental concerns are leading to increased interest in renewable resource utilisation. Agricultural and forestry biomass, specifically by-products of these industries, hold great potential in the production of higher value products from low value sources.

Wheat is one of the major agricultural crops cultivated in South Africa for human consumption with production of around 1.7 million tons per annum (Grain SA, 2015). Approximately 0.3 million tons of bran is produced annually from wheat milling which is mainly sold as livestock feed (Faurot et al., 1995; Hollmann & Lindhauer, 2005; Schooneveld-Bergmans, van Dijk, Beldman, & Voragen, 1999; Vitaglione, Napolitano, & Fogliano, 2008). This bran fraction contains around 44% dietary fibre (Stevenson, Phillips, Sullivan, & Walton, 2012) which is indigestible for monogastrics. Better utilization of the feedstock can be achieved through fractionation and extraction of the fibre component for application in the food industry.

Accumulating evidence demonstrates the beneficial effects of increased dietary fibre intake against chronic diseases, such as cardiovascular diseases, diabetes and colon cancer (Stevenson et al., 2012; Vitaglione et al., 2008). In view of the health promoting potential of dietary fibre, more food products are being developed with increased fibre content. The addition of dietary fibre to food products contribute to the development of value-added foods or functional foods, which have physiological benefits. Moreover, due to their functional properties, fibre components can attribute texturizing, emulsifying, gelling and stabilizing effects in certain foods (Ebringerová & Hromádková, 1999; Inglett, 1998; Rose, Inglett, Liu, & Wiley, 2010).

As bread is one of the world's most regularly consumed processed food (Stevenson et al., 2012), there is a continuous demand for improvement of production processes and product quality. The process of bread-making has been a topic of research for decades, and will continue to do so for as long as bread remains the staple food of the world (Caballero, Gómez, & Rosell, 2007; Dobraszczyk & Morgenstern, 2003; Gan, Ellis, & Schofield, 1995; Hansen et al., 2002; Janssen, Vliet, & Vereijken, 1996). With knowledge of the principals of bread-making, it is possible to alter and improve on the final product properties. The latter is of great interest in today's consumer driven market where nutrition and functionality is key. Producing nutritional alternatives whilst utilising renewable by-products may be an attractive option. The addition of dietary fibre obtained from milling by-products can improve the nutritional properties of bread while also potentially improving final product properties (Biliaderis, Izydorczyk, & Rattan, 1995; Courtin & Delcour, 1998; Izydorczyk & Biliaderis, 1992b; Michniewicz, Biliaderis, & Bushuk, 1991).

Arabinoxylans (AX) are the major dietary fibre component in wheat bran accounting for approximately 28% of the total composition (Maes & Delcour, 2002). AX are branched polymers consisting of a xylan backbone substituted with arabinose side chains (Courtin & Delcour, 2002).

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The physiochemical properties of AX make this group of polymers diverse in its application potential which may be exploited even further using modification processes. Catalysed by oxidative enzymes, AX can form three dimensional networks (gels) in the presence of water (Carvajal-Millan, Guigliarelli, Belle, Rouau, & Micard, 2005). The process of gel formation is highly influenced by the presence of ferulic acid residues bound to AX polymers, which are cross-linked upon oxidation by free radicals-generating enzymes (Figueroa-Espinoza, Morell, Surget, & Rouau, 1999). The ability of AX to form gels and increase water binding capacity may be of interest in the baking industry as water absorption is a governing factor affecting final product characteristics (Scanlon & Zghal, 2001). The use of enzymes to improve bread properties is not a novel concept as amylases are widely used in commercial bread making and other enzymes such as xylanases and oxidases have been intensively studied (Flander et al., 2008; Labat, Morel, & Rouau, 2001; Orel, Utio, Lander, Ouau, & El, 2008; Primo-Martín, Valera, & Martínez-Anaya, 2003; Selinheimo, Kruus, Buchert, Hopia, & Autio, 2006; Selinheimo, Autio, Kruus, & Buchert, 2007; Trogh et al., 2004; Zhou et al., 2010).

Numerous studies have been conducted on AX and their role during bread making (Biliaderis et al., 1995; Courtin & Delcour, 1998; Courtin & Delcour, 2002; Rattan, Izydorczyk, & Biliaderis, 1994; Shiiba, Yamada, Hara, Okada, & Nagao, 1994; Zhang et al., 2011). The physiochemical characteristics of AX determine their functional properties and due to their high water holding capacities they have the potential to act as partial flour replacers in the bread making process. With the water holding capacity of AX being substantially higher than flour (10 and 3 g/g, respectively) (J. Wang, Rosell, & Benedito de Barber, 2002), it is proposed that less AX is required to replace a larger amount of flour. The application of AX as partial flour replacer could hold economic value in potentially reducing bread production cost.

The aim of this study was to obtain a crude AX extract from alkaline extraction of wheat bran and apply it in the bread making process. The purpose was to maximise flour replacement with minimal AX addition i) to maintain/improve final bread properties and ii) for potential economic benefit.

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1.1 Thesis layout

Chapter 2 reviews the literature relating to the structure, extraction and characterisation of wheat AX and their functional role in bread making. AX are identified as a major constituent of the non-starch polysaccharide content of wheat, with bran a particularly good source of AX. The structures of wheat bran AX are examined in relation to how this affects their extractability. Extraction methods and techniques for the characterisation of AX are identified. An overview of the bread making process and the role of flour constituents and bread improvers are reviewed. The functional role of AX as an ingredient in bread dough is analysed which leads to the objectives of the current study, to extract AX from wheat bran and to investigate the functional performance of crude AX extracts in bread as a partial flour replacer.

Chapter 3 describes the optimisation of AX alkaline extraction at lab scale and characterisation of the crude AX extracts obtained. Large scale extraction with inclusion of addition purification steps were investigated and compared to extracts obtained on small scale. Final extract properties were evaluated for application in the bread making process. The production cost for AX extraction was estimated to determine the economic impact of AX addition in bread production.

Chapter 4 presents the trials conducted to determine the effect of AX addition and flour removal levels on bread properties as well as analyse the functional role of AX in bread making.

Chapter 5 concludes the thesis by summarising the main findings from the current work and makes recommendations about how extraction of AX from wheat bran could be progressed in a research based capacity for industrial application.

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2 LITERATURE REVIEW

2.1 Cereal bran composition

Cereals are primarily cultivated for their starchy grain which is processed for human consumption, animal feed or industrial use. The most common cereal grains include wheat, rye, oat, barley, maize and rice. The cereal grain consists of three major portions: the germ, responsible for the production of new plants, the endosperm which serves as food for the germinating seed and the bran consisting of various layers to protect the grain. Although the proportions vary, all cereal grains follow the same general pattern. Wheat grain consists of approximately 85% endosperm, 13% bran and 2% germ (Goesaert et al., 2005).

The first stage of cereal processing is milling, during which the grain is ground to expose the various components. Milling is followed by multiple sieving steps to separate the endosperm from the bran and germ. To improve this separation process, some cereals are polished to reduce contamination of the endosperm with bran and germ (Stevenson et al., 2012).

Bran comprises the outer part of the grain and includes the tissues that make up the pericarp (fruit coat), testa (seed coat) and the aleurone layer, which is part of the endosperm (Apprich et al., 2014). Bran consists mainly of non-starch polysaccharides (NSP) (46%), protein and starch. The main NSP are xylan-type polymers consisting of a xylose backbone (**Figure 2.1**) (Maes & Delcour, 2002; Schooneveld-Bergmans, van Dijk, Beldman, & Voragen, 1999). In wheat bran arabinoxylan (AX) comprises approximately 24% of the total wheat bran (**Table 2.1**). Bran is therefore a good source of AX, which is found as part of the complex xylans in the cell walls of each of the tissue types in differing proportions. To perform the function of protecting the kernel, cell walls in bran tissues are thick, hydrophobic and formed primarily of cellulose, complex xylans and lignin. This bran fraction produced as a by-product during the milling process is generally used for animal feed with only a fraction (~18%) going to human consumption.

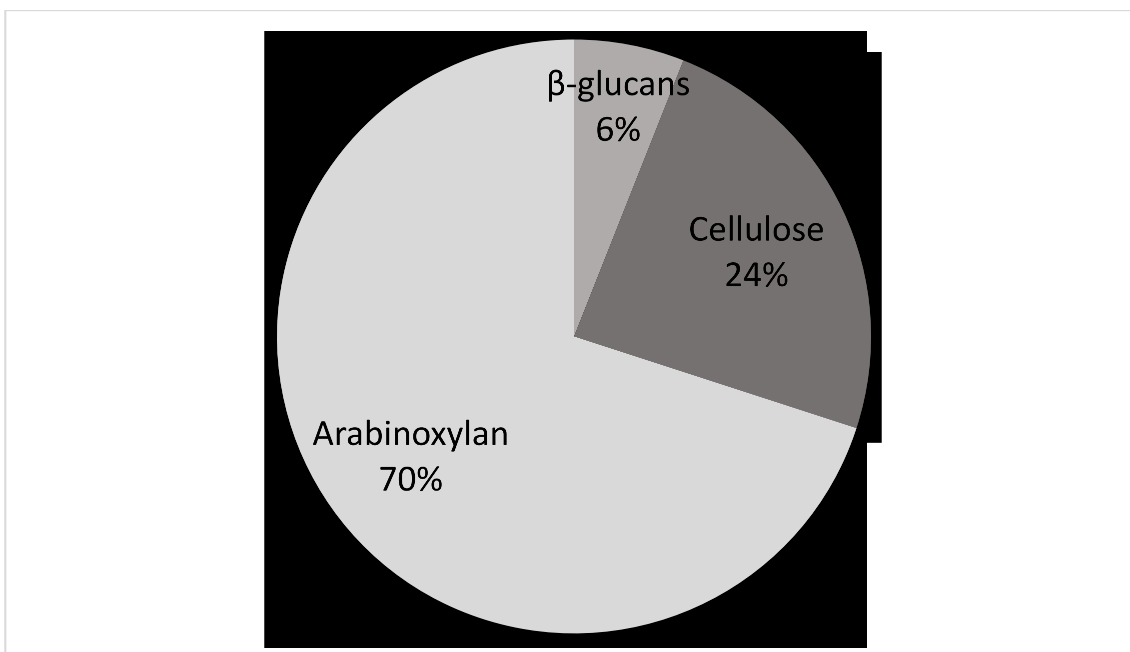

Figure 2.1 Components that make up the non-starch polysaccharides in the wheat grain (Schooneveld-Bergmans, van Dijk, Beldman, & Voragen, 1999).

Table 2.1 Arabinoxylan content in wheat bran expressed as % dry weight

Arabinoxylan (%)	Source
19.0	Bataillon, Mathaly, Cardinali, & Duchiron (1998)
22.6	Maes & Delcour (2002)
25.0	Hollmann & Lindhauer (2005)
29.0	Aguedo, Fougnies, Dermience, & Richel (2014)

2.2 Cereal bran, co-product of the milling industry: Utilization and potential applications

With an increase in consumer awareness and emphasis on healthy living, there has been tremendous interest in the production of functional foods (Vitaglione et al., 2008), with a focus on the most regularly consumed foods such as bakery and dairy products and functional drinks. Research has been aimed on finding new sources of functional ingredients such as under-valued plant food co-products. These products may be suitable for inclusion in functional foods by use of fractionation and/or purification methods to obtain fractions of interest. However, in some cases these research efforts have not yet been extrapolated to an industrial scale to utilize the application potential of these co-products.

Cellulose (from maize bran) has already found its niche in the commercial market in the form of a fibre gel. It is used as a fat mimetic in baked goods, dairy foods, condiments and processed meats (Rose et al., 2010).

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The process of cellulose gel production was developed by Inglett (Inglett, 1998) and involves a two-stage high-shear process. The first stage involves treatment with alkali to disintegrate the cell wall structure of the bran. After the solids are recovered they are treated with hydrogen peroxide as bleaching agent to produce a colourless product. The commercial success of this product is promoted by the increasing incidence of diet related disorders, such as type 2 diabetes and the metabolic syndrome, and was developed to counteract these growing medical conditions (Rose et al., 2010).

Ferulic acid (FA) is a phenolic compound commonly found in bran linked to xylan and lignin. It is an antioxidant, which is a bioactive substance which the human body cannot produce. Due to its antioxidizing properties it plays a role in the prevention of chronic diseases caused by oxidative stress (Goñi & Hervert-Hernández, 2011). FA also possesses antimicrobial activity which can protect food products against spoilage and pathogenic microorganisms (Rose et al., 2010). Because of these properties, FA has great potential for use in the food industry as additive in functional foods and/or as preservative. Furthermore, FA is a precursor of vanillin and can be used for the natural production of vanillin, which is a phenolic aldehyde used in the food, pharmaceutical and cosmetic industry as an important flavour and aroma compound (Dignum, Kerler, & Verpoorte, 2001). Ferulic acid can be extracted from various milling by-products such as maize, wheat and rice bran and brewers spent grain (Hansen et al., 2002; Mussatto, Dragone, & Roberto, 2007; Tilay, Bule, Kishenkumar, & Annapure, 2008). The optimum extraction conditions vary depending on the source but generally the process involves alkaline treatment at elevated temperatures. NaOH concentrations of 2 M to 4 M has been reported and extraction temperatures of 40 to 120°C (Tilay, Bule, Kishenkumar, & Annapure, 2008; Mussatto, Dragone, & Roberto, 2007). Adsorption chromatography and high-performance thin-layer chromatography has been successfully applied for purification resulting in final product purity of 95.3% (Tilay et al., 2008)

When it comes to functional foods, dietary fibre still remains the most popular subject of interest for researches and food industries alike. Dietary fibre (DF) is a collective term referring to cellulose, lignin and hemicellulose which are not digested or absorbed in the human small intestine (Vitaglione et al., 2008). These components are typically divided into two categories: soluble- and insoluble fibre. Soluble DF has the ability to interact with water and provides fermentable carbon sources for bacteria that inhabit the large intestines. Soluble DF slows down digestion and also affects blood sugar levels, which in turn has a beneficial effect on insulin sensitivity (Peressini & Sensidoni, 2009). Insoluble DF adds bulk to the diet as they do not dissolve in water and pass through the gastrointestinal tract relatively intact subsequently speeding up the passage of food and waste through the gut. DF-rich fractions from numerous cereal grains and bran have been investigated as additives in staple foods to improve daily fibre intake (Goñi & Hervert-Hernández, 2011).

Fibre addition has shown to have a pronounced effect on bread and dough properties and these effects vary depending on the fibre source and composition. Collar, Santos, & Rosell (2007) investigated the effect of three types of fibre: soluble, partly soluble and insoluble on dough properties. They found that the insoluble

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fibre resulted in shortening and hardening of the dough whereas the partly soluble fibres, with good hydration properties, had the most beneficial effect on dough texture and no adverse effects on the mechanical properties as assessed by texture profile analysis and texturometer measurements. Similarly, Wang, Rosell, & Benedito de Barber (2002) reported that two commercial fibres (carob and pea fibre) had no adverse effects on dough rheology and improved the softness of the final product with the disadvantage of reducing loaf volume. After sensory analysis the authors also concluded that the fibre-rich breads where acceptable as determined by a sensory panel.

In general, DF addition to bread products has a pronounced effect on dough properties. Dough hardening or a reduction in extensibility is often observed due to the dilution of the gluten protein content and disruption of the crumb structure which is a result of impaired gas retention (Collar et al., 2007). The increase in soluble and insoluble fibre content is largely responsible for the disruption in the gluten network and the resulting low resistance to extension (Wang et al., 2002; Gómez, Ronda, Blanco, Caballero, & Apesteguía, 2003). An increased water absorption, due to increase in water binding capacity of the added fibres, has been shown to increase stickiness of the dough which negatively affects machinability (Collar et al., 2007). The hydroxyl groups in the fibre structure are responsible for the increased water absorption, which allows more interaction with water through hydrogen bonding. Furthermore, longer dough development time and tolerance to over mixing has been observed due to increased water absorption (Peressini & Sensidoni, 2009; Gómez et al., 2003).

The effects of fibre addition are also evident in the final product with a reduction in loaf volume and increased crumb firmness. The effect on loaf volume can be attributed to the fibre-gluten interaction which leads to decreased gas retention and finally results in lower loaf volumes (M. Wang, Vliet, & Hamer, 2004). As for the crumb firmness, this is caused by the thickening of the walls surrounding the air bubbles of the bread crumb (Peressini & Sensidoni, 2009; Gómez et al., 2003). Even though fibre addition has adverse effects on bread properties, decreased staling is one of the major advantages. The high water binding capacity of fibre results in better water retention over the storage period and therefore softer crumb structure which is possibly enhanced by fibre-starch interactions that delay starch retrogradation (Gómez et al., 2003).

Similar to DF, purified AX also affects dough and bread properties as would be expected because of its high water holding capacity. In the past, research has been aimed in understanding the functional role of endogenous AX (present in the flour) and the effect of modification on their function. The extraction of AX from cereals however has been aimed at different applications than functional foods. The potential industrial application of AX is currently focused in the packaging industry for the production of biodegradable films and as additive in papermaking to replace other cationic polymers (Egues, Sanchez, Mondragon, & Labidi, 2012). It also has potential in the biomedical and pharmaceutical industries for adhesion and drug delivery (Da Silva et al., 2012; Ebringerova & Heinze, 2000). The major obstacle for these potential applications is production of highly pure AX and the development of a commercially viable extraction and purification process.

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Application of AX in the food industry, however, may require lower purity than required in other industries such as pharmaceuticals.

2.3 Wheat bran arabinoxylan as high value co-product

Extraction of AX from the low value animal feed produced during wheat milling has the potential to provide a high value co-product. This is only achievable if a market is available for the AX extract produced. The functional properties of AX opens up a wide range of possibilities for its use in both food and non-food applications (Courtin & Delcour, 2002; Maes & Delcour, 2002).

Not only can AX be used as a functional food ingredient (Biliaderis et al., 1995), it also has potential as a nutritional food additive by exhibiting prebiotic effects (Topping, 2007). In the large intestine, undigested AX is thought to change the composition of the microbial flora, which affects the activity of the bacterial enzymes present, influencing the end products of bacterial fermentation, promoting colonic health (Ebringerová & Hromádková, 1999; Weickert et al., 2005). Potential uses of AX as a prebiotic in the food industry could be the production of health promoting cereal-based food products, including bread, biscuits and pasta (Broekaert et al., 2011).

With both technological and nutritional functional properties, it is clear that many opportunities become available for differing industries to take advantage of the beneficial properties of AX, thus making it a sought after, high value product.

The indigestibility of AX is associated with high molecular weight (MW), which exhibit high viscosities in solution, preventing the breakdown of nutrients and their uptake (Courtin et al., 2008). Monogastric animals are deficient in the necessary enzymes to degrade AX, but improvements to digestibility can be made by supplementing animal feeds with particular microbial xylanases, which depolymerise AX, reducing their viscosity and increasing nutrient uptake (Courtin et al., 2008). Removing AX as a high value functional food ingredient, rather than degrading them as an inconvenience in animal feed, offers a commercially beneficial alternative.

2.4 Arabinoxylan extraction from wheat: Structural and physiochemical properties

AX can be classified according to their extractability as either water-extractable (WEAX) or water-unextractable (WUAX), with different functional properties being displayed by the AX obtained from the different extraction techniques.

The extractability of AX is based on their physical interactions, the degree of ester linkages between FA and other cell wall components and the degree and substitution patterns of arabinose residues (Izydorczyk & Biliaderis, 1995). Due to the high ferulic acid content, WUAX molecules are physically and chemically

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associated with each other through cross linking and readily form a network matrix of covalent (e.g. ester and ether bonds and diferulic acid bridges) and non-covalent (e.g. hydrogen bonds) linkages with other cell wall components such as proteins, β-glucans, lignin and cellulose (Biliaderis et al., 1995; Ebringerova & Heinze, 2000). Due to these interactions WUAX cannot be extracted with water and must be physically, chemically or enzymatically treated to render them water-soluble (Schooneveld-Bergmans, Hopman, Beldman, & Voragen, 1998). In contrast to this WEAX is only partially associated with other cell wall components due to incomplete cross-linking and is therefore easily solubilised in water (Maes & Delcour, 2002).

The majority of wheat bran AX is WUAX with only around 4-6% being WEAX (Maes & Delcour, 2002). This indicates that wheat bran AX requires specific treatment for extraction. In literature, alkaline extraction is the most commonly used method to extract these polymers from the cell wall matrix to render them water soluble.

2.4.1 Alkaline extraction

WUAX can be extracted using mild alkaline conditions at mild temperatures. Berlanga-Reyes et al (2009) extracted AX from wheat bran using 0.5 M NaOH at 25°C and obtained a final yield of 17% and purity of 73%. Similarly Bataillon et al (1998) extracted wheat bran AX with 0.5 M NaOH at 40°C and produced a final product with a yield of 13% and 75% purity. For wheat straw NaOH concentrations of 2.5 M at an extraction temperature of 40°C resulted in an extract containing 45% xylan (García et al., 2013).

The purification of AX from lignified tissue, such as husks and bran, may require additional chemical treatment to produce purer fractions. Höije et al. (2005) demonstrated that the use of chlorite as delignification agent resulted in higher yields of AX with less lignin contamination. Due to the hazardous nature of chlorite, hydrogen peroxide has been used as alternative delignifying agent during alkaline extraction. The inclusion of a delignification step using 2% hydrogen peroxide has been shown to increase the purity of AX extracted from wheat bran to 81% (Hollmann & Lindhauer, 2005) and 92.4% (Bergmans, Beldman, Gruppen, & Voragen, 1996). However, an anti-foaming agent is required during this process for effective extraction without excessive foaming.

In addition to the classic alkaline extraction method, alternative methods have been investigated to reduce extraction time and alkaline usage. The use of ultrasound assisted extraction has been shown to reduce the extraction time from 60 min to 5min resulting in a similar final AX yield and purity, also using 60% less NaOH for the extraction process (Hromádková, Koštalova, & Ebringerová, 2008). A similar study showed a decrease of extraction time from 240 min to 10 min using ultrasound-assisted extraction without affecting maximum yield (Juergen Hollmann, Elbegzaya, Pawelzik, & Lindhauer, 2009).

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One of the major obstacles for industrial scale AX extraction is the construction of a high throughput process. Some research has pointed to the use of an extruder type twin screw reactor and compared the effectiveness to the more commonly used batch extraction. The co-extrusion of wheat straw and bran resulted in a lower extraction rate but had the advantage of reducing chemical and water consumption (Zeitoun, Pontalier, Marechal, & Rigal, 2010).

2.4.2 Purification

Apart from the initial extraction steps, the extract must also be purified to separate the AX from soluble contaminants including, proteins, β-glucans, lignin and starch (Izydorczyk & Biliaderis, 1995). Berlanga-Reyes et al. (2009) and Carvajal-Millan et al. (2007) reported that treatment of wheat bran and maize bran with 80% ethanol prior to extraction with water resulted in isolated AX with a very low protein content (2.7% and 2.5% respectively). A study conducted by Cleemput, Roels, Van Oort, Grobet, & Delcour (1993) used heat treatment (90°C) to precipitate soluble protein but resulted in much higher protein contamination (7-13%). Izydorczyk et al. (1990) demonstrated that heat treatment (90-95°C for 5min) followed by treatment with Vega Clay as adsorbent, removed residual proteins and accomplished a protein content as low as 1.7% in wheat flour extracts. In more recent studies the use of bacterial proteases have resulted in a protein contents of 3% (Elizabeth Carvajal-Millan et al., 2005) using Pronase (from *Streptomyces griseus*) and 10% (Dervilly, Saulnier, Roger, & Thibault, 2000) using a protease from *Bacillus licheniformis*, in AX purified from wheat endosperm. α-Amylases or amyloglucosidases are routinely used to remove residual starch contaminants (Carvajal-Millan et al., 2005; Cleemput et al., 1993; Izydorczyk et al., 1990; Maes & Delcour, 2002; Rattan, Izydorczyk, & Biliaderis, 1994). For the purification of AX on a large scale, enzyme treatments can be costly and may not deliver reproducible results. An alternative is water treatment which consists of consecutive washing and filtering steps using distilled water to remove the endosperm starch (Zeitoun et al., 2010; Aguedo et al., 2014; Sun, Cui, Gu, & Zhang, 2011).

After these purification steps, additional purification processes are required to produce a product of acceptable purity. The extract may contains lignin and β-glucan contaminants which were solubilised during the initial extraction process. For the removal of β-glucans, lichenase is often used for the degradation of the polymers into monomers which can be removed by centrifugation. These enzymes require a specific pH and temperature for optimum efficacy (Hollmann & Lindhauer, 2005). Depending on the initial content of this hemicellulose in the raw material and the application of the final fraction, purification processes are adapted to include or exclude this step. The next and final purification step involves precipitation of the AX fraction to separate it from the remaining soluble lignin. Throughout literature, ethanol is the most popular solvent used for precipitation and has been reported at concentrations from 60 to 80% v/v (Hollmann & Lindhauer, 2005; Jacquemin, Zeitoun, Sablayrolles, Pontalier & Rigal, 2012; Sun, Cui, Gu & Zhang, 2011; Kale, Hamaker & Campanella, 2013; Hollmann, Elbegzaya, Pawelzik & Lindhauer, 2009). Schooneveld-Bergmans et al. (1998)

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compared the use of ethanol, methanol and acetone on the recovery of AX extracted from wheat bran. They found that acetone resulted in the highest sugar recovery but also the highest lignin content whereas methanol resulted in low lignin contamination but also low sugar recovery. This would explain the general use of ethanol as it results in adequate sugar recovery with less lignin contamination. The major drawback this purification step, is a decrease in final yield. Juergen Hollmann et al. (2009) reported a 4% decrease in yield after 80% v/v ethanol precipitation of wheat bran AX. Low yields have also been reported in other studies for extraction of AX from wheat bran (Sun et al., 2011; Zeitoun, Pontalier, Marechal, & Rigal, 2010).

Due to the high cost of ethanol precipitation and the difficulties encountered during purification and recycling of the ethanol, alternative purification methods have been reported in literature. In recent studies ultrafiltration has been investigated as an alternative purification method. Zeitoun et al. (2010) performed ultrafiltration using a hollow fibre polyethersulfone membrane with a molecular weight cut-off (MWCO) of 30 kDa. They reported an increase in purity from 77% to 92% for AX extracted from wheat bran. Egues et al (2012) investigated the effect of MWCO (1, 5 and 10kDa) on the purity and yield of an extract from a maize waste stream. Their results showed that the 10kDa retentate liquid had the highest AX concentration. In some studies ultrafiltration has been implemented as a concentration step to decrease the amount of ethanol required for precipitation and does not replace precipitation (Hollmann & Lindhauer, 2005).

In general, during the purification process, there is a trade-off between yield and purity and therefore the optimum conditions vary depending on the properties of interest for each individual study.

The extraction and purification methods discussed in this section thus far, has focussed on the production of highly pure products for research purposes. The aim of these research efforts was mainly to determine the structural and physiochemical properties of AX which attributes to their functionality.

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Table 2.2. Reagents used for arabinoxylan extraction

Reagent	Role	Reference
NaOH	Alkaline extraction	Berlanga-Reyes et al (2009)
Chlorite	De-lignification	Höije et al. (2005)
Hydrogen peroxide	Delignification	Hollmann & Lindhauer (2005); Bergmans, Beldman, Gruppen, & Voragen (1996)
Protease	Protein removal	Elizabeth Carvajal-Millan et al. (2005); Dervilly et al. (2000)
α-Amylase	Starch removal	Carvajal-Millan et al. (2005); Cleemput et al., 1993; Izydorczyk et al. (1990); Maes & Delcour (2002); Rattan, Izydorczyk, & Biliaderis (1994)
Lichenase	B-glucan removal	Hollmann & Lindhauer (2005)
Ethanol	Arabinoxylan precipitation	Hollmann & Lindhauer (2005); Jacquemin et al. (2012); Sun et al. (2011); Kale, Hamaker & Campanella (2013); Hollmann et al (2009)

2.4.3 Structure of wheat bran arabinoxylan

AX is made up of a linear backbone of xylose, unsubstituted, mono- or di-substituted with arabinose residues singly at C(O)-3 or doubly at C(O)2-3 on the xylose backbone (**Figure 2.2** (B)) (Ebringerova & Heinze, 2000). Additionally, ferulic acid can be covalently linked through ester linkages to some of the arabinose side-chains at position C(O)-5 and create cross-links with other cell wall components, such as β-glucan, cellulose, glucose and protein to yield insoluble complexes. Wheat bran AX polymers may also contain uronic acid, mainly glucuronic acid at the C(O)-2 position (**Figure 2.2** (A)), along with short side chains of α-(1-2)- and α-(1-3)-linked arabinose (Bataillon et al., 1998; Hollmann et al., 2009; Hopman, Beldman, & Voragen, 1998; Schooneveld-Bergmans, van Dijk, et al., 1999).

The ratio of arabinose to xylose (A/X) is an indication of the degree of substitution and differs between AX populations present in different tissue types. The general A/X ratio of wheat bran AX populations range from 0.54 to 0.71 (Izydorczyk & Biliaderis, 1995).

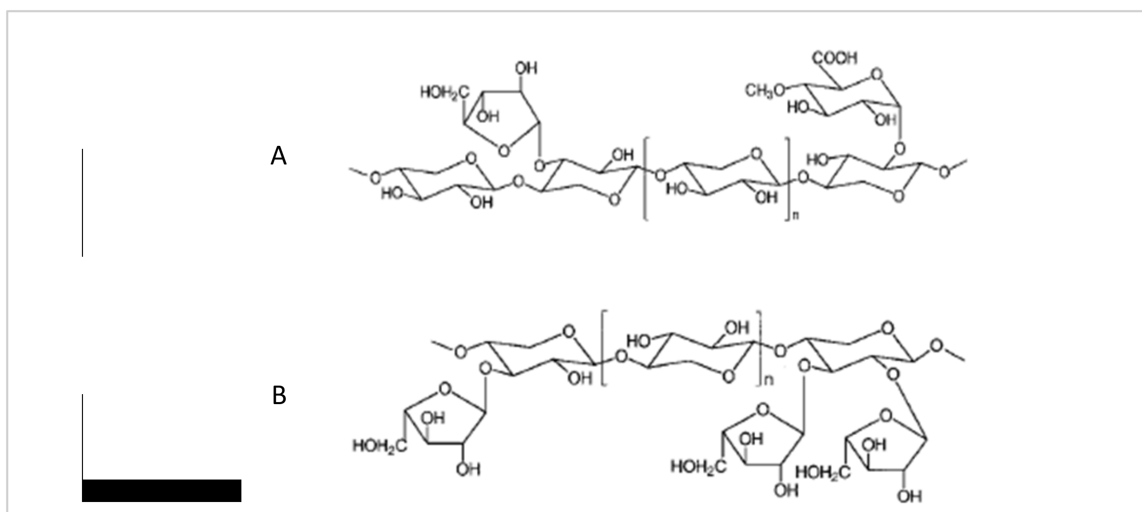

Figure 2.2 Chemical structure of xylans. (A) Arabinoglucuronoxylan (AGX), (B) Arabinoxylan (AX) (Ebringerova & Heinze, 2000).

2.4.4 Physiochemical properties of wheat arabinoxylan

AX possess a variety of physiochemical characteristics that influences their functional properties. Understanding the underlying properties influencing AX functionality is crucial to fully utilize the application potential of these polymers.

The highly substituted backbone of AX results in a stiff structural confirmation and is partly responsible for the high viscosity of AX in aqueous solutions (Izydorczyk & Biliaderis, 1995; Izydorczyk & Biliaderis, 1992a). Aggregation of these molecules are limited by steric hindrance caused by the arabinose side chains. This was demonstrated in a study by Izydorczyk & Biliaderis (1992a) who revealed that AX from wheat endosperm with high intrinsic viscosities had high A/X ratios. They also found other factors effecting viscosity such as ferulic acid content and the content of doubly substituted Xyl*p*. An increase in viscosity was observed with an increase in FA content and decrease in doubly substituted Xyl*p*.

The ability of AX to solubilise in water is also mainly dependent on the degree of substitution of the molecule. Fractions with a low A/X ratio are insoluble in water due to the aggregation of unsubstituted regions of the AX molecule. These insoluble aggregates are stabilised by hydrogen bonds and result in a more flexible configuration that is able to align with each other (Courtin & Delcour, 2002). Along with the substitution degree, the substitution pattern also affects the solubility of AX. Long stretches of unsubstituted xylose residues favour aggregation, whereas substitution prevents it. Molecular weight is another factor affecting solubility: lower molecular weight favours solubility (Izydorczyk & Biliaderis, 1992b).

An important property of AX is its capability of forming three dimensional networks (gels) in the presence of radical-generating agents (peroxidase/ H_2O_2 and laccase/O_2 systems). FA associated with AX has shown to be responsible for the oxidative gelation (Figueroa-Espinoza, Morell, Surget, & Rouau, 1999; Izydorczyk et al., 1990; Schooneveld-Bergmans et al., 1999). These phenolic acids can undergo oxidative coupling reactions,

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cross-linking AX chains through a dimerisation reaction. The disappearance of FA and formation of dehydrodiferulic acid (Di-FA) demonstrated the central role of FA in AX gelation (Izydorczyk, Biliaderis, & Bushuk, 1991).

Further investigation into the properties affecting gel formation resulted in the discovery of numerous other factors which also contribute to the gelling phenomenon. Izydorczyk & Biliaderis (1992a) observed differences in gelling potential of AX fractions from wheat endosperm with different molecular sizes. The fractions with a higher molecular size had a higher potential for gel formation. Some fractions with similar intrinsic viscosities did not however have the same gel forming potential. Therefore, other structural properties may also contribute to AX gelling capacity. AX molecules with less substitutions are more flexible, allowing the formation of a continuous gel network by facilitating cross-linking of FA of neighbouring chains. On the other hand, in the highly substituted AX the flexibility of the backbone may be limited and as a result limit the accessibility of the FA to cross-link. Dervilly-Pinel, Rimsten, Saulnier, Andersson, & Åman(2001) also found that in samples with the same intrinsic viscosity, stiffer gels were obtained with increasing FA content.

The solubility and gelling properties of AX play an important role in their application potential. Because it is a covalently cross-linked gel, AX gels form quickly, bind strongly and is very heat stable (Niño-Medina et al., 2010). The storage stability of a laccase induced gel was recorded over a six day period and the results indicate that after thermal inactivation of laccase the gel only lost 5% hardness and AX molecular weight rendering it more stable, compared to 43% and 20% decrease observed without this treatment (Carvajal-Millan, Guigliarelli, Belle, Rouau, & Micard, 2005).

These physiochemical characteristics of AX influence their functional properties which is of interest for application purposes. Endogenous water-soluble AX have been ascribed many functional properties in cereal grains, particularly in wheat flour, where their high water holding capacity is one of the most important characteristics affecting dough and bread properties (Izydorczyk, Biliaderis, & Bushuk, 1990).

2.5 Wheat flour constituents and bread improvers: Their interactions and role in bread making

During the bread-making process flour, water, salt, yeast and other specified ingredients are mixed in appropriate proportions into a viscoelastic dough which is subjected to fermentation and baking to deliver a final product. The quality of the final product can be measured by its manageability during mixing and development, dough consistency, loaf volume, crumb texture and finally taste. The desired properties can be acquired by understanding the underlying chemical and physical processes involved in bread-making and the interactions between the participating components.

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2.5.1 Starch

Starch consists of two major polymers, amylose and amylopectin. Amylose is a slightly branched molecule consisting of α-1,6 and 1,4-linked-D-glucopyranosyl units (Selinheimo et al., 2007) opposed to amylopectin which is very large and highly branched. Wheat starch commonly used in baking contains approximately 25% amylose and 75% amylopectin (Goesaert et al., 2005).

In the presence of ample water and at room temperature, starch granules absorb up to 50% of their dry weight of water, but will only swell to a limited extent. This process is reversible, but only below a specific temperature (the gelatinisation temperature). When a starch suspension is heated above the gelatinisation temperature, i.e. baking, it leads to irreversible loss of the molecular order of the starch granules in a process known as gelatinisation (Goesaert et al., 2005). When the suspension is cooled the starch polymers reassociate to a more ordered crystalline state. This process is termed retrogradation (Goesaert et al., 2005).

During storage, bread progressively loses its freshness and stales. Staling is influenced by a combination of various aspects including loss of moisture and flavour, crust toughening and an increase in crumb firmness (Dobraszczyk, 2003) which are all associated with starch.

2.5.2 Gluten

Gluten proteins are the major storage proteins in wheat. They form part of the endosperm of wheat grain and form a continuous matrix around the starch granules. Gluten proteins can be distinguished into two groups based on their functionality: monomeric gliadins and polymeric glutenins, which account for approximately 80% of the wheat proteins (Goesaert et al., 2005). These proteins play an important role in dough development and functionality, determining final bread quality with regard to crumb structure and loaf volume (Selinheimo et al., 2007). Due to the viscoelastic nature of these proteins they are able to form a continuous protein network during dough mixing (Selinheimo et al., 2007). The protein network is formed after hydration of the dough, via breaking and reforming of both covalent (disulphide) and non-covalent (hydrophobic and hydrogen) bonds between wheat proteins (Singh & MacRitchie, 2001).

During dough mixing, the resistance of the dough increases to an optimum level, where after it decreases during what is called over-mixing. The quality and quantity of gluten proteins largely determine dough mixing requirements and sensitivity to over-mixing. The quantity of gluten proteins refers to the gliadin/glutenin ratio (Weegels, Groot, Verhoek, & Hamer, 1994). In the protein network, gliadins and glutenins fulfil different roles, glutenin polymers form a continuous network that provides strength and elasticity to the dough whereas gliadins act as plasticisers of the glutenin system. For good quality bread-making, the right balance between dough viscosity and elasticity/strength is required. The quality of gluten proteins refers to the composition, structure and/or size distribution of the glutenin polymers (Scanlon & Zghal, 2001; Weegels et al., 1994). During fermentation and the initial stages of baking, carbon dioxide is produced. The gluten

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network plays an important role in retaining carbon dioxide by allowing gas cells to expand without rupturing. During this process glutenins provide strength and elasticity while gliadins provide extensibility in the dough (Dobraszczyk, 2003).

2.5.3 Gluten-starch network

Dough can be divided into two phases, the gas phase which consists of gas cells form during mixing and the solid phase which is made up of the gluten-starch matrix. During proving no new gas cells can be formed but subdivision of existing cells is possible though sheeting and moulding. During fermentation, the ability of these gas cells to remain intact is critical for gas retention which influences final loaf volume. Gas cells are enclosed with a thin liquid film layer which is stabilised by surface active components and sustained by the gluten-starch matrix (**Figure 2.3**). The surface active compounds such as endogenous polar lipids, proteins and AX dissolved in the dough liquid phase may have a positive effect on gas retention by stabilising the films allowing them to expand without rupturing (Gan et al., 1995).

During the advanced stages of fermentation the gluten-starch matrix cannot separate the gas cells completely, due to the expansion of the cells, this results in areas containing only the liquid film layer between the cells. During the baking process, the rate of cell expansion increases until the films is unable to enclose the cells and ruptures. This converts the foam structure of dough into the open sponge structure of bread (Gan et al., 1995).

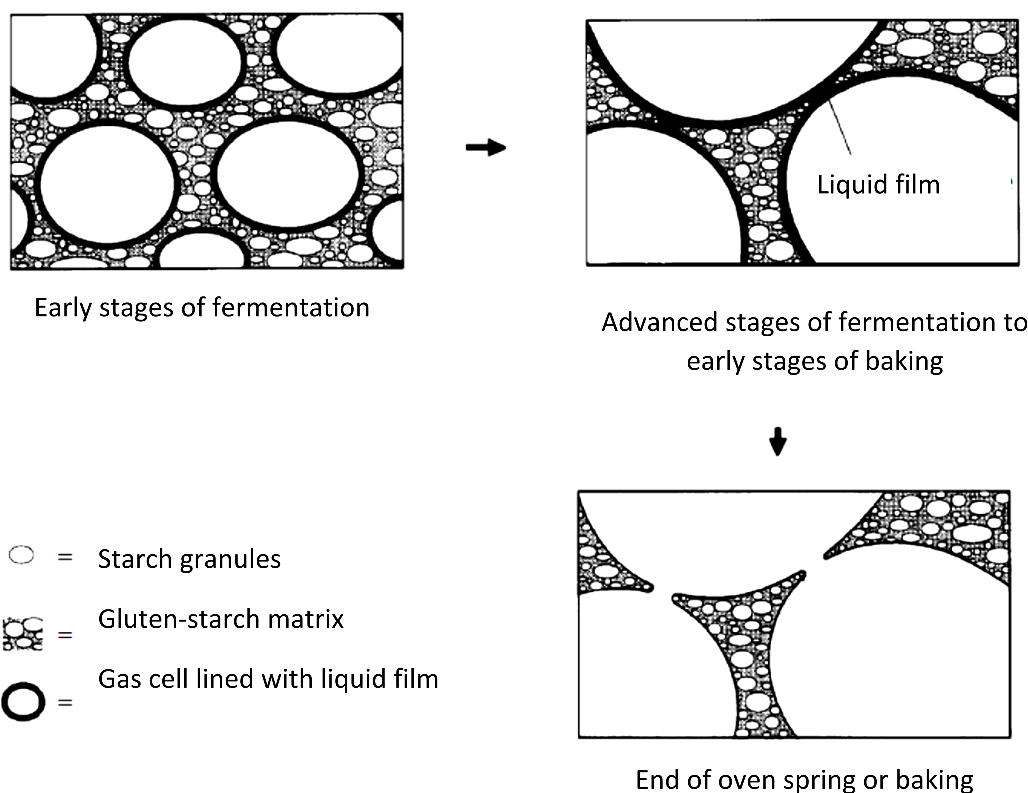

Figure 2.3 A model of dough expansion (Gan et al., 1995).

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2.5.4 Arabinoxylans

AX are of particular interest due to their water holding capacity, ability to form viscous solutions and gels in the presence of oxidising agents/enzymes (Izydorczyk & Biliaderis, 1995). In literature, the effect of AX on dough rheology and final bread properties are contradicting. These differences may occur due to differences in AX extraction methods used, starting material, nature of the AX (WEAX or WUAX), MW and purity as well as the bread making technique and quality evaluation methods implemented (Courtin & Delcour, 1998).

The extractability of the AX greatly determines its role in the bread-making process. Higher content of water-unextractable AX (WUAX) results in decreased extensibility of dough and an increase in resistance to extension. This supports the hypothesis that WUAX interfere with optimum gluten formation during dough mixing by competing for water (Goesaert et al., 2005). This effect can be corrected for by adding more water or by addition of xylanase (Wang et al., 2004). Xylanase act by making the WUAX water soluble and reducing its water binding capacity. AX can also directly affect the gluten network through linkages of bound ferulic acid to gluten proteins resulting in a decrease in dough extensibility (Wang et al., 2004). During fermentation and baking water-extractable arabinoxylan (WEAX) reduces the diffusion rate of carbon dioxide leading to better gas retention which results in prolonged oven rise and increased loaf volume (Gan et al., 1995).

Starch, gluten and AX have different water-holding capacities and will therefore compete for the available water in the dough. It then follows that the addition of AX will affect the availability of water for gluten development and the extent of the decrease in availability will be determined by the molecular weight/intrinsic viscosity of the added AX (Wang et al., 2004). Literature has demonstrated that soluble AX with higher intrinsic viscosities and therefore higher water binding capacities will require more water to correct for their negative effect on the gluten network (Wang et al., 2002).

During bread staling, the interactions between AX and starch play an important role in retarding the starch retrogradation process (Goesaert et al., 2005). AX may interfere with starch intermolecular structure and also has the potential to affect the water distribution in the dough which can slow down the staling process (Biliaderis et al., 1995).

2.5.5 Enzyme active soy flour as bleaching agent

Untoasted soy flour contains active enzymes which are useful during bread making processes containing yeast. Enzyme active soy flour has been shown to improve dough handling properties and improve crumb texture and colour and is included in some commercial white bread formulations. Lipoxygenase is an enzyme present in soy flour and reacts with flour lipids and atmospheric oxygen resulting in a bleaching effect of flour carotenoids. Only a small percentage (0.5%) of soy flour addition is required for effective bleaching and can be used to replace chemical bleaching agents (Brown, 1993). Soy flour is also rich in the essential amino acid, lysine, which can improve the protein quality of breads (Figoni, 2004).

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2.5.6 Oxidising agents: Improvement of bread quality

In the gluten network, disulphide bonds are the most pronounced linkages as they hold together the glutenin subunits. By interfering with these linkages, oxidising and reducing agents can affect polymerisation of the gluten network, thereby changing the mechanical and rheological properties of the dough (Bonet et al., 2006). Chemical oxidising agents, such as potassium iodate and potassium bromated, are able to reform intermolecular disulphide bonds of the gluten proteins increasing the dough strength (Bonet et al., 2006). Due to the toxic nature of these chemicals, determining functional use of alternative oxidising agents have been the basis of many recent studies (Labat et al., 2001; Primo-Martin & Martinez-Anaya, 2003; Emilia Selinheimo et al., 2007). Exogenous oxidising enzymes such as tyrosinase, laccase and glucose oxidase have received increasing interest due to their impact on gluten (Goesaert et al., 2005).

Laccase (benzene-diol:oxygenoxidoreductase) is a multi-copper enzyme catalysing a one electron transfer mechanism with accompanying reduction of molecular oxygen to water. The resultant oxidation products are reactive radicals that may undergo further, non-enzymatic reactions, such as cross-linking of monomers or degradation of polymers. Laccase addition in bread-making results in increased dough strength, stability and reduced stickiness (Labat et al., 2001; Orel et al., 2008). In addition an increase in loaf volume and improved crumb structure have been observed (Flander et al., 2008; Selinheimo et al., 2006). Although in contrast, Selinheimo et al. (2007) reported no significant improvement of laccase addition on loaf properties.

Glucose oxidase oxidises β-D-glucose to D-glucuronic acid which produces hydrogen peroxide. Similarly to laccase, reactive radicals are formed which can then react with each other non-enzymatically (Bonet et al., 2006; Primo-Martín et al., 2003). Glucose oxidase treatment of bread dough modifies the gluten proteins through the formation of disulphide bonds and the high molecular weight glutenin subunits were shown to be the most susceptible to oxidation. Dough strengthening and improved bread quality can be acquired with glucose oxidase addition in wheat bread making. However, inverse effects were observed with excessive enzyme addition (Bonet et al., 2006).

Oxidative enzymes are useful in hampering the softening effects of xylanases on dough and can therefore contribute to further improvements of bread quality (Martinez-Anayal & Jimenezz, 1997).

2.5.7 Xylanase: counteraction of adverse effects of water insoluble arabinoxylan

AX functionality in bread-making can be optimised through the use of endoxylanases. These enzymes are able to hydrolyse the xylan backbone internally. Endoxylanases are routinely used in bread-making to improve dough and bread properties such as softness, extensibility, oven spring and loaf volume. The impact of endoxylanases on AX functionality is strongly dependent on their selectivity toward WUAX and WEAX as substrates (Courtin & Delcour, 2002). The use of endoxylanases that preferentially attack WUAX, causing a reduction in the WUAX level and inceasing the level of solubilised AX, has a positive effect on dough and

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bread-making properties (Courtin & Delcour, 2002). In contrast, endoxylanases that preferentially hydrolyse WEAX, reducing their molecular weight, results in no improvement or has a negative impact on dough and bread properties. In excess, both of these enzymes result in slack and sticky dough and loaves with poor crumb structure, due to extensive degradation of all AX and the accompanying loss of water-holding capacity of the dough (Cleemput et al., 1997; Wang et al., 2004).

2.5.8 Synergetic effect of enzyme combinations on dough and bread properties

As discussed earlier, laccase leads to the oxidation of AX solutions through the cross-linking of FA residues. The hydrogen peroxide generated by glucose oxidase can oxidise thiol groups into disulphide bonds in gluten, but the enzyme may also lead to the oxidative gelation of AX. Possibly acting through different mechanisms, the combined application of both oxidising enzymes has proven to act in a synergistic way to improve bread quality (Primo-Martin & Martinez-Anaya, 2003; Primo-Martín et al., 2003). Primo-Martín et al. (2003) established that the use of xylanases in combination with oxidising agents (glucose oxidase and laccase) led to improved bread properties including improved loaf volume, decreased firmness and retarded staling. These results correlated to WEAX with higher molecular weight and arabinose and xylose content. Similar results were obtained by Selinheimo et al. (2007) with the combined addition of xylanase with either laccase or tyrosinase.

Another study conducted by Selinheimo et al. (2006) demonstrated the positive effects of enzyme combination on dough properties. The authors found that addition of laccase alone increased dough strength and decreased dough extensibility resulting in hardening of doughs. It was concluded that the effects of laccase were mainly due to the cross-linking of AX resulting in a strong AX network. In contrast to laccase, xylanases decreased dough strength and increased extensibility. Combined addition of laccase and xylanase resulted in a decrease of the hardening effects of laccase with an increase of xylanase concentration. This is due to the hydrolysing effect of xylanase, resulting in a decrease in the availability of AX molecules to form cross-linking networks.

The effects of oxidising agents and xylanase on the gluten-network, is also of interest because changes in this network significantly influences the quality of the dough and final product. The presence of WEAX associated with gluten, has been reported to lower the extensibility of dough (M. Wang et al., 2002). The negative effect of WEAX was attributed to direct linking of FA to gluten proteins (M. Wang, Vliet, et al., 2004). This effect was decreased by a reduction of WSAX content, in the gluten-network, by addition of xylanase alone, as well as in combination with oxidising agents (Primo-Martín et al., 2003).

These results were contradicted by the studies of (Labat et al., 2001). The authors reported that no covalent complexes were detected between AX and gluten proteins in oxidised doughs. Labat et al. 2002 proposed that free FA is more likely to react with proteins rather than feruloylated AX.

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2.6 Influence of physiochemical properties of arabinoxylans on their functionality in bread making

The overall effects of AX on the functional performance of dough and the subsequent bread quality, depend on their structural features and physiochemical characteristics (Selomulyo & Zhou, 2007).

2.6.1 Molecular weight

Molecular weight (MW) of AX vary depending on the isolation method used for extraction. Comparison of the effects of high molecular weight (HMW) and low molecular (LMW) AX on dough water absorption revealed that both increase absorption but HMW AX increased absorption to a greater extent which resulted in increased loaf volumes (Izydorczyk & Biliaderis, 1995). In contrast to this, Courtin & Delcour (1998) observed a decrease in water absorption with LMW AX addition but AX addition still resulted in increased loaf volume. This suggests that high water binding capacity resulting in higher baking absorption cannot be the determining factor for LMW AX functionality.

2.6.2 Viscosity and foam stability

Addition of AX to bread dough increases the viscosity of the aqueous phase between the gluten molecules and the gas cells (dough liquid phase), resulting in positive effects on dough structure and its stability, especially during early baking when high pressures are formed in the gas cells. The increased stability of the film at this stage beneficially prolongs oven rise and prevents coalescence, leading to a higher loaf volume and improved crumb structure (Courtin & Delcour, 2002). The positive effect of AX in bread making is dependent on the concentration of AX in the system, with greater than optimum levels causing excessively high viscosity and detrimental effects (Biliaderis et al., 1995). The optimum concentration is dependent on MW of the AX and characteristics of the base flour used (Biliaderis et al., 1995).

The ability of AX to retain gas in dough and protect protein foam against thermal disruption was related to their viscosity and film forming properties (Courtin & Delcour, 2002; Ebringerova & Heinze, 2000). It is thought that the high viscosity of AX will add to the strength and elasticity of gluten-starch films surrounding the gas cells and thus slow down the rate of carbon dioxide diffusion from dough during baking (Izydorczyk & Biliaderis, 1995).

2.6.3 Water solubility

One of the main factors affecting AX solubility is the degree of substitution. The presence of a high numbers of arabinose molecules prevents aggregation of unsubstituted xylose residues. The substitution pattern of arabinose side chains on the xylan backbone also effects the interaction of AX molecules both with one another and other cell wall constituents. This will in turn affect the solubility of the AX polymer. Areas of the

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AX molecule where there are large amounts of unsubstituted xylose residues allow intermolecular aggregation. The presence of noncovalent interactions between AX molecules with low arabinose substitution patterns and fragments of β-glucans also contribute to the formation of insoluble aggregates. This suggests that in the plant cell wall, poor water solubility of AX molecules with low A/X ratios could be due to these interactions.

Water solubility of AX is therefore linked to the degree of substitution (A/X ratio), substitution pattern and chain length of the polymer (Izydorczyk & Biliaderis, 1992b). Insoluble AX have few arabinose side chains and long regions of unsubstituted xylan chains which form a flexible twisted threefold ribbon-like structure (Izydorczyk & Biliaderis, 1995), which is insoluble due to intermolecular H-bond linkages. With the introduction of arabinose side chains on the xylan backbone, the ability for this intermolecular linking is reduced, enhancing water solubility, meaning AX molecules with higher degrees of substitution are expected to be more water soluble (Izydorczyk & Biliaderis, 1995).

2.6.4 Water holding capacity

AX absorbs water well due to hydrogen bonding of the water molecules to OH-groups on the polymer chain (Biliaderis et al., 1995; Courtin & Delcour, 1998, 2002; Wang et al., 2002). Courtin and Delcour (2002) reported WUAX being able to hold 7-10 times their weight in water, with the water holding capacity of WEAX slightly lower at 4-6 times their weight. Other studies have shown that AX addition to bread dough results in increased resistance to mixing can be compensated for by the addition of 2-10 times their weight in water depending on the solubility and source of the AX (Courtin et al., 2008; Guttieri, Souza, & Sneller, 2008). Studies into the water absorption capacity of arabinoxylan have found an increase in Farinograph absorption when arabinoxylan is added to flours at different levels. Izydorczyk & Biliaderis (1995) observed that increasing the AX content from 0.5 to 1.3% resulted increased water absorption. Similarly, Michniewicz et al. (1991) demonstrated a linear increase in water absorption with an increase in the amount of AX added.

2.7 Improvement of nutritional properties

Apart from improvement of final product quality research is also aimed at increasing nutritional value of bread. Because bread is one of the most regularly consumed processed food, it has great potential as a source of added nutrition. Numerous studies have been dedicated to increasing dietary fibre intake to address increasing diet associated medical conditions (Goñi & Hervert-Hernández, 2011; Inglett, 1998; Izydorczyk & Dexter, 2008; Vitaglione et al., 2008; Weickert et al., 2005).

Most studies aim to investigate the combination of white bread flour with other flours such as barley (Cavallero, Empilli, Brighenti, & Stanca, 2002) and oat (Flander et al., 2008) to increase the dietary fibre content of the bread and improve its nutritional value. These studies result in final bread products with reduced desirable properties and therefore require further development and improvement, usually these

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studies include enzyme additions to reduce the negative effects of the dietary fibre addition (Flander et al., 2008).

The addition of dietary fibre as a function to deliver antioxidants such as FA have also been investigated (Vitaglione et al., 2008). AX polymers or oligosaccharides also have the potential to function as prebiotics in the human intestinal tract which can decrease the occurrence of chronic colon diseases (Topping, 2007)

2.8 Conclusion

Purification of AX from wheat bran can be achieved through alkaline extraction. Due to the physiochemical characteristics of AX they contribute certain functional properties to dough and bread. The high water-holding capacity of AX leads to increased dough absorption allowing AX to act as a partial flour replacer. With the water-holding capacity of AX being substantially higher than flour (10 vs 3 g/g, respectively) it is proposed that less AX is required to replace a larger amount of flour, while maintaining bread properties.

Compared to the studies found in literature, where nutritional value is the main factor motivating functional food development, the current study will take a different approach. First and foremost, the industrial application of the AX, extracted from wheat bran, will be investigated. The application of AX as partial flour replacer to reduce the amount of flour required for bread production is the main objective to potentially reduce production costs, with the added advantage of increasing the nutritional value.

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2.9 Research statements

Arabinoxylan can be extracted from wheat bran to produce a crude AX extract using mild alkaline extraction.

Arabinoxylan, extracted from wheat bran, can be implemented as a partial flour replacer in the bread making process while maintaining bread properties.

The application process holds economic implications for the bread industry.

2.10 Research objectives

The objectives of this study was to:

- optimise alkaline extraction from wheat bran to produce a crude AX extract for application in the bread making process
- evaluate the effect of AX addition on dough water absorption and physical bread properties
- determine to what extent AX addition can replace flour during the bread making process
- evaluate the effect of oxidative enzyme addition (laccase) on bread properties
- evaluate the effect of AX-flour replacement and laccase addition on the economics of bread making

2.11 Thesis scope

AX extraction and partial characterisation was conducted. Only chemical compositional analysis was conducted to determine the main properties of interest, AX and FA content of the extracts. Complete characterisation was not conducted. Production cost of AX was determined according to estimated costs.

Application of the extracted AX in bread making was conducted only on small scale (100 g) and not commercial scale (700 g). Farinograph properties and physical bread properties were investigated as response for AX addition and flour removal. Farinograph water absoption was used to determine water addition during baking trials. Economic impact of AX-flour replacement on bread industry was determined according to an industrial template used in the research and development sector.

Finally, this thesis serves as a starting point for investigation and optimisation of both AX extraction and functional application in the baking industry.

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3 ARABINOXYLAN EXTRACTION FROM WHEAT BRAN: OPTIMISATION AND CHARACTERISATION

3.1 Abstract

The major dietary-fibre component of wheat bran, arabinoxylan (AX), has application potential in the baking industry as a partial flour replacer. In this chapter the extraction of AX was optimised to produce a crude AX fraction for application in the bread making process.

Alkaline extraction was investigated to determine the effect of three extraction parameters: alkaline concentration, extraction time and temperature, on AX extract yield and purity. Final extracts were obtained after alkaline extraction, centrifugation (1000 *g*, 15 min), neutralisation (pH 5.5), dialysis and lyophilisation. With extraction parameters of 0.5 M NaOH, 4 hours and 80°C, AX content of the final fraction obtained on small scale was maximised at 44.3% also taking into account ferulic acid content, which is of interest for the application process. On large scale, with inclusion of a discolouration and precipitation step, a final extract containing 49.3% AX was obtained. The two extracts obtained on small scale (E1) and large scale (E2) both had high average molecular weights (620 000 and 470 000 Da, respectively) and A/X ratios of 0.7 and 0.6. Using anion-exchange chromatography the whiteness index of the final extract was increased from 33 to 93. For the application purpose, the lighter extract colour will have a less prominent effect on bread colour and is therefore advantageous.

In conclusion, the crude AX fraction obtained by alkaline extraction was optimised for application in the bread-making process.

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3.2 Introduction

Wheat bran is a by-product of the milling industry and comprises mainly dietary fibre and protein. Due to the relatively high protein content wheat bran has commercial value as animal feed.

In monogastrics, however, the viscoelastic properties of AX reduces the rate of digestion and absorption of nutrients (Schooneveld-Bergmans, van Dijk, Beldman, & Voragen, 1999). This undervalued fibre fraction may hold potential in other applications where properties such as viscosity and high water binding capacity are of value and not of detriment.

Fractionation of wheat bran has been investigated for more than two decades, specifically for the production of arabinoxylan (AX) (Ebringerová & Hromádková, 1999; Faurot et al., 1995; J Hollmann & Lindhauer, 2005; Juergen Hollmann et al., 2009; Mandalari et al., 2005). The major drawback of industrialisation of AX extraction is the purification processes required to produce highly pure AX as well as the costs associated with the processes (Hollmann & Lindhauer, 2005). The viscosity of wheat bran, once in alkaline solution, may also impede the efficiency of the process requiring the use of very low solid loadings for optimal extraction (Schooneveld-Bergmans, van Dijk, Beldman, & Voragen, 1999).

AX extraction hold two distinctly different benefits. Firstly, removal of AX from animal feed has the potential to render it more digestible to monogastric animals, while secondly, AX could be sold as a high value functional ingredient for use in the bread making industry.

Literature has shown that the physicochemical properties of AX have an effect on its functional behaviour. One of the key functional properties of AX is their ability to form highly viscous solutions, which increases with increasing molecular weight (MW) and degree of substitution. High MW also affect foam stability due to increased viscosity (Goesaert et al., 2005; Izydorczyk & Biliaderis, 1992a, 1992b). Water solubility and water holding capacity are also affected by the conformation of the polymer, with the degree of substitution and degree of polymerisation being of particular importance, whilst oxidative cross linking and gel formation are affected by the presence of ferulic acid (Berlanga-Reyes, Carvajal-Millan, Lizardi-Mendoza, Islas-Rubio, & Rascón-Chu, 2011; Robertson, Faulds, Smith, & Waldron, 2008). These physicochemical properties all play a role in the functional effect of AX on the bread making process.

In addition to the physiochemical properties of AX, the physical and chemical properties of the extract containing the AX is important for its application in the baking process. Firstly, high AX content is important to reduce the amount of extract required for the application. Secondly, extract colour should be taken into account as this may affect the appearance of the final product.

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The aim of this study was to produce an AX extract with the required properties for the application process whilst using cost effective methods for obtaining these fractions. Therefore no enzyme hydrolysis or purification steps were included in the optimised process conditions used for economic evaluation of AX production.

Crude AX fraction was extracted from wheat bran, on small and large scale, for application in the baking process. The objectives were therefore to:

- Optimise AX extraction under alkaline conditions
- Produce an extract with the required chemical and physical properties for successful application
- Assess the cost implications of in-house production of a crude AX fraction from wheat bran

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3.3 Materials and methods

3.3.1 Materials

Commercial wheat bran (9% moisture) was provided by Essential Grains, Pioneer Foods (Paarl, South Africa). Sodium hydroxide pellets (ACS reagent, >98%), potassium hydroxide pellets (ACS reagent, >85%), 72% sulphuric acid (standard solution), acetic acid (ACS reagent, >99.7%), ethyl acetate (LC-MS CROMA SOLV)) and ethanol (>96% v/v) were obtained from Sigma Aldrich (Germany). Wheat arabinoxylan (high viscosity) was purchased from Megazyme (Bray, Ireland).

3.3.2 Wheat bran sample preparation

A representative sample of wheat bran was obtained using the coning and quartering method as described by the British Standards DD CEN/TS 14780:2005 “Solid biofuels - Methods for sample preparation” (British Standards, 2005). The sample was milled using a laboratory Retch ZM200 mill equipped with a 2 mm circular blade to produce samples of approximately +425 µm, which were further milled to approximately +250 µm. The sample was screened in a Retch AS200 shaker to obtain wheat bran fractions between 250 and 425 µm, which were used for compositional analyses (NREL/TP-510-42620).

3.3.2.1 Compositional analysis of wheat bran

The compositional analysis of the wheat bran samples was done using analytical methods provided by the NREL (National Renewable Energy Laboratory, 2010). The different analytical methods used are given in **Table 3.1**. The starch content was determined using the K-TSTA 09/14 assay kit obtained from Megazyme (Bray, Ireland)

Table 3.1 Standard NREL methods used for compositional analysis of wheat bran

Analysis	Analytical method
Moisture content	NREL/TP-510-42621
Ash content	NREL/TP-510-42622
Water and solvent extractives	NREL/TP-510-42619
Klason lignin and carbohydrate composition	NREL/TP-510-42618

3.3.3 Wheat bran pre-treatment for arabinoxylan extraction

Starch removal was conducted prior to alkaline extraction at 40°C for 15 min at a water to bran ratio of 1:10 (w/v) in a shaking water bath. The starch milk and bran were separated by filtration (500 µm) where after the bran was washed with three volumes of water and dried in a benchtop oven (EcoTherm, Labotec) at 40°C for 48 h. Samples were milled using a laboratory Retch ZM200 mill equipped with a 2 mm circular blade.

3.3.4 Optimisation of arabinoxylan extraction on small scale (275 ml)

Alkaline extraction conditions were optimised using conditions obtained from literature as set out in **Table 3.2**. A full factorial design was used with three factors at two levels (2^3) using Statistica 64 software.

3.3.4.1 Experimental design

Table 3.2 Arabinoxylan alkaline extraction optimisation conditions

Run	Alkaline concentration (M)	Extraction time (min)	Extraction temperature (°C)
1	0.5	90	60
2	1	90	60
3	0.5	240	60
4	1	240	60
5	0.5	90	80
6	1	90	80
7	0.5	240	80
8	1	240	80

Approximately 10 g of destarched wheat bran was mixed with 275 ml NaOH (0.5 or 1 M) and incubated at various temperatures (60 or 80°C) for a set time (90 or 240 min) (**Table 3.2**) with agitation (30 *g*). After the extraction period the supernatant was recovered by centrifugation (10 000 *g*, 15 min). The supernatant was neutralised with acetic acid to pH 5.5 and dialysed (seamless cellulose, retention 99.99%, MWCO 12400) against distilled water at room temperature to remove the sodium ions. The samples were stored at -80°C and lyophilised (VirTis benchtop freeze dryer, SP Industries). The sample obtained at the optimum condition is referred to as extract 1 (E1) in this thesis.

3.3.5 Large scale production and purification of arabinoxylan (27 l)

The extraction and purification process was adapted from the methods of Zeitoun et al. (2010). Briefly, destrached bran was treated with 0.5 M NaOH (S/L 1:27) at 50°C for 240 min under continuous stirring (30 *g*) (**Figure 3.1**). After alkaline extraction the sample was centrifuged (2000 *g*) to obtain the liquid fraction (**Figure 3.2a**). The liquid was concentrated by a factor of 2 using an ultrafiltration system fitted with a hollow fibre polyethersulfone membrane (MWCO 30 kDa, 1.15 m^2). After concentration some salts and small impurities were removed by diafiltration using a TIA filtration unit (**Figure 3.2b**). The retentate was discoloured by anion-exchange chromatography using FPA91Cl resin (Rohm and Haas) by batch treatment with over-head agitation. The sample was neutralised with acetic acid to pH 5.5 and precipitated with 3 volumes of 96% ethanol. After precipitation at 4°C for 24 h the sample was filtered, redissolved in water and freeze dried. The final sample will be referred to as extract 2 (E2).

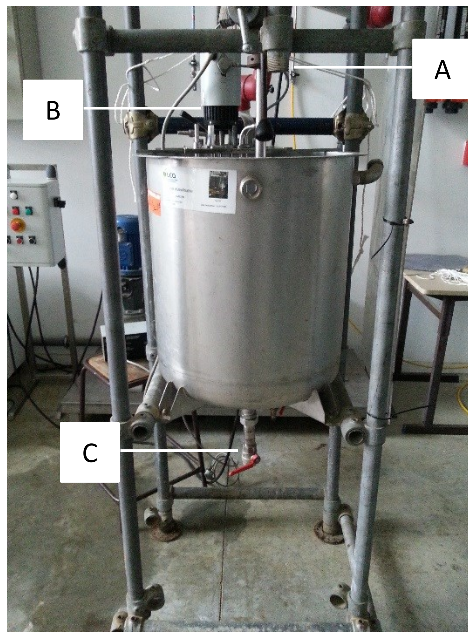

Figure 3.1 Batch extraction unit used for large scale AX extraction. (A) Over-head stirrer (B) heating unit and (C) emptying valve.

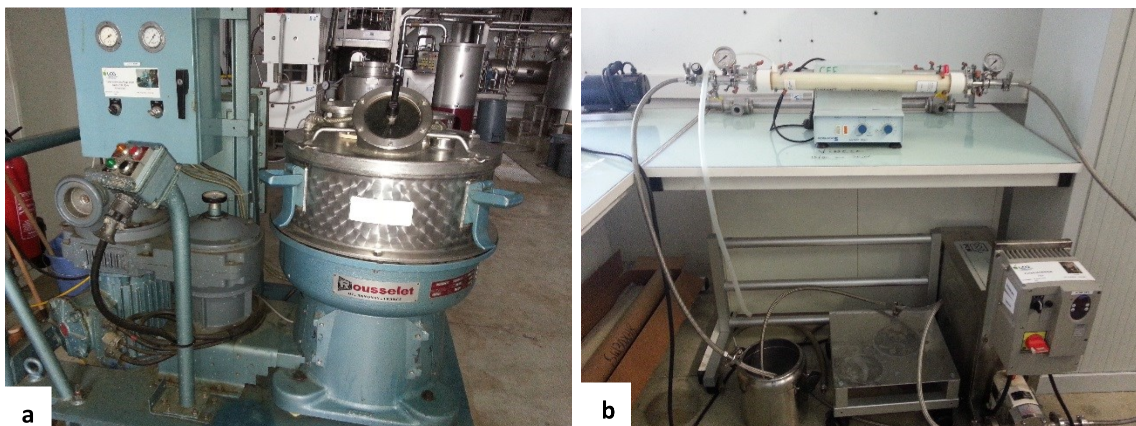

Figure 3.2 Centrifuge (**a**) and ultrafiltration unit (**b**) used for large scale extraction of AX from wheat bran.

3.3.6 Chemical and physical characterisation of the arabinoxylan extract

Moisture and ash content was determined using the NREL methods outlined in **Table 3.1**. The protein content was determined with a nitrogen analyser (Truspec® N Elemental Determinator, Leco, St. Joseph, Michigan) according to the Dumas combustion method, a factor of 3.7 was used for conversion of nitrogen to crude protein (AOAC 992.15 method).

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3.3.6.1 Monosaccharide content determination

The extracted samples were milled using a laboratory Retch ZM200 mill equipped with a 2 mm circular blade. Using an adapted method from Izydorczyk & Biliaderis (1992b), duplicate samples were hydrolysed with 1 M H_2SO_4 at 121°C for 90 min where after the pH was increased to 3-4 using 7 M KOH. The samples were filtered with 0.22 µm filters and analysed by high performance liquid chromatography (HPLC) to determine the monosaccharide composition of the extracts.

The system was equipped with a Biorad Aminex H-column (300 × 7.8 mm). Isocratic separation was performed using 0.005 M H_2SO_4 at 65°C. The elution was monitored with a refractive index detector. Detection was done by UV absorbance of 215 – 285 nm. After hydrolysis and filtration the remaining solid was dried and represented the insoluble, Klason lignin.

3.3.6.2 Ferulic acid content determination

Approximately 50 mg sample was saponified with 3 ml 4 M NaOH under nitrogen for 18 h. Samples were centrifuged (3000 *g*, 10 min) and acidified to pH 2 using 2 M HCl. The ferulic acid was extracted with 3 times 3 ml ethyl acetate. Ferulic acid was used as an external standard. The ethyl acetate phases were combined and dried under nitrogen. The residue was redissolved in 2 ml H_2O:MeOH (1:1) and filtered with 0.22 µm filters. Solutions (20 µl) were separated on a PPS Suprema 3000 column (300×8 mm) by elution with H_2O at 70°C. The elution was monitored with ELSD detection.

3.3.6.3 Determination of molecular weight (MW)

Molecular weight distribution was determined using gel permeation chromatography (GPC). Solutions obtained were separated on a Dionex 3000 column by elution with H_2O at 70°C. Molecular weight markers were Pulin standards with Mw 400 Da – 800 kDa.

3.3.6.4 Infrared spectroscopy for determination of functional groups

The infrared (IR) spectra of E1, E2 and highly pure AX was measured with a NEXUS model FTIR instrument (Thermo Scientific, Nicolet, USA). ATR was used in conjunction with the IR spectroscopy. The IR spectra were accumulated using 32 scans and a resolution of 4 cm^{-1}, representing data point spacing of just under 2 cm^{-1}. The final format of the spectra was in absorbance over the range of 4000 – 650 cm^{-1}. The spectrometer was equipped with a Ge-on-KBr beamsplitter and DTGS/CsI detector and was not purged with HP nitrogen gas during recording. The operating and data manipulating software was done with OMNIC (Version7).

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3.3.6.5 Determination of arabinoxylan extract colour

The colour of the final crude AX extracts (E1 and E2) was measured using a Konica Minolta Spectrophotometer (London, UK). Whiteness of samples were compared and evaluated for International Commission on Illumination (CIE) standard illuminant D65 using the whiteness index (WI) CIE where a WI closer to zero indicates a whiter sample. A second measure of colour is represented by the lightness of the sample (L^*) which represents the vertical coordinate of a three dimensional system of colours and has values ranging from 0 (black) to 100 (white). The other two coordinates are a^* (green and red) and b^* (blue and yellow).

3.3.7 Arabinoxylan production cost estimation

AX production cost was estimated by taking into account the material and chemical costs as well as running costs (energy requirements) but not labour or set-up costs. The evaluation of production cost was only aimed to serve as an estimation for economic evaluation.

3.3.8 Statistical Analysis

Analysis of variance (Statistica 64) was conducted to determine the effect and interaction of factors on the response during optimum extraction conditions determination on small scale. Further Fisher least significant difference (LSD) post-hoc test was performed to identify statistical differences between samples.

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3.4 Results and discussion

3.4.1 Composition of wheat bran and destarched bran

The wheat bran contained approximately 7% fermentable starch (**Table 3.3**) which is mainly found on the outside of the bran particles and originates from the endosperm which is not effectively removed during the milling process. For the alkaline extraction step, prior starch removal is required to prevent increased viscosity during extraction at elevated temperatures. Starch affects the viscosity of solutions due to its swelling capacity and will therefore affect the efficiency of the extraction process (Schooneveld-Bergmans et al., 1998). Furthermore, during ultrafiltration the starch particles block the pores of the membrane resulting in poor flux.

The residual starch was successfully removed during the destarching step, as determined using a total starch content assay kit, with the destarched bran containing less than 1% starch (**Table 3.3**). The ash content was also slightly decreased during this process. The remaining glucose found in the destarched bran originates from the cellulose component and possibly some resistant starch. Arabinoxylan (AX) is a major constituent in wheat bran accounting for approximately 30% of the total composition of the destarched bran. The wheat bran composition correlates well with what has been reported in literature (Bergmans et al., 1996; Hollmann & Lindhauer, 2005; Maes & Delcour, 2002).

From the composition results it can be deduced that arabinoxylan constitutes the majority of the wheat bran composition. The aim is to extract and separate this component from the other bran components, and to use this crude AX extract in the bread making process.

Table 3.3 Chemical composition of wheat bran (% dry weight)

Component	Wheat bran[b]	Destarched bran[b]
Ash	6.9 ± 0.1	4.8 ± 0.2
Glucose	29.0 ± 1.2	21.9 ± 0.5
Xylose	22.1 ± 0.7	23.2 ± 0.6
Arabinose	10.7 ± 0.2	13.2 ± 0.3
Lignin	8.9 ± 0.8	9.5 ± 1.2
Protein	18.9 ± 0.2	22.4 ± 0.7
Starch	6.8 ± 0.3	0.8 ± 0.5
Arabinoxylan (AX)[a]	26.2 ± 0.9	29.1 ± 0.9

[a]AX - Arabinoxylans = 0.88 (%Xylose + %Arabinose)
Values ± SD with n = 3 independent samples

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3.4.2 Optimum conditions for arabinoxylan production from alkaline extraction

Of the eight conditions investigated, condition 4 (1 M NaOH, 240 min, 60°C) delivered a crude AX fraction with the highest AX yield (44.7%) and purity (49.7%) (**Table 3.3**).

From the extraction optimisation results it was determined that NaOH concentration and extraction time significantly affected the extract properties of interest. The extraction temperature however did not have a significant effect on either AX content or yield of the extract (**Figure 3.4**). For AX yield, NaOH concentration and extraction time as well as NaOH concentration and temperature showed interaction (**Figure 3.4a**). AX content was only affected by the interaction of NaOH and temperature (**Figure 3.4b**). The optimum results (condition 4) indicate that higher NaOH concentration and a longer extraction time is advantageous for the production of an extract with the desired characteristics (**Figure 3.3**). In contrast to this, condition 1 (0.5 M NaOH, 90 min, 60°C) produced an extract with the lowest purity (33%) and low yield (30.6%) caused by low NaOH concentration and a short extraction time. Thus both alkaline concentration and extraction time aid in the release of AX from other fibre constituents.

When water-unextractable arabinoxylans (WU-AX) are treated with alkali, bridges between AX molecules are broken resulting in the release of a large part of the WU-AX molecules from the cell wall matrix which renders them water-soluble (Courtin & Delcour, 2002). This process involves disruption of the hydrogen and covalent bonds in the cell wall matrix (Fincher & Stone, 1986). Hydrogen bonds between cellulose and AX can also be broken by hydroxyl ions, which cause hydrolysis of ester linkages allowing solubilisation of part of the WU-AX (Cyran, Courtin, & Delcour, 2004). This was reflected by the higher AX content (49.7 and 46.7%) in the AX extracts obtained by longer extraction times (240 min) and higher alkaline concentrations (1 M) (**Table 3.4**).

Similar yields and purities have been reported in literature for samples obtained from wheat bran by alkaline extraction. Jacquemin et al. (2012) obtained a final extract with a 36.5% yield and 48.4% purity. Protein contamination of the final extract was 12.9% protein which is similar to the values obtained in the current study. Without hydrolysis and proper removal of protein, it remains the major contaminant in the final extract. A yield of 20.8% and 24% and purity of 50.6% and 52% was reported by Aguedo et al. (2014) and Zeitoun et al. (2010), respectively. A higher purity of 74.8% was obtained with inclusion of a delignification pre-treatment step but yield was much lower at 13.3% and a significant amount of protein (7%) was still present in the final extract (Bataillon et al., 1998). Enzyme hydrolysis of protein resulted in lower protein contents (3%) of the final extract and increased the purity to 81% while maintaining a yield of 12.8% (Hollmann & Lindhauer, 2005). For the purpose of this study however, additional pre-treatment and enzymatic purification steps were not included as cost efficiency of production process was of interest, resulting in production of a crude AX extract.

Table 3.4 Chemical composition of the alkaline extracted crude AX fractions (dry basis) obtained from the eight optimisation conditions

Component[a,b]	1 0.5 M NaOH 90 min 60°C	2 1 M NaOH 90 min 60°C	3 0.5 M NaOH 240 min 60°C	4 1 M NaOH 240 min 60°C	5 0.5 M NaOH 90 min 80°C	6 1 M NaOH 90 min 80°C	7 0.5 M NaOH 240 min 80°C	8 1 M NaOH 240 min 80°C
Ash	2.0 ± 0.5[ab]	8.5 ± 0.4[c]	5.8 ± 0.2[abc]	2.6 ± 0.7[ab]	6.9 ± 0.8[bc]	8.8 ± 0.2[c]	4.3 ± 0.6[ac]	8.1 ±1.1[bc]
Lignin	13.7 ± 0.4[ac]	13.6 ± 0.8[c]	15.5 ± 0.1[b]	13.5 ± 0.9[c]	14.8 ± 0.6[ab]	15.4 ± 0.2[b]	17.5 ± 0.3[d]	13.4 ± 0.3[c]
Glucose	15.7 ± 0.2[a]	10.2 ± 0.1[c]	9.5 ± 0.3[cde]	10.5 ± 0.3[c]	7.3 ± 0.1[b]	7.8 ± 0.4[b]	8.2 ± 0.8[bd]	8.7 ± 0.4[be]
Xylose	23.2 ± 0.8[a]	28.0 ± 0.6[bd]	26.5 ± 1.0[abc]	37.1 ± 0.5[e]	25.1 ± 0.1[ab]	23.7 ± 0.8[a]	30.0 ± 0.5[cd]	31.8 ± 0.5[de]
Arabinose	14.3 ± 1.0[a]	20.0 ± 0.6[bc]	19.2 ± 0.5[bc]	19.4 ± 0.9[bc]	18.6 ± 1.1[bc]	18.3 ± 0.7[b]	21.1 ± 1.2[c]	21.3 ± 0.8[bc]
AX[c]	33.0 ± 1.8[a]	42.2 ± 1.2[ef]	40.2 ± 1.5[cf]	49.7 ± 1.4[g]	38.4 ± 1.2[bc]	37.0 ± 1.5[b]	44.3 ± 1.7[de]	46.7 ± 1.3[dg]
Protein	23.4 ± 0.6[a]	18.6 ± 0.7[bc]	20.9 ± 0.2[ac]	13.2 ± 0.2[de]	18.0 ± 0.4[b]	16.5 ± 0.6[b]	13.7 ± 0.0[d]	9.3 ± 0.2[e]
FA (mg/g)[d]	1.52 ± 0.04[a]	0.98 ± 0.03[c]	1.37 ± 0.04[b]	0.42±0.07[d]	1.53 ± 0.08[a]	0.91 ± 0.04[c]	1.47 ± 0.02[ab]	0.41 ± 0.02[d]
Total	92.3 ± 0.8	98.9 ± 0.7	97.4 ± 1.2	96.3 ± 1.1	90.7 ± 1.2	90.5 ±1.0	94.8 ± 1.6	93.3 ± 1.8
AX yield (%)[e]	30.6 ± 1.1[ab]	32.0 ± 1.3[ab]	29.6 ± 0.1[b]	44.7 ± 0.8[d]	30.8 ± 0.3[ab]	32.7 ± 0.9[a]	37.1 ± 0.5[c]	40.6 ± 1.5[c]

[a]Values ± SD with n = 2 experimental duplicates
[b]Different letters in the same row (for each component) represents statistical differences between samples (Fisher LSD, Statistica 64)
[c]AX - Arabinoxylans = 0.88 (%Xylose + %Arabinose)
[d]FA – Ferulic acid
[e]AX yield - percentage of AX present in extract relative to the available AX in wheat bran

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3.4.3 Effect of extraction conditions on arabinoxylan extract properties for application in the bread making process

Alkaline extraction condition 7 (0.5 M NaOH, 240 min, 80°C) resulted in the production of a crude AX extract with the highest combined content of required functional components (AX and FA) for the application process.

Apart from high AX content, ferulic acid (FA) content is an additional criteria for application in the bread making process. Cross-linking of FA bound to AX occur in the presence of oxidative enzymes (Schooneveld-Bergmans, Dignum, et al., 1999) such as laccase. This is important for the application chapter (Chapter 4) where FA is required to determine the effect of laccase addition on the bread making process. Therefore FA content of the crude AX extract is a critical outcome for the extraction process. It was observed that AX extraction condition 4 (1 M NaOH, 240 min, 60°C) and 8 (1 M NaOH, 240 min, 80°C) result in the lowest FA content (**Figure 3.5a)**. Both conditions have high alkaline concentrations and longer extraction times. For all cases, extraction temperature did not affect FA content (**Figure 3.5b**). When comparing the crude AX extract FA content at the lower alkaline concentration (condition 1, 3, 5 and 7) (**Figure 3.5a**) it was observed that extraction time did not affect FA content. All four conditions resulted in an extract with similar FA contents (1.52, 1.37, 1.53, 1.47 mg/g) irrespective of the extraction time (**Figure 3.5a**). On the other hand, at high alkaline concentrations (condition 2, 4, 6 and 8) longer extraction times result in lower FA content.

During alkaline extraction the release of AX results in the release of FA as FA is bound to AX and lignin in the cell wall matrix which is disrupted during the extraction process (Snelders, Dornez, Delcour, & Courtin, 2014). With increased alkaline concentration and extraction time, more FA is released and less remain bound to the AX polymer (Aguedo et al., 2014). Mandalari et al., (2005) demonstrated the effect of alkaline concentration on FA content. Wheat bran FA content of 1.03% was decreased with 0.5 M KOH to 0.25% and even further to 0.05% with 1 and 4 M KOH. This is in agreement with the results obtained in the current study. Alkaline concentrations of 0.5 M resulted in FA contents of 0.14-0.15% whereas increased alkaline concentrations of 1 M reduced the FA content to 0.04-0.1%.

Table 3.5. Mass balance of the feedstock (wheat bran) and extract 1 (E1)

g/100g	Ash	Glucose	Xylose	Arabinose	Lignin	Protein	Total	Residual
Wheat bran	6.9±0.1	29.0±1.2	22.1±0.7	10.7±0.2	8.9±0.8	18.9±0.2	96.5±0.6	3.5±0.6
Arabino-xylan	4.3±0.6	8.2±0.8	30.0±0.5	21.1±1.2	17.5±0.3	13.7±0.0	94.8±0.9	5.2±0.9

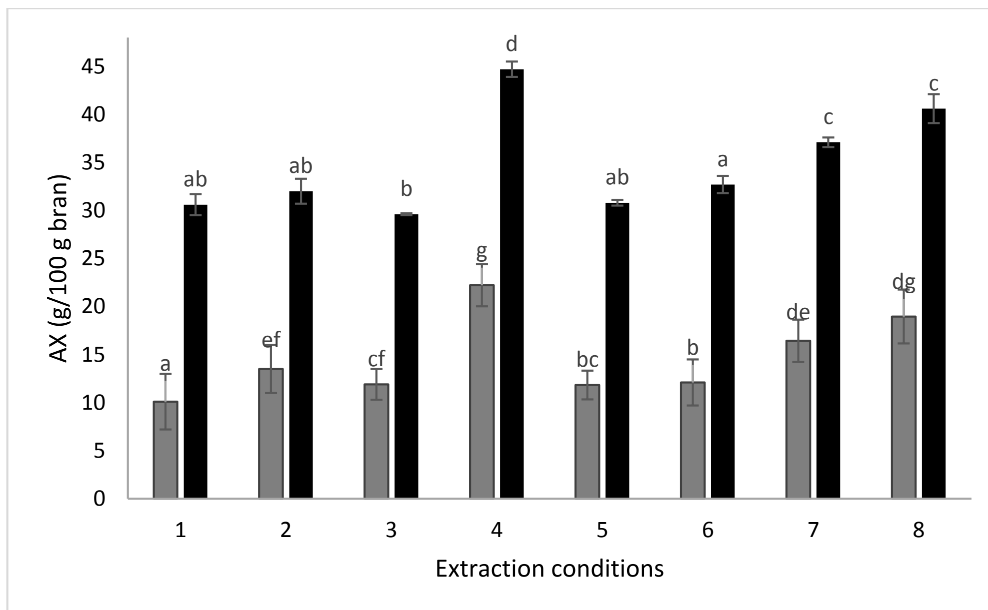

Figure 3.3 Pure AX in the crude AX extract (■) and AX yield (■) in terms of total AX available in wheat bran. (1) 0.5 M NaOH, 90 min, 60°C (2) 1 M NaOH, 90 min, 60°C (3) 0.5 M NaOH, 240 min, 60°C (4) 1 M NaOH, 240 min, 60°C (5) 0.5 M NaOH, 90 min, 80°C (6) 1 M NaOH, 90 min, 80°C (7) 0.5 M NaOH, 240 min, 80°C (8) 1 M NaOH, 240 min, 80°C. The error bars represent the standard deviation of experimental duplicates. Different letter represent significant differences ($p<0.05$) between samples as determined using a test for least significant differences (Statistica 64)

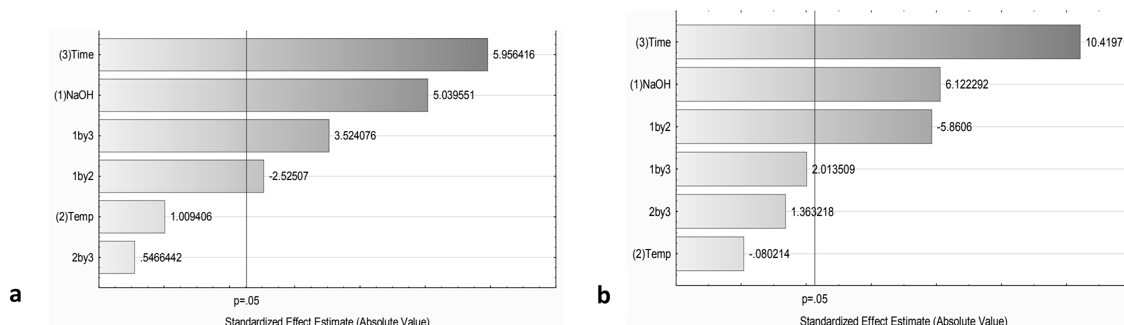

Figure 3.4 Pareto chart of the standardised effects of AX yield (**a**) and AX content (**b**) of the final extracts obtained from the eight extraction conditions. The solid line indicates significance at $p = 0.05$

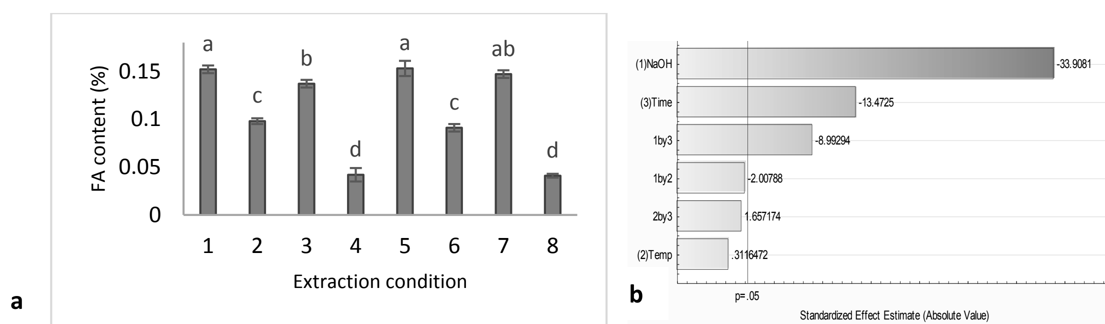

Figure 3.5 Ferulic acid content of the crude AX extracts (1) 0.5 M NaOH, 90 min, 60°C (2) 1 M NaOH, 90 min, 60°C (3) 0.5 M NaOH, 240 min, 60°C (4) 1 M NaOH, 240 min, 60°C (5) 0.5 M NaOH, 90 min, 80°C (6) 1 M NaOH, 90 min, 80°C (7) 0.5 M NaOH, 240 min, 80°C (8) 1 M NaOH, 240 min, 80°C. The error bars represent the standard deviation of experimental duplicates. Different letter represent significant differences (p<0.05) between samples as determined using a test for least significant differences (Statistica 64) (**a**). Significance and size of effect of extraction condition on ferulic acid content and the effect of interaction of extraction conditions. The solid line indicates significance at $p = 0.05$ (**b**).

Apart from the effect on extract composition, the extraction conditions also influenced the extract colour (**Figure 3.6**). In this case all three factors play a role in the final extract colour; higher alkaline concentration, longer extraction time and higher temperature resulted in a darker extract colour.

High temperatures and alkaline pH can lead to the formation of sugar-degradation compounds resulting in brown/red tones. In addition lignin and phenolics (ferulic acid) were also extracted and contribute to the darkening of the final extracts (Bergmans et al., 1996).

To determine the optimum extraction conditions from the conditions investigated, the individual results were combined to identify the appropriate AX extract for application in the bread making process. Although condition 4 (1 M NaOH, 240 min and 60°C) (**Table 3.4**) resulted in an extract with the highest AX content and yield it also contained the lowest ferulic acid content and produced a dark coloured extract (**Figure 3.6**). Alternatively, condition 7 (**Table 3.4**) produced an extract with high ferulic acid content. In comparison to the other conditions resulting in high ferulic acid content (condition 1, 3 and 5) condition 7 (0.5 M NaOH, 240 min and 80°C) had the highest AX content and yield. Thus, condition 7 was chosen as the optimum extraction process for production of a crude AX extract (E1). In addition to producing the required characteristics for application in bread making, condition 7 also uses lower alkaline concentrations which is more cost effective and reduces downstream processing.

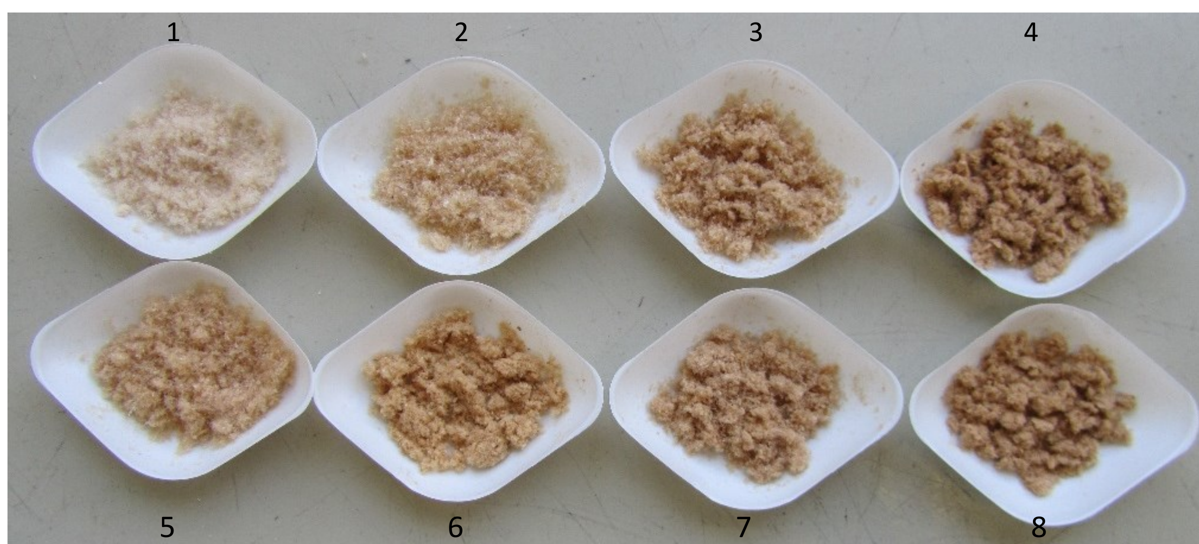

Figure 3.6 Crude AX extracts obtained from the eight extraction conditions. (1) 0.5 M NaOH, 90 min, 60°C (2) 1 M NaOH, 90 min, 60°C (3) 0.5 M NaOH, 240 min, 60°C (4) 1 M NaOH, 240 min, 60°C (5) 0.5 M NaOH, 90 min, 80°C (6) 1 M NaOH, 90 min, 80°C (7) 0.5 M NaOH, 240 min, 80°C (8) 1 M NaOH, 240 min, 80°C.

3.4.4 Optimisation for alkaline arabinoxylan extraction with respect to extraction time

Extraction time was optimal at 4 hours for maximum AX extraction at 0.5 M NaOH and 80°C.

Further optimisation was conducted in terms of extraction time, as analysis of variance results indicated that time had the most significant effect on both AX content and yield (**Figure 3.4**). AX content of the liquid fraction (obtained after extraction and centrifugation) increased with an increase in extraction time and reached a maximum between 4 and 5 hours (**Figure 3.7**). No significant difference was observed between the AX extracted in the liquid fraction at 4 and 5 hours. Therefore the shorter extraction time was chosen as the optimum to keep the energy consumption at a minimum and consequently reduce production cost.

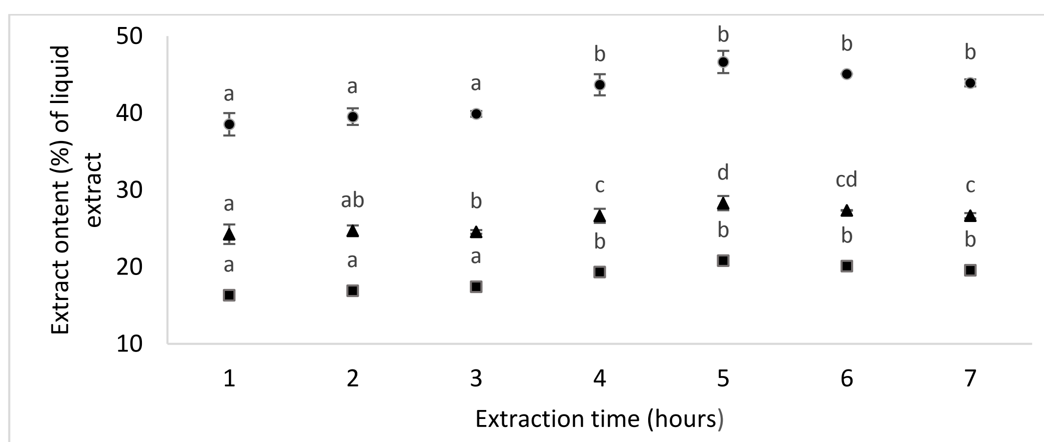

Figure 3.7 Arabinose (■), Xylose (▲) and Arabinoxylan (●) content of the liquid fraction separated after alkaline extraction of wheat bran using 0.5 M NaOH at 80°C for 1 to 7 hours. The error bars represent the standard deviation of duplicate results. Different letter represent significant differences ($p<0.05$) between samples as determined using a test for least significant differences (Statistica 64)

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3.4.5 Comparison of properties of crude AX fractions obtained from small scale (E1) and large scale (E2) extraction

The final crude AX extracts obtained from small scale and large scale extraction contained 44.3 and 49.3% AX, respectively (**Table 3.6**).

IR spectra of (extract 1) E1, (extract 2) E2 and highly pure AX are shown in **Figure 3.8**. The spectra is typical of polysaccharides in the 1200-800 cm^{-1} region with a band maximum at 1030-1040 cm^{-1}which is characteristic to xylans and a band characteristic of β- (1-4) linkages at ~895 cm^{-1}. The shape of the spectra is greatly dependent on the substitution degree and the presence of arabinose or glucuronic units on O-3. In AX substituted with arabinose on O-3, the intensity of the band assigned to the glycosidic bond at ~1160 cm^{-1} decreases together with the band at ~990 cm^{-1} (Kaeurakova, Ebringerova, Hirsch, & Hromadkova, 1994). It was observed that E1 had a higher substitution of O-3 arabinose compared to E2 and AX (**Figure 3.8**). Another noticeable difference was observed at ~ 1406 cm^{-1} between the two extracted samples and the highly pure AX sample. This may be indicative of the protein contamination of the extracted samples as that band is characteristic of amide II (N-H) present in protein but not AX.

The major compositional and physical differences observed between E1 and E2 are as a result of the extraction and purification methods used to obtain these fractions. E1 was produced using less purification processes. The temperature for the large scale extraction was reduced from 80°C (optimum at small scale) to 50°C as the equipment used during large scale extraction was not accurate at higher temperatures and statistical analysis determined that temperature did not significantly affect AX or FA content of the final extract. However, the interaction of temperature and alkaline extraction did affect both AX and FA content.

Small scale extraction resulted in a final crude AX fraction with high protein and lignin contamination but with high average molecular weight and A/X ratio (**Table 3.6**). The A/X ratio is directly correlated to the solubility of the extract. A decrease in arabinose substitution leads to a decreased solubility in water (Courtin & Delcour, 2002). This insolubility can be attributed to increased aggregation of the unsubstituted regions in the AX molecule. Solubility is an important factor for the application process as insoluble aggregates will result in a non-uniform mixture which may affect the functionality of the AX extract in the bread making process and interpretation of the results.

In contrast to E1, E2 had a slightly higher AX content with less lignin and protein contamination due to inclusion of an ultrafiltration and precipitation step, removing low molecular weight contaminants and increasing the purity of the extract.

An additional chromatographic step was also included in the purification of E2 to remove colour components and consequently producing a lighter extract. **Figure 3.9** illustrates the effect of purification on the liquid extract. After ultrafiltration the concentrated liquid appear much darker than the original sample after

centrifugation. With inclusion of a chromatographic discolouration step, the colour of the extract improved and appeared lighter.

In **Table 3.7** the colour of the final AX extracts (E1 and E2) are compared in terms of lightness and the whiteness index (WI). The lightness measures the appearance of the extract on a scale of 0 (black) to 100 (white). The lightness (*L**) is only one coordinate of a three-dimensional system which includes two more scales; a* representing the scale from green (-80) to red (+80) and b* representing the scale of blue (-80) to yellow (+80). E1 was more red and yellow compared to E2 and also appeared darker (lower *L** value). E2 was significantly whiter than E1 as measured by the WI. These results indicate that the discoloration and precipitation steps significantly improved the colour of the final extract. Similar improvement in final extract colour was observed by Zeitoun et al. (2010). The strong anionic resin combines ion exchange and adsorption with the main interaction of the extract with resin being hydrophobic. Using spectrophotometric measurements, Zeitoun et al. (2010) indicated that the resin retained a significant part of the molecules involved in colouring such as Maillard compounds. The extract colour is of significance for the application process as it could affect the appearance of the final product.

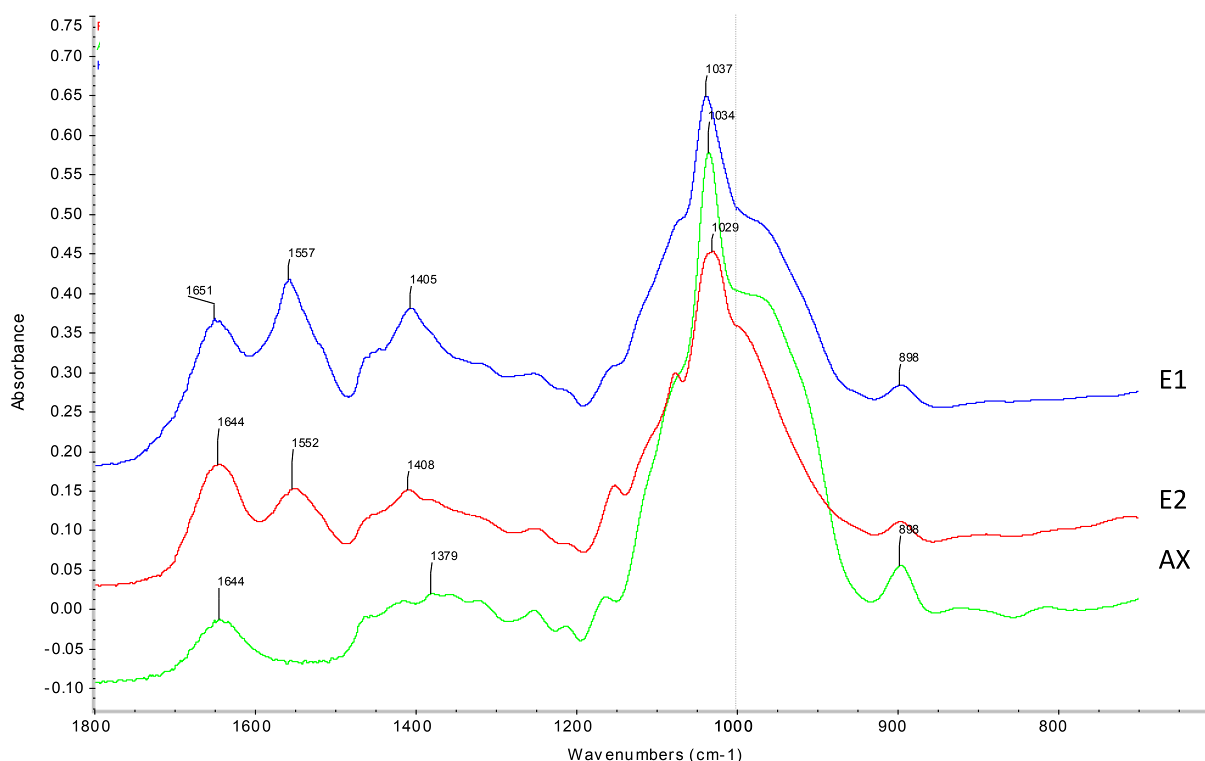

Figure 3.8 IR spectra of extract 1 (E1) (top), extract 2 (E2) (middle) and commercial AX (bottom).

Table 3.6 Physiochemical properties of highly pure AX, extract 1 (E1) and extract 2 (E2)

Component	**AX[a]**	**E1[b]**	**E2[c]**
AX (%)	95	44.3	49.3
A/X	0.6	0.7	0.6
Lignin (%)	0	17.5	7.3
Protein (%)	0.9	15.0	13.3
Average Mw (Da)	370 000	618 547	468 486

[a]Highly pure wheat arabinoxylan
[b]Small scale extraction (275 ml)
[c]Large scale extraction (27 l)

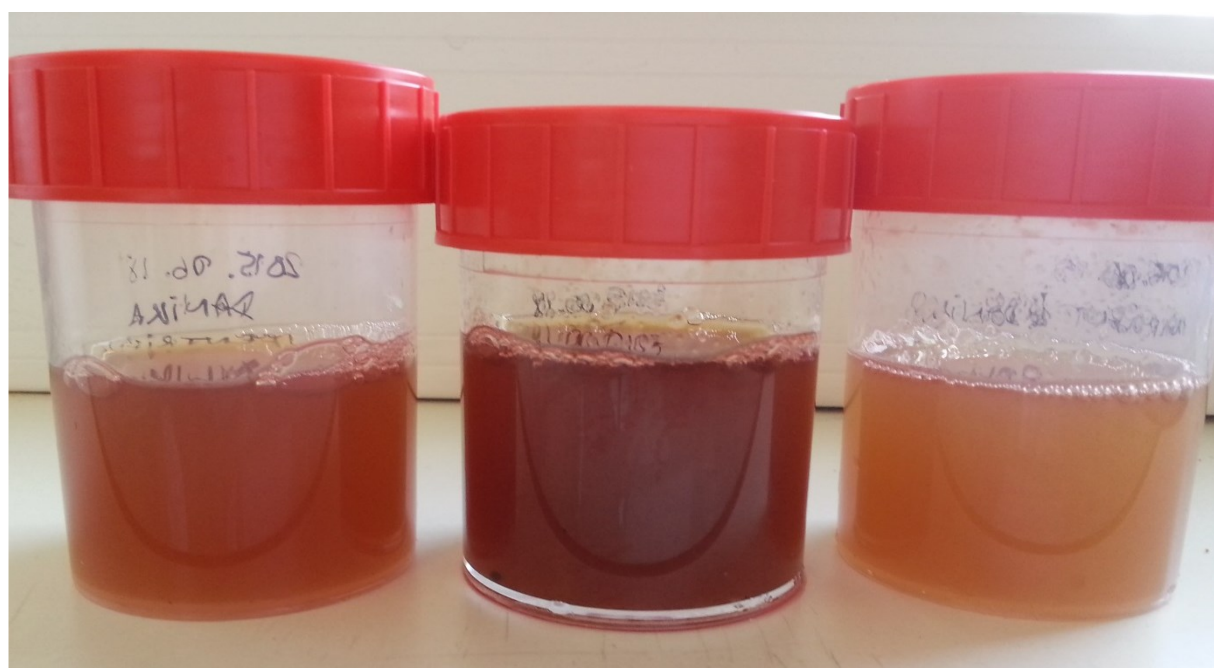

Figure 3.9 Liquid fractions obtained after alkaline extraction of AX from wheat bran on large scale and centrifugation (left), ultrafiltration (centre) and chromatographic discolouration (right).

Table 3.7 Final extract colour of extract 1 (E1) and extract 2 (E2)

	L*[a]	***a****[a]	***b****[a]	**WI**[a]
E1[b]	61.89±0.01 [c]	9.14±0.01 [c]	19.90±0.01 [c]	93.24±0.01 [c]
E2[c]	75.98±0.00 [b]	4.08±0.01 [b]	15.38±0.01 [b]	33.19±0.03 [b]

[a]Different letter next to values represent significant differences ($p<0.05$) between samples as determined using a test for least significant differences (Statistica 64)
[b]E1 (extract 1) from small scale (275 ml) extraction
[c]E2 (extract 2) from large scale (27 l) extraction

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3.4.6 Estimation of scaling up cost for arabinoxylan production using small scale production conditions

The final cost of AX production at small scale production conditions was estimated at R110.66 per kg (**Table 3.9**). The estimation of production cost is of interest for the application of AX in bread making to determine the implications it may have on bread pricing.

From the process outlined in **Table 3.8** it is clear that freeze drying is the most costly step during AX extraction, being responsible for nearly 41% of the energy usage of the entire process. This is mainly due to the longer time required to freeze dry small volumes of extract. Spray drying can be used as alternative method as it may require less time to produce the same amount of product. Further investigation into alternative drying methods is required and design of specific equipment for this process may hold great potential for reduction of production costs.

Table 3.8 Outline of the extraction process steps for the production of a crude AX fraction from wheat bran

Process steps	Weight (g)	Volume (l)	Water (l)	Electricity (kWh)
De-starching	1000		40	0.22
Alkaline extraction			27	3.58
Centrifugation		22		5.75
Concentration		11		
Dialysis		16	10	6
Freeze drying		16		10.8
Final extract	300			
NaOH	540			
Total			77	26.35

Table 3.9 Estimation of the cost for production of a crude arabinoxylan extract from wheat bran.

	Unit[a]	R/unit	R/kg AX
Water	R/kl	14.23	3.65
Sewage	R/kl	10.97	2.81
Electricity	R/kWh	0.99	86.88
NaOH	R/kg	6.00	10.79
Bran	R/kg	1.96	6.53
Total			**110.66**

R – Rand; kl – kilolitre; kg - kilogram

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3.5 Conclusion

Alkaline extraction conditions were determined on small scale for the production of an AX extract from wheat bran with high AX and FA content. Optimum conditions of 0.5 M NaOH at 80°C for 4 h resulted in the production of an extract containing 44% AX and 1.15 mg/g FA. The adapted method investigated on large scale resulted in the production of an extract with a higher purity (49%) and a lighter final product colour due to inclusion of additional ion exchange and ethanol precipitation steps.

The production cost for the crude AX extract was estimated at R110/ kg. The production cost, together with the optimum AX addition and flour removal levels (Chapter 4), will determine the commercial viability of AX application in the bead making industry.

In conclusion, the two extracts produced (E1 and E2) possess the required properties for successful implementation in the bread making process as partial flour replacers.

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4 EFFECT OF ARABINOXYLAN ADDITION ON THE BREAD MAKING PROCESS

4.1 Abstract

Arabinoxylan (AX) extracted from wheat bran can be implemented as a partial flour replacer in the bread making process. The aim of this study was to maximise flour removal while using the minimum AX addition possible while maintaining physical bread properties.

The crude AX extract (E1) obtained in Chapter 3 was added to dough and bread formulations at 0.8% and 1.2% addition levels (based on flour weight). Both AX addition levels resulted in increased dough water absorption. Together with AX addition, a flour removal range (0-4%) was investigated to determine the maximum amount of flour removal possible. AX addition at flour removal levels of 2% and 2.5% did not alter bread weight, height or volume when compared to a control. Final bread moisture content and crumb structure was influenced by both AX addition levels. A maximum flour removal of 2.5% was achieved with 0.8% and 1.2% AX addition, showing no significant effect on bread weight, height or volume. Comparison of extract performance was done against highly pure AX and the second extract obtained in Chapter 3 (E2) at the optimum application conditions. No significant difference was observed in the performance of pure AX, E1 or E2 with the exception of final product colour which was directly affected by the initial extract colour. E1 resulted in the darkest loaf colour followed by E2 and AX. The effect of laccase addition was also investigated at the optimum conditions. The enzyme addition had no significant effect on bread physical properties but did decrease slice firmness. Results from the economic analysis estimated that cost for loaves with AX addition was significantly higher than the control loaf. To maintain profit margins the selling price needs to be increased by 9.6%.

In conclusion, crude AX extracts obtained by alkaline extraction was able to act as partial flour replacers. At the optimum, 0.8% AX addition was able to replace 2.5% flour and increase dough water absorption by nearly 2%. The performance of this crude extract was comparable to that of highly pure AX.

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4.2 Introduction

Bread is one of the major staple foods in the western diet. Because bread constitutes such a large part of the human diet, it has the potential to deliver many of the required daily nutrients. Most commercial white breads are fortified to some extent, with added vitamins and minerals. To further improve the nutritional value of bread it is important to also increase the fibre content and decrease the glycaemic index (GI). The addition of wheat bran arabinoxylans (AX) could be a potential candidate to improve the fibre contents of bread.

During the bread making process AX have been proven to influence dough water absorption and mixing and rheological properties due to their high water binding capacity, high solution viscosities and their ability to undergo oxidative gelation (Gan et al., 1995; Izydorczyk & Biliaderis, 1995; Michniewicz, Biliaderis, & Bushuk, 1991). The high water binding capacity of AX can be advantageous during the bread making process. The addition of AX results in a higher water requirement for dough development, as AX will compete for water with the other flour constituents (Goesaert et al., 2005). It may therefore be possible to omit a fraction of flour and replace it with AX. AX can hold more water compared to protein and starch and therefore, less AX can be added while removing a larger fraction of flour. This replacement process may hold commercial value by reducing the amount of flour required for bread making whilst improving the nutritional value by replacing flour (starch and protein) with fibre (AX).

In addition, ferulic acid bound to the AX polymers may be altered using oxidative enzymes. These enzymes, such as laccase, cause cross-linking of the FA residues which could result in further improvement of the final product.

This study aims to establish broad effects of AX extract addition on bread properties as the basis for potential commercial application. Crude AX was used in all experiments to determine as a starting point if AX extracts could have notable effects without further purification, as this would be of major benefit for commercial production of AX.

Taking into account the amount of AX available from extractions for the baking trials, and the purity of the extract, a range of trials were devised to optimise the application of AX in bread making.

The objectives of this study were therefore to:

- Determine to what extent AX addition can increase wheat flour dough water absorption
- Evaluate the effect of AX addition on final bread properties
- Determine the minimum amount of AX addition to replace the maximum amount flour while maintaining final product properties (bread volume and weight)
- Evaluate the effect of oxidative enzyme (laccase) addition on AX functionality in bread
- Determine the effect of AX and laccase addition on the economics of bread-making

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4.3 Materials and methods

4.3.1 Materials

A single batch of commercial white bread flour (13.4% moisture and 10.5% protein) was supplied by Essential Foods, Pioneer Foods (Paarl, South Africa). Chemically pure salt (NaCl) and ascorbic acid were purchased from Labchem (Johannesburg, South Africa). Fresh compressed yeast was acquired from Anchor Yeast (Johannesburg, South Africa). The plant based shortening (palm oil) was purchased from Chipkins Bakery Supply (Montague Gardens, South Africa). Enzyme active soy flour was obtained from Impilo Products (Pretoria, South Africa). Highly pure arabinoxylan (wheat arabinoxylan, high viscosity) was purchased from Megazyme (Bray, Ireland). The crude AX extracts, extract 1 (E1) and extract 2 (E2) was obtained by extraction from wheat bran as described in Chapter 3 of this thesis. Laccase (purified from *Trametes versicolor*) was purchased from Sigma Aldrich (Germany). The activity was measured with Syringaldazine where one unit (U) corresponds to the oxidation of 1mmol/min at 30°C and pH 6.5

4.3.2 Moisture content assessment

Moisture content was measured according to the AACC Approved Methods 44-15A (AACC, 2000) and was performed in duplicate. Briefly, samples were air-dried, milled and oven dried at 130°C for one hour to determine dry weight.

4.3.3 Rheological measurements of dough samples

The water absorption and dough properties was determined using a Brabender Farinograph (Duisburg, Germany). The mixing was carried out at 30°C in a 300 g bowl according to AACC Approved Method 54-21 (AACC, 2000). The control for this analysis consisted of only flour and water and the samples tested against the control contained the crude AX extract (extracted in Chapter 3). The water absorption values obtained by the farinograph measurements were used during the bread making process.

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4.3.4 The bread making process

The optimised straight-dough bread making method was used as described in the AACC Approved Method 10-10B (AACC, 2000). The basic formulation is outlined in **Table 4.1**. The ingredients comprised chemically pure salt, fresh compressed yeast, sugar, shortening, chemically pure ascorbic acid, distilled water and commercial white bread flour. The water absorption was optimised for the control sample (based on the moisture and protein contents of the flour). A pin type mixer (National Manufacturing Company, Lincoln, Nebraska, USA) was employed for dough mixing, until optimum dough development (**Figure 4.1**). The optimum mixing time was manually determined by a test baker. After mixing, the weight of the dough was recorded and the dough was rounded by hand before placing it in a proofer cabinet for 52 min at 33±2°C and 85% relative humidity (RH). The dough was passed through a sheeter (National Manufacturing Company, Lincoln, Nebraska, USA) (**Figure 4.2**) and allowed to proof for another 25 min. This was followed by a second sheeting and 13 min fermentation step. The dough was then sheeted, hand moulded, placed into fully greased pans (141 x 81 x 54 mm) and proofed for a final 33±2 min. After final proofing the height of each loaf was recorded using a graded height meter. Finally, the loaves were baked in a convection oven (Macadams, South Africa) for 20 min at 170±2°C. The loaves were removed from the pans directly after baking and subsequently weighed. The loaves were then left to cool for 1 h at room temperature where after the height and volume was measured. Loaves were placed in plastic bags (Proton Packaging, Paarl) and stored for 24 h at 21°C where after quality evaluations (crumb texture, crumb softness and slice brightness) were performed.

Table 4.1 The formulation used for the baking trials according to the AACC Approved Method 10-10B (AACC, 2000)

Ingredients	Flour basis (%)
Flour (14% moisture basis)	100
Water	Variable
Crude arabinoxylan extract	Variable
Yeast (compressed)	5.3
Sucrose	6
Salt (chemically pure)	1.5
Shortening	3
Ascorbic acid (ppm)	40

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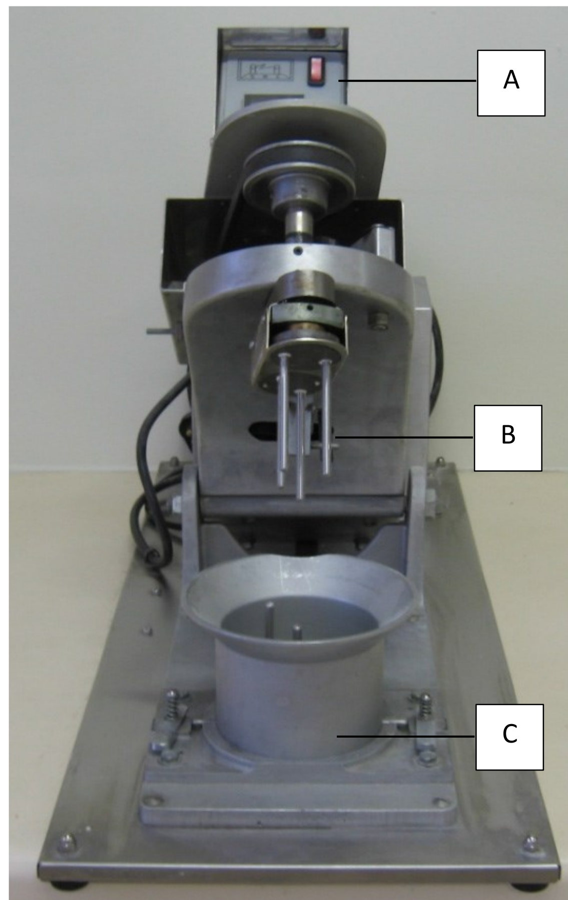

Figure 4.1 The pin type mixer used for dough mixing during the bread making process. (A) Control panel. (B) Mixer head consisting of rotating mechanism and four mixing pins. (C) Mixing bowl with two stationary pins and bowl clasps.

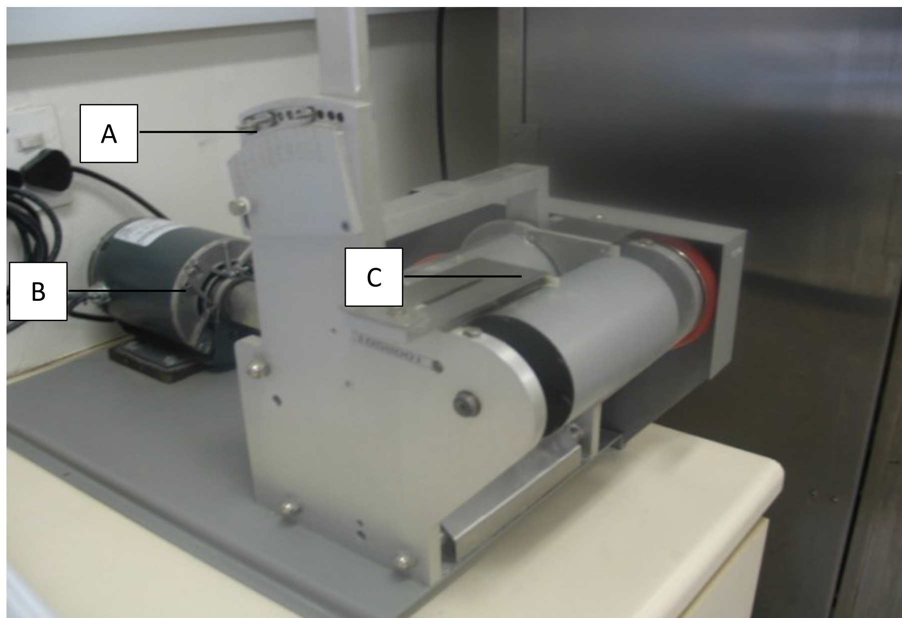

Figure 4.2 The sheeter used after the fermentation steps during the bread making process. (A) Settings panel for sheet thickness. (B) Sheeter motor that runs the rolling pins. (C) The tow rolling pins where the dough is sheeted through.

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4.3.5 Bread quality assessment

Physicochemical characteristics of the bread loaves including weight (g), height (mm) volume (cm^3), moisture, crumb texture, slice firmness and colour were assessed. Loaf volume was measured 1 h after baking using the rapeseed displacement method with the aid of a loaf volumeter. The specific loaf volume refers to the volume of the loaf relative to the amount of flour used to produce the loaf. The texture analysis of the bread was performed after cooling and 24 h of storage at 21°C. The loaves were cut using an automatic, adjustable bread slicer (182 Master, Graef, Germany) to slices of ±12 mm thickness. Crumb grain characteristics were assessed using a C Cell digital image analysis system (Colibre Control International, Appleton, UK). The analysis was performed on 2 separate slices obtained from each loaf. The crumb grain characteristics recorded were: number of cells, area of cells, cell volume and cell diameter. Crumb firmness was measured (grams of force) using an Instron Universal Testing Machine (Model 4404, Apollo Scientific, South Africa). The Instron was equipped with a 21 mm diameter aluminium cylindrical probe at a cross head speed of 100 mm/s. Two stacked bread slices (25 mm total height) were compressed to a depth of 40%. Slice colour was measured using a Konica Minolta Spectrophotometer (London, UK). Whiteness was evaluated for CIE standard illuminant D65 using the whiteness index (WI).

4.3.6 Baking trial 1: Determining the optimum flour removal range

Crude AX was used in all experiments to determine, as a starting point, if AX extracts could have notable effects without further purification, as this would be of major benefit for commercial production of AX. One level of AX addition, 0.8% AX extract 1 (small scale extraction), (on flour basis) was used in a single factor experimental design to determine the flour removal level range for Baking trial 2. The AX addition of 0.8% (using extract 1 obtained in Chapter 3) was calculated on the flour weight of the control sample (no flour removal) and was kept constant for all the flour removal levels. AX was included in the bread formulation in the water phase. The extract was suspended in water by heating the mixture to 40°C under continues stirring for 15 min. The sample was allowed to cool to room temperature before adding to the other ingredients. The mixing time was adjusted for samples containing the crude AX extract in reference to farinograph dough development time and the expertise of a test baker.

4.3.7 Baking trial 2: Determining optimum arabinoxylan addition at the maximum flour removal level

After the preliminary trial, Baking trial 2 was conducted using a factorial design with two factors, one at three levels and the other at four levels (**Table 4.2**).

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Table 4.2 Factorial design for baking trial 2

Arabinoxylan addition (%)[a]	0		0.8	1.2
Flour removal (%)[a]	0	2	2.5	3

[a]Expressed as weight percentage of flour

4.3.8 Baking trial 3: Determining the effect of laccase addition on physical bread properties

Using the optimum conditions obtained from Baking trial 2, Baking trial 3 compares the effect of crude AX extract addition alone, using extract 1 (obtained by small scale extraction) and in combination with laccase. Laccase (1U/g of flour) was added to the water phase just before mixing.

Table 4.3 Fractional factorial experimental design for laccase addition

Arabinoxylan	Laccase	Flour removal
-	-	+
-	+	-
+	+	+
+	-	-

4.3.9 Baking trial 4: Comparison of performance of extracted and highly pure arabinoxylan in bread making

Extracted AX (extract 1 (E1) – small scale) addition was compared to commercial AX and a second extracted sample (extract 2 (E2) – large scale) at the optimum flour removal level determined in Baking trial 2. For all conditions AX was added equivalent to the 0.8% (based on flour weight) of the crude AX extract (E1) and 2.5% flour was removed. The control had no AX addition or flour removal. It was assumed that the physical properties observed with AX (E1) addition was due to the AX present in the extract. This assumption was tested during baking trial 4 by comparing the crude AX extract (E1) with purified AX (E2) and commercial AX and determining the effect of purity on the performance of AX in bread making.

4.3.10 Statistical Analysis

Statistica 64 Software was used for all statistical analysis. For all baking trials univariate (one-way) ANOVA was conducted and regression model used to determine the effects of factors on the various responses analysed. Fisher least significant difference (LSD) post-hoc test was performed for baking trial 1, 2 and 4 to identify statistical differences between samples.

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4.3.11 Evaluation of arabinoxylan-flour replacement on bread making costs

The optimum flour replacement level determined in Baking trial 2 was implemented to determine the impact of the application on bread production costs. The costs were based on the 100 g baking formula and prices for ingredients were determined from commercial prices available. The wheat bran and flour prices were obtained from Sasko mills (Malmesbury, South Africa) and the AX price was based on the production costs determined in Chapter 3, section 3.4.6. The bread prices were obtained from commercial prices available.

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4.4 Results and discussion

4.4.1 Effect of arabinoxylan addition on dough water absorption and dough properties

AX addition resulted in a linear increase in water absorption at a fixed dough consistency (500 BU), thus a higher AX addition level lead to higher water absorption.

The increased water absorption of the dough observed with AX addition, means more water was required to obtain a specific dough consistency (500 BU). The maximum water absorption of 64.6% was obtained with 1.2% AX addition but no flour removal where after water absorption decreased with an increase in flour removal and decrease in AX addition (**Table 4.4**). The decrease in water absorption can be directly correlated to a decrease in the available flour constituents (gluten and starch) and AX to bind the water.

From the absorption values in **Table 4.4** it was observed that an AX addition level of 0.4% was not sufficient to increase water absorption. The water absorption was only higher than the control at 1.5% flour removal. At a flour removal level of 2% the water absorption for samples containing 0.4% AX addition was approximately the same as the control (60.8 and 60.7%, respectively). If no additional water is added but flour is removed it would be expected that the weight of the dough will decrease which will result in a loaf of lower weight and volume. Therefore, during Baking trial 2 only 0.8% and 1.2% AX addition levels were used.

In addition to water absorption, the Farinograph also measures a variety of dough properties including, development time, stability and softening.

The time taken to reach peak resistance is expressed as the development time and indicates the point at which the flour has become hydrated. The stability of a dough is represented as the difference between the arrival and departure time, and signifies the time during which maximum dough consistency does not change. Softening represents the difference in BU between development time and 12 minutes of mixing, meaning the greater the softening, the less tolerant the dough is to overmixing.

Table 4.5 shows the effect of AX addition on the above mentioned dough properties. A significant increase in dough development time and dough stability was observed for samples with AX addition (5.8 and 7.1 min, respectively) compared to the control (2.9 and 6.5 min, respectively). This indicates that doughs with AX addition required more mixing time to obtain a specific dough consistency but was more tolerant to over mixing. This is due to the increased water absorption which results in longer mixing times for AX to bind the added water. The higher water absorption also contributes to increasing dough extensibility and softness and therefore increasing the time it takes for the dough to become over-mixed and stiff (Denli & Ercan, 2001). The degree of softening was however decreased with AX addition for the specific parameters used (0.8% crude AX addition, 2.5% flour removal).

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Table 4.4 Farinograph water absorption of samples with varying flour removal and AX addition levels

Flour removal level (%)	Arabinoxylan addition (%)			
	0	0.4	0.8	1.2
	Water absorption (%)			
No flour removal	60.8	62.3	63.6	64.6
1.5	59.9	61.5	62.6	63.4
2	59.2	60.8	62.8	63.1
2.5	58.7	n.d	62.7	62.8
3	58.2	n.d	62.3	62.3
4	57.3	n.d	62.0	62.1

n.d - not determined

Table 4.5 Comparison of farinograph properties between the control dough and sample containing 0.8% crude arabinoxylan extract and with 2.5% flour removal

Farinograph properties	Control[a]	0.8% AX, 2.5% flour removal
Water absorption (%)	60.8±0.1[b]	62.7±0.1[c]
Development time (min)	2.9±0.1[d]	5.8±0.1[e]
Stability (min)	6.5±0.1[f]	7.1±0.1[g]
Degree of softening (BU)	76.5±2.1[h]	63.0±2.8[i]

[a]Control – no flour removal or AX addition
Different letters represent significant differences ($p<0.05$) between samples as determined using a test for least significant differences (Statistica 64)

The increase in water absorption obtained with AX addition can be attributed to the high water binding capacity of AX, which is caused by hydrogen bonding of the water molecules to OH-groups on the polymer chain (Biliaderis et al., 1995; Courtin & Delcour, 1998, 2002; Wang et al., 2002). Due to the significantly higher water binding capacity of AX compared to protein and starch (10, 2 and 1 g/g respectively), the addition of AX results in increased viscosity which affects the availability of water for gluten development. This affects the distribution of moisture among the dough components, which causes changes in dough rheological properties (J. Wang et al., 2002).

Studies on water absorption of AX and their effect on dough development have been conducted (Biliaderis et al., 1995; Cleemput et al., 1993; Michniewicz et al., 1991). A linear increase in water absorption and dough development time was observed with increased water addition. This is in agreement with the results obtained in the current study. In contrast to this, Courtin & Delcour (2002) observed a decrease in dough development time which may be a result of low water absorption.

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AX addition not only affects water absorption and dough development time but also the dough structure, which becomes evident during mixing and proofing. The role of AX in the dough structure is affected by a combination of factors including its binding of water, interaction with gluten and presence of ferulic acid (FA) (Courtin, Roelants, & Delcour, 1999; Michniewicz et al., 1991; Wang et al., 2002; Wang et al., 2004). The mechanism for the interaction of AX with gluten is not yet fully understood. Wang et al. (2002) proposed that AX interfere with gluten formation both directly and indirectly. AX indirectly competes with gluten for water, causing the conditions for gluten formation to change, resulting in increased resistance of gluten to extension and a decreased gluten yield. This effect can however be corrected for by increasing water addition during mixing. On the other hand, AX can affect the gluten network directly through cross linking of gluten proteins with AX via FA bound to the AX polymers (Figueroa-Espinoza et al., 1999; Labat, Rouau, & Morel, 2002; Wang et al., 2002). This results in the formation of a highly viscous aqueous solution which leads to a lower extensibility of gluten and cannot be corrected by water addition, however, addition of free FA does restore the extensibility and gluten yield by competing with the AX bound FA (Wang et al., 2004).

Molecular weight (MW), which is linked with viscosity is also suggested to be linked to the strength and elasticity of the gluten-starch network which develops during mixing (Biliaderis, 1995). High MW AX enhances resistance to extension and decreases extensibility, due to the higher viscosity of the mixture. AX of high MW (201 000 and 555 000, respectively) have been shown to have higher development times than AX of lower MW (134 000 and 50 000, respectively) (Biliaderis et al., 1995; Courtin & Delcour, 1998). This is in agreement with the current study as addition of AX with a high MW (618 547) resulted in longer development times when compared to the control.

In general, the effect of AX addition on dough properties observed in this study concur with numerous previous investigations. The discrepancies in the findings reported in literature may be due to variations in starting materials and the isolation methods used for AX extraction, as well as the impurities present. These factors must be kept in mind when comparing results from various sources.

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4.4.2 Effect of arabinoxylan addition on bread properties

4.4.2.1 Effect of arabinoxylan addition on dough and bread height and weight and final loaf volume

All five responses (dough weight and height, bread weight and height, and loaf volume) were significantly affected by the factors, flour removal and AX addition levels (ANOVA, $p < 0.05$). When the addition of the crude AX extracts were compared to commercial AX no significant differences were observed on final product quality.

In Baking trial 1, a maximum dough weight was achieved at 0% flour removal (and 0.8% AX addition), which can be attributed to the increased water absorption obtained with AX addition (**Figure 4.3**). Dough and bread weight exceeded that of the control for samples with 0% and 1.5% flour removal. At 2% and 2.5% flour removal levels the weight was similar to the control indicating that the water addition compensates for the flour removal. When removing more flour (3% and 4%) the water addition was no longer sufficient to compensate for the flour removal resulting in lower dough and bread weights.

Proof height was kept constant by varying fermentation time of the final proof step with ± 2 min. The optimum proof height was determined by the height of the control. Flour removal did however influence proof height (ANOVA, $p < 0.05$) specifically for samples with AX addition but no flour removal, where proof height was significantly higher than the control even with reduced fermentation time (**Figure 4.4**). On the other hand, proof time was not sufficient to obtain an optimum height at flour removal of 4% indicating that the amount of flour available was not able to produce the same volume of dough. These results can be extrapolated to the final bread weight and volume (**Figure 4.3 and 4.5**). Flour removal of 1.5-3% resulted in a similar final bread height when compared to the control (**Figure 4.4**). Whereas no flour removal resulted in a significantly higher loaf due to the increased water absorption caused by AX addition.

The final volume obtained from flour removal levels 0-2.5% was similar to the control but 3-4% was significantly lower due to excessive flour removal which could not be compensated for by water addition (**Figure 4.5**).

The flour removal range investigated in Baking trial 1 demonstrated an optimum between 2 and 3%. This optimum was obtained due to a balance between water absorption and flour removal which resulted in loaves with comparable characteristics to the control. At both lower and higher flour removal levels loaf properties were significantly altered. At the lower flour removal level, bread weight and height was higher than the control. This may be beneficial for a different application but for this study, the aim was to determine the maximum flour removal level while maintaining control bread properties. On the other hand, at higher flour removal levels the dough water absorption decreased which resulted in decreased bread weight and volume. The next trial investigated the effect AX addition level (0.8% and 1.2%) using the optimum flour removal range obtained in Baking trial 1.

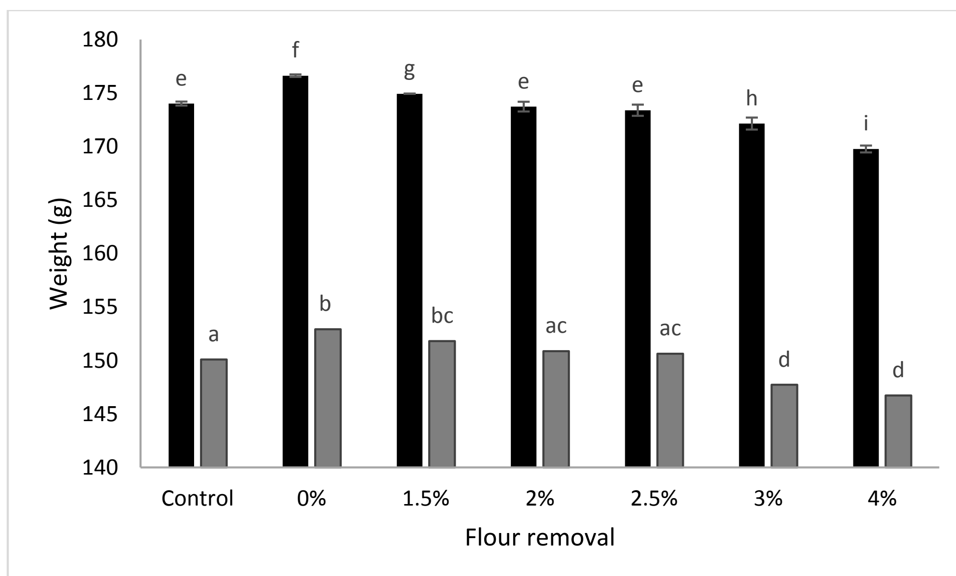

Figure 4.3 Dough weight (■) and bread weight (■) of samples containing 0.8% crude AX extract (E1) at varying flour removal levels. Control has no flour removal or arabinoxylan addition. The error bars represent the standard deviation of duplicate results. Different letter above each bar represent significant differences between samples as determined using a test for least significant differences (Statistica 64).

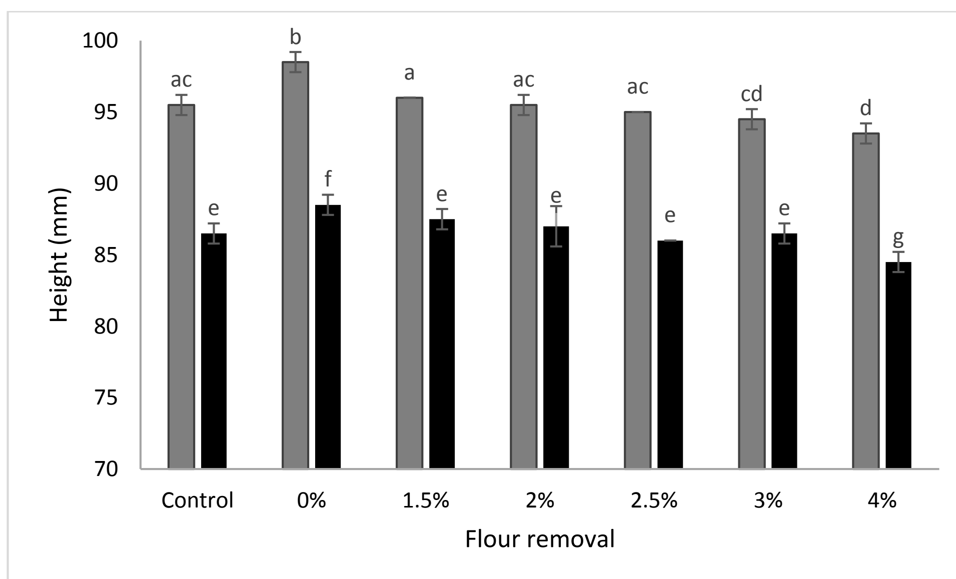

Figure 4.4 Proof height (■) and bread height (■) of samples containing 0.8% AX (E1) at varying flour removal levels. Control has no flour removal or arabinoxylan addition. The error bars represent the standard deviation of duplicate results. Different letter above each bar represent significant differences between samples as determined using a test for least significant differences (Statistica 64).

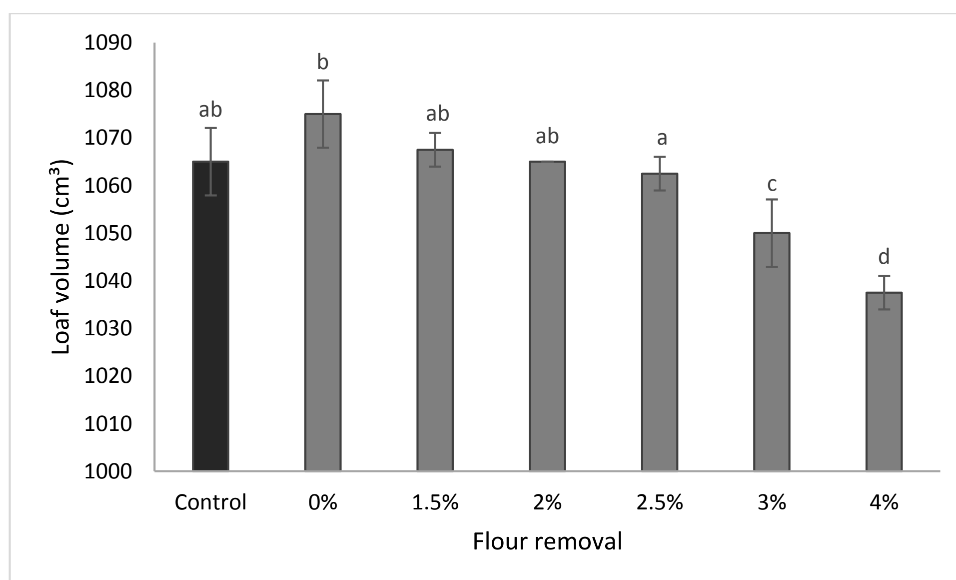

Figure 4.5 Bread volume (in cm^3) of samples containing 0.8% AX (E1) at varying flour removal levels. The error bars represent the standard deviation of duplicate results. Control has no flour removal or arabinoxylan addition. Different letter above each bar represent significant differences between samples as determined using a test for least significant differences (Statistica 64).

In Baking trial 2, both flour removal and AX addition levels significantly affected dough and bread weight (ANOVA $p < 0.05$). The general trend observed was a decrease in weight with an increase in flour removal and a significant increase in weight with AX addition (**Figure 4.6 and 4.7**). Both 0.8% and 1.2% AX addition levels resulted in similar dough and bread weights compared to the control at 2 and 2.5% flour removal levels. No significant differences were observed between 0.8% and 1.2% AX addition at these conditions. The higher flour removal level of 3% resulted in a decreased dough and bread weight, once again indicating insufficient water addition to counteract the amount of flour removed.

AX addition of 1.2% resulted in significantly higher loaves at 2 and 2.5% flour removal levels, when compared to the control (**Figure 4.8**). This however did not translate to the loaf volume which was similar to the control and 0.8% AX addition level.

Loaf volume significantly decreased (ANOVA $p < 0.05$) with an increase in flour removal for samples with no AX addition. With AX addition of 0.8% and 1.2% loaf volumes were similar to the control at flour removal levels of 0 to 2.5%, whereas 3% flour removal resulted in significantly lower volumes (**Figure 4.9**). Both AX addition and flour removal played a role in loaf volume, however, the interaction of the factors did not affect the response.

Specific loaf volume (**Figure 4.10**) can be correlated to loaf volume (**Figure 4.9**) to some extent. In both cases AX addition of 0.8% and 1.2% at flour removal levels 2-2.5% result in similar volumes. The main difference is the higher specific volume of these conditions compared to the control indicating that normalising the data by inclusion of flour weight magnifies the effect of hemicellulose addition. With decreased flour content, the

specific volume for samples with AX addition (at 2-2.5% flour removal) is significantly higher than the control. This indicates that a higher loaf volume could be obtained with AX addition using the same amount of flour. It can also be observed that the specific volumes for the samples with no AX addition but with flour removal (**Figure 10**) remains similar to the control although the actual volume is significantly lower (**Figure 4.9**).

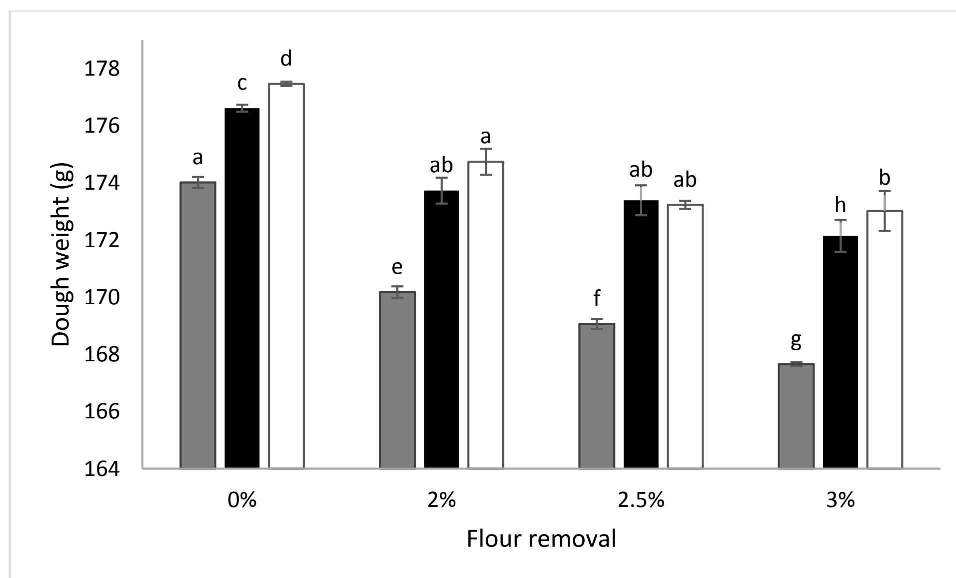

Figure 4.6 Dough weight of samples containing 0% (■), 0.8% (■) and 1.2% (□) crude AX extract (E1) at varying flour removal levels. The error bars represent the standard deviation of duplicate results. Different letter above each bar represent significant differences between samples as determined using a test for least significant differences (Statistica 64).

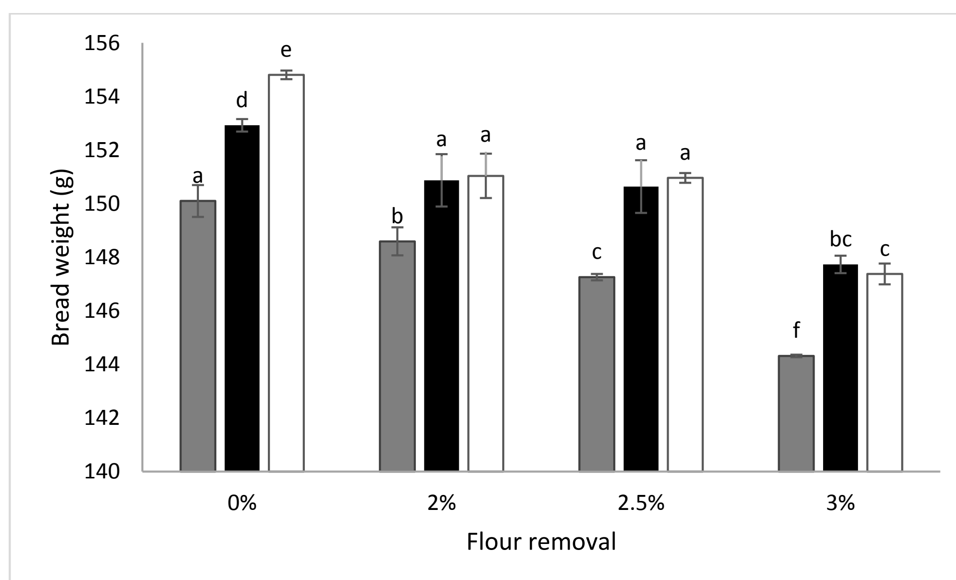

Figure 4.7 Bread weight of samples containing 0% (■), 0.8% (■) and 1.2% (□) crude AX extract (E1) at varying flour removal levels. The error bars represent the standard deviation of duplicate results. Different letter above each bar represent significant differences between samples as determined using a test for least significant differences (Statistica 64).

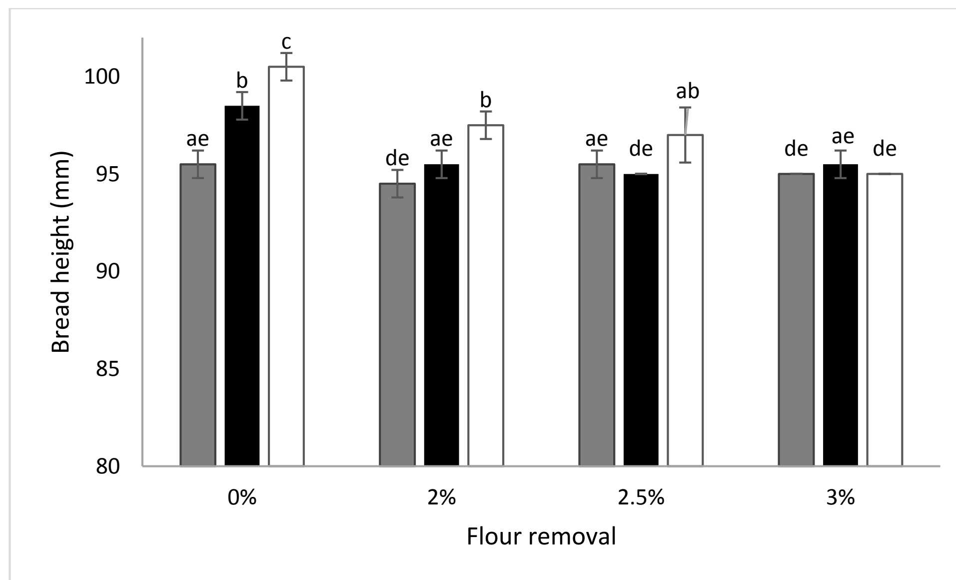

Figure 4.8 Bread height of samples containing 0% (■), 0.8% (■) and 1.2% (□) crude AX extract (E1) at varying flour removal levels. The error bars represent the standard deviation of duplicate results. Different letter above each bar represent significant differences between samples as determined using a test for least significant differences (Statistica 64).

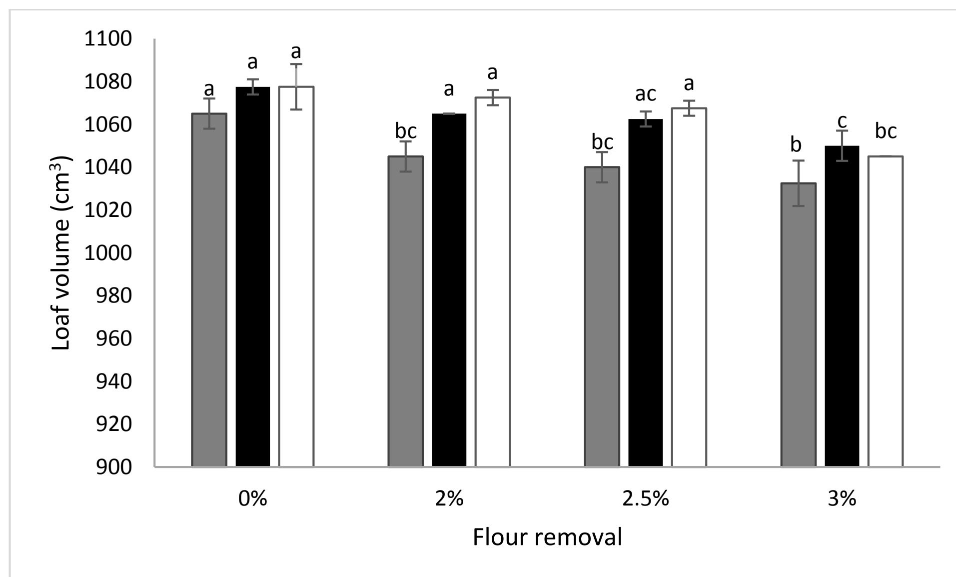

Figure 4.9 Loaf volume of samples containing 0% (■), 0.8% (■) and 1.2% (□) crude AX extract (E1) at varying flour removal levels. The error bars represent the standard deviation of duplicate results. Different letter above each bar represent significant differences between samples as determined using a test for least significant differences (Statistica 64).

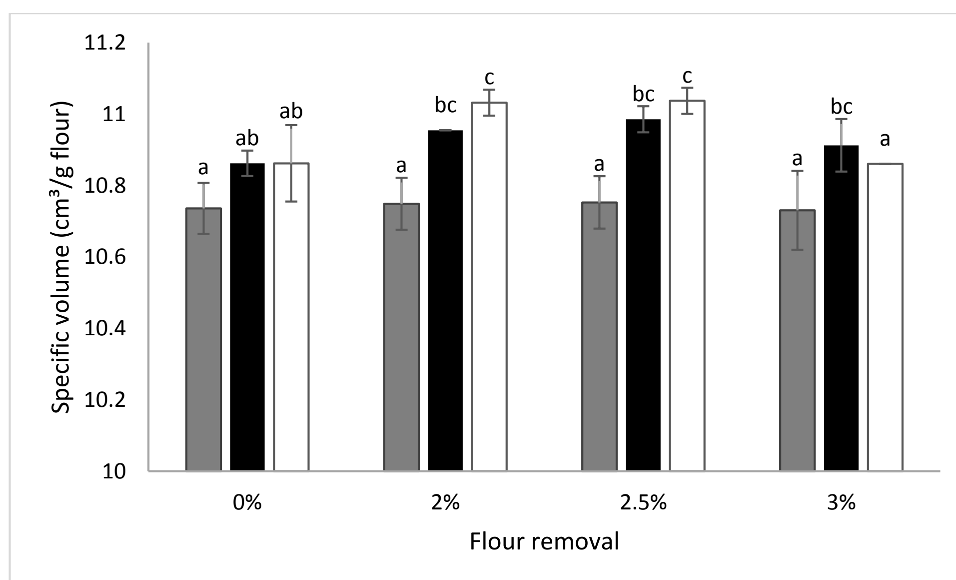

Figure 4.10 Specific loaf volume of samples containing 0% (■), 0.8% (■) and 1.2% (□) crude AX extract (E1) at varying flour removal levels. The error bars represent the standard deviation of duplicate results. Different letter above each bar represent significant differences between samples as determined using a test for least significant differences (Statistica 64).

The final baking trial was conducted to compare the performance of the crude AX extracts (E1 and E2) to commercial AX (AX). All samples had the same pure AX addition level (equivalent to the AX present in 0.8% of the crude extract), flour removal level (2.5%) and water absorption (62.7%). A control sample was also included in the trial containing no AX addition or flour removal with water absorption of 60.8%.

The results indicate that neither dough nor bread weight (**Figure 4.11**) was significantly affected by AX addition. The measured weight, for all samples (E1, E2 and AX), was comparable to the control. Similar results were observed for the proof height and final bread height (**Figure 4.12**) with the exception of pure AX, which resulted in a significantly higher bread loaf. This could be an indication that the impurities found in the extracted AX samples may have an adverse effect on the application potential of the extracts. This result was however not supported by the specific loaf volumes obtained (**Figure 4.13**) where all the AX containing samples show significantly higher volumes compared to the control. With all the samples (AX, E1 and E2) containing the same percentage of AX with the same flour removal level and water absorption, the purity of the samples demonstrated no significant effect on the physical properties analysed in this study as the two crude extracts performed similarly to the highly pure highly pure AX.

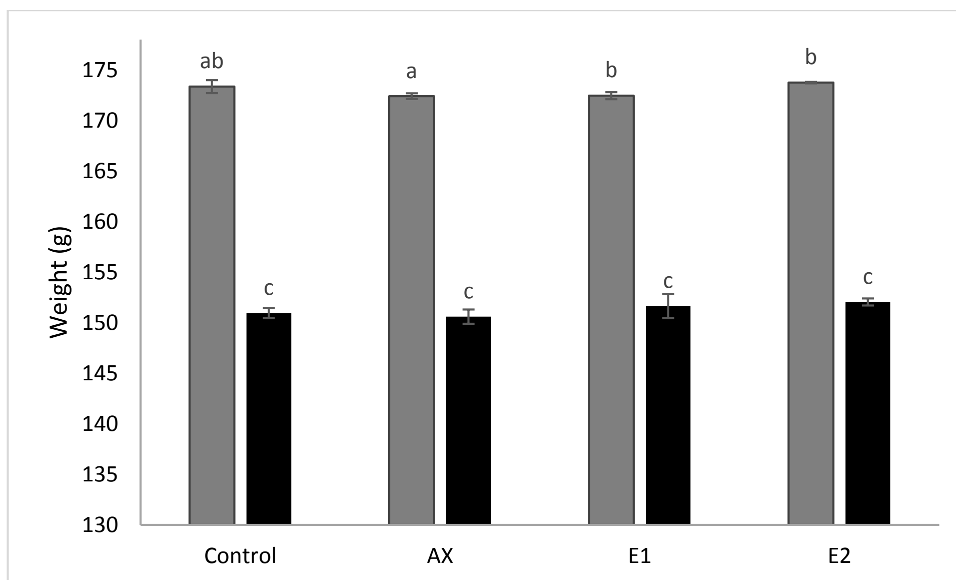

Figure 4.11 Dough weight (■) and bread weight (■). The control refers to samples with no AX addition or flour removal. AX – commercial AX, E1 – extract 1 (small scale), E2 – extract 2 (large scale). The error bars represent the standard deviation of duplicate results. Different letter above each bar represent significant differences between samples as determined using a test for least significant differences (Statistica 64).

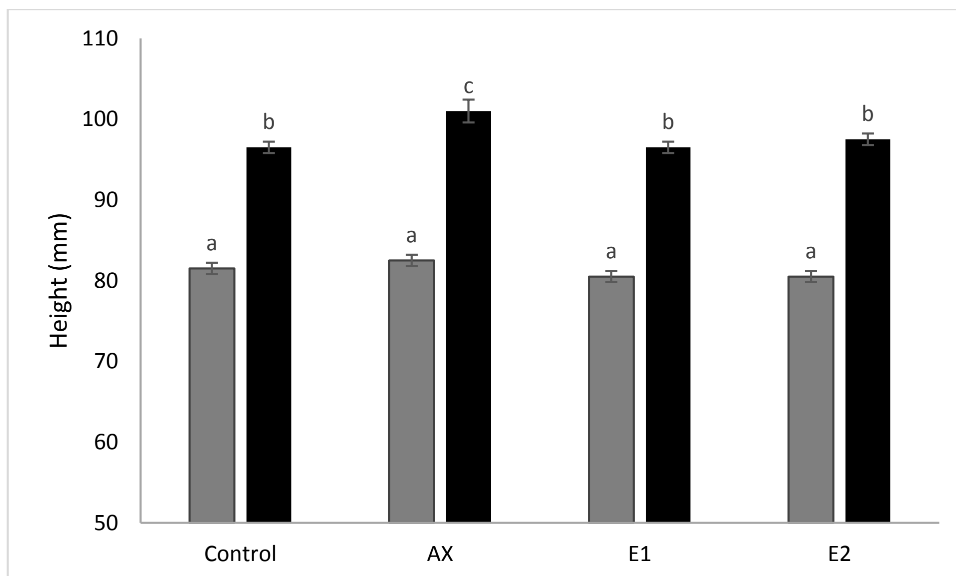

Figure 4.12 Proof height (■) and bread height (■) from baking trial 4. The control refers to samples with no AX addition or flour removal. AX – commercial AX, E1 – extract 1 (small scale), E2 – extract 2 (large scale). The error bars represent the standard deviation of duplicate results. Different letter above each bar represent significant differences between samples as determined using a test for least significant differences (Statistica 64).

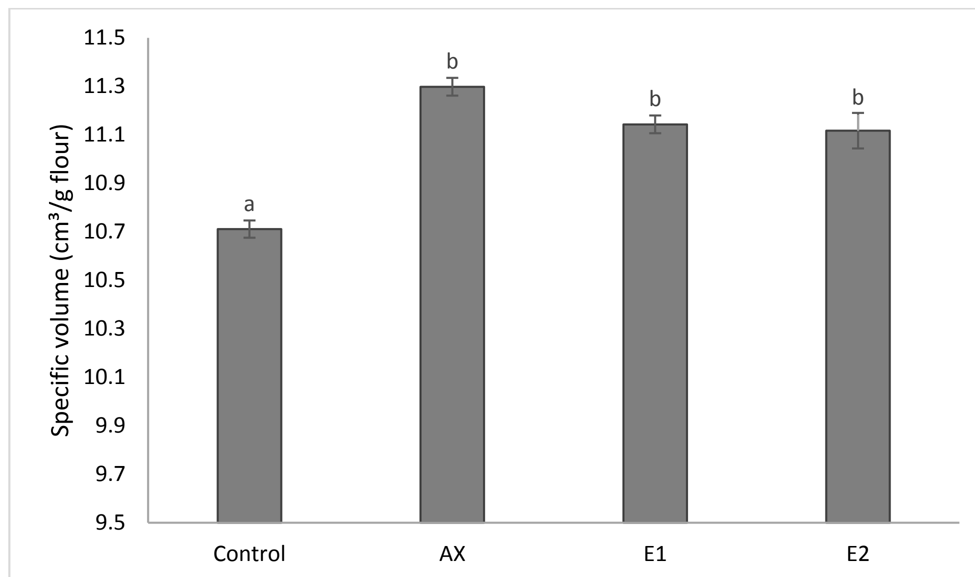

Figure 4.13 Specific loaf volume of samples obtained from baking trial 4. The control refers to samples with no AX addition or flour removal. AX – commercial AX, E1 – extract 1 (small scale), E2 – extract 2 (large scale).The error bars represent the standard deviation of duplicate results. Different letter above each bar represent significant differences between samples as determined using a test for least significant differences (Statistica 64).

The results from studies examining AX mediated effects on bread making have been very contradicting. On one hand, increased loaf volumes for samples with AX addition has been reported. Courtin & Delcour (1998) demonstrated the effect of wheat flour water-extractable arabinoxylan (WEAX) and a wheat pentosan concentrate (WPC) on loaf volume by removing a weight fraction of flour equal to the weight fraction of material added. Addition levels of 1-3% was used resulting in an increase in volume with an increase in flour substitution. Similarly Biliaderis et al. (1995) observed a maximum increase in loaf volume with 0.5% water-soluble AX addition. In contrast to this, some authors reported reduction in loaf volume with AX addition (Gan et al., 1995; Maes & Delcour, 2002)

To determine the functional role of AX in bread making, differentiation must made between WEAX and water-unextractable AX (WUAX). It is postulated that these polymers act differently on the dough network exerting opposite effects on gas cell properties. WEAX stabilize gas cells which leads to prolonged oven rise resulting in increased loaf volume. On the other hand, WUEX causes loss of gas retention which encourages gas cell coalescence which in turn results in lower loaf volumes (Gan et al., 1995).

These explanations for AX functionality are in contrast to the findings of this study where WUAX addition resulted in improved specific loaf volumes. Gan et al. (1995) made no distinction between water-extractable and water-soluble AX. AX can be extracted by either water (WEAX) or alkaline (ASAX), both of which render

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them water-soluble (WSAX). This solubility is mainly influenced by the AX substitution degree of the polymer backbone (arabinose to xylose ratio). With an average ratio of 0.43 the polymer is rendered insoluble (Courtin & Delcour, 2002). In this study an average ratio of 0.7 was obtained indicating that the extracted AX was indeed water-soluble. As the water-soluble AX extracts in this study was able to increase specific loaf volume it can be postulated that the viscosity of the dough aqueous phase was increased, due to the high average MW, which in turn stabilised the gas cells liquid films resulting in the higher specific volumes observed. Therefore solubilisation of AX must also be considered.

4.4.2.2 Effect of arabinoxylan addition on crumb texture, firmness and moisture content

In general, crumb texture and moisture content, but not firmness was significantly affected by AX addition.

For Baking trial 1, an increase in flour removal did not significantly affect firmness (neither increasing nor decreasing firmness) and the results are inconclusive with some higher and some lower firmness values for the flour removal levels when compared to the control (**Table 4.6**). This may be due to the differences in loaf volume which affects the interpretation of firmness. Therefore the analysis was repeated for Baking trial 2, using various AX addition levels and comparing firmness at one flour removal level were the loaf volumes were similar.

Table 4.6 Crumb firmness measured over time for Baking trial 1

Sample	**Crumb firmness (gram of force)[a]**				
	Day 0	**Day 1**	**Day 2**	**Day 3**	**Day 4**
Control[b]	65 a	230 ab	329 bc	464 b	594 a
0%	73 a	266 a	437 a	573 a	578 a
1.5%	62 a	239 ab	315 bc	475 b	490 bc
2%	70 a	240 ab	366 ab	495 b	539 ac
2.5%	63 a	205 b	282 c	465 b	486 bc
3%	65 a	217 ab	284 c	446 b	470 b
4%	69 a	209 b	285 c	483 b	504 bc

[a] Different letter next to values represent significant differences between samples (column) as determined using a test for least significant differences (Statistica 64)
[b] Control refers to samples with no flour removal or AX addition

Figure 4.14 illustrates moisture content as a function of storage time (loaves stored at 21°C in plastic bread bags). For all cases moisture content decreases over time with significantly higher moisture contents in loaves containing AX (0.8%) compared to the control. The higher final moisture content reflect the higher water absorption values obtained by samples with AX addition (**Table 4.4**). Higher moisture content may have a positive effect on bread staling by slowing down starch retrogradation through changes in water distribution and the ability of AX to bind water, making it unavailable for distribution or loss to the atmosphere (Izydorczyk & Biliaderis, 1995)

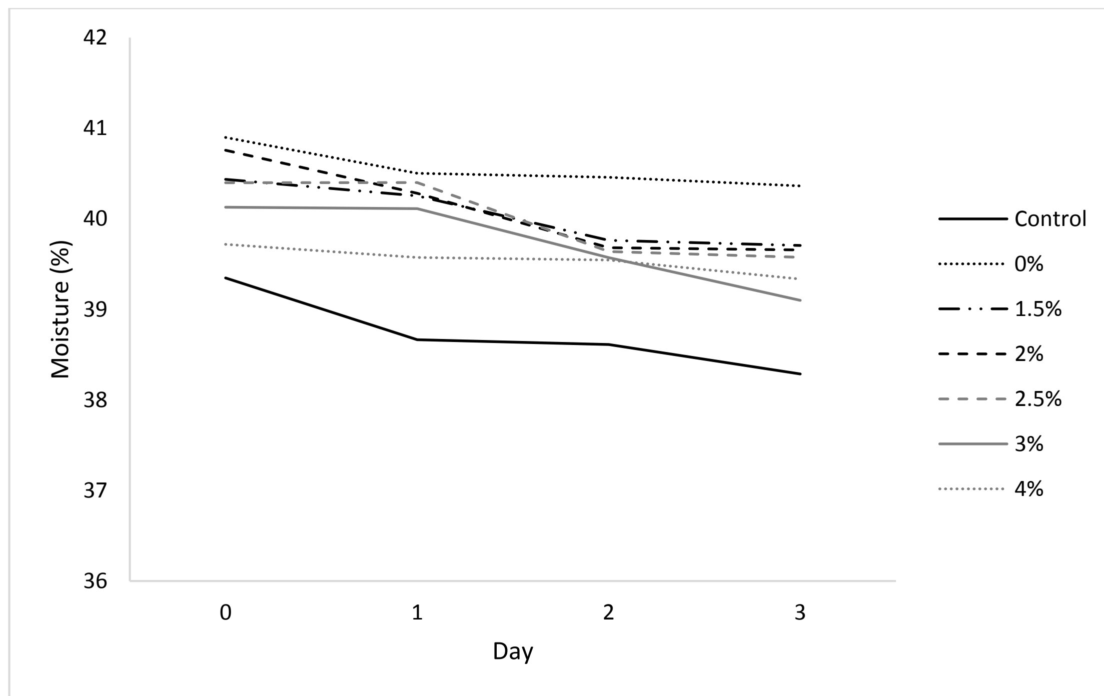

Figure 4.14 Bread moisture (%) measured over time for samples containing 0.8% crude AX extract at varying flour removal levels. The control refers to a samples with no flour removal or AX addition.

For Baking trial 2, samples containing 0.8% and 1.2% AX addition, at a flour removal level of 2.5% was chosen for crumb firmness evaluation, due to the comparable loaf volumes. In general, firmness increases over time, with the control showing slightly higher firmness values compared to samples containing AX. These differences however were not statistically significant ($p > 0.05$) (**Figure 4.15**).

The crumb structure, for the control and samples containing 0.8% and 1.2% AX addition with 2.5% flour removal, can be observed in **Figure 4.16b**. The area and depth of each cell indicates its prominence which is colour coded. Small cells are dark blue in colour while larger ones are displayed in lighter shades of blue, green and yellow. Cells large enough to be categorised as holes are outlined in red. The cells in the control (left) has a more regular structure and size compared to the slices containing 0.8% AX addition (right) and 1.2% AX addition (centre).

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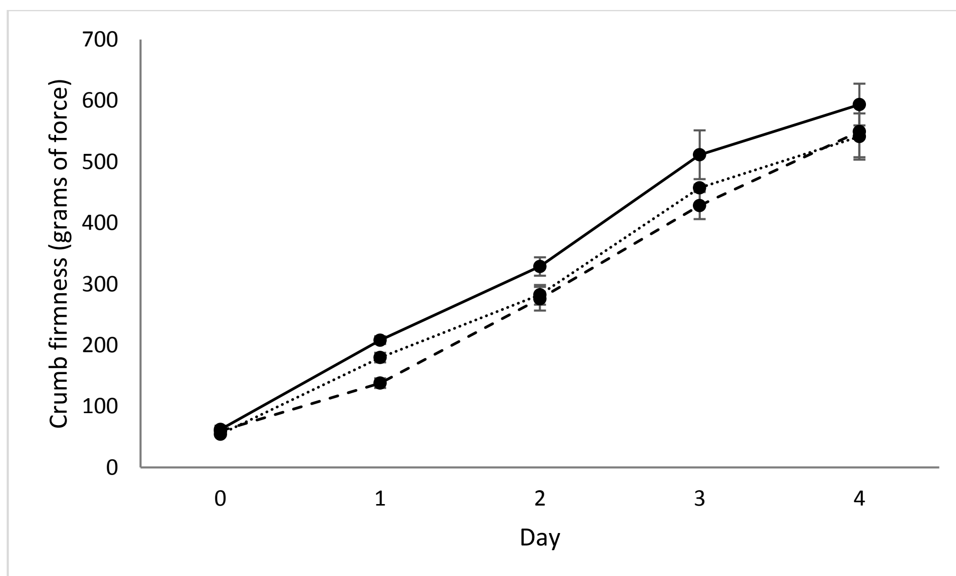

Figure 4.15 Crumb firmness of the control (–), 0.8% AX addition (···) and 1.2% AX addition (---). 2.5% flour is removed in samples with AX addition. The error bars represent the standard deviation of duplicate results.

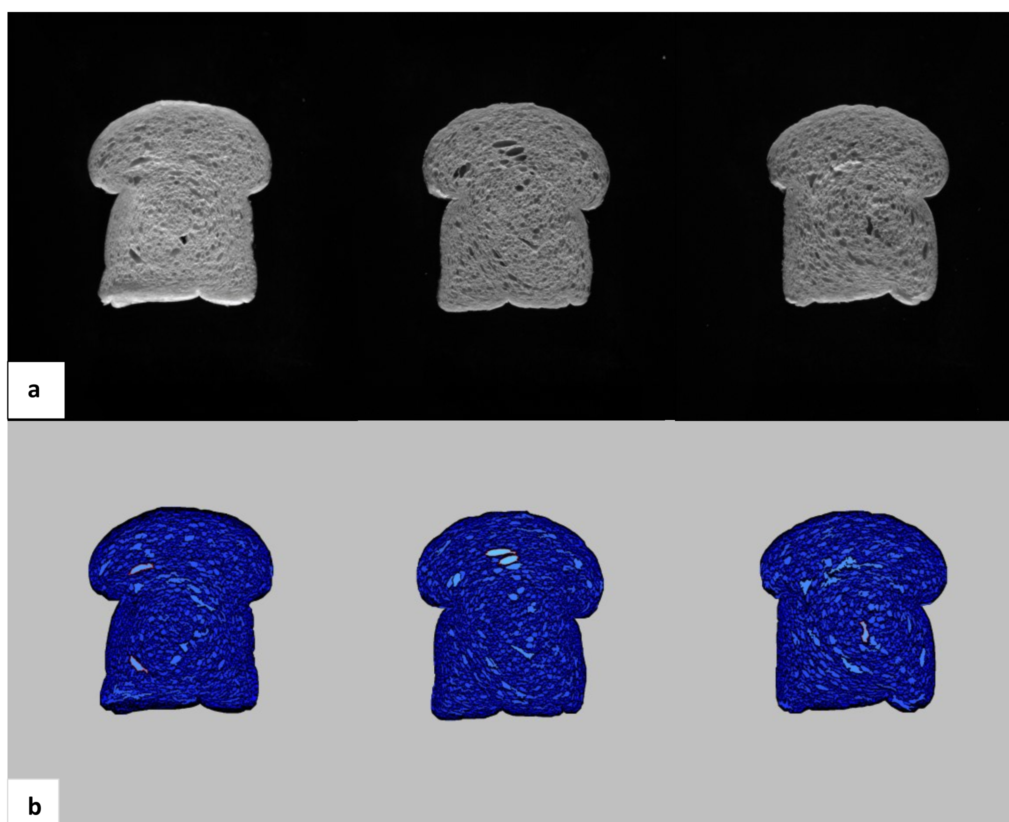

Figure 4.16 C Cell raw, unprocessed image for the control (left), 1.2% AX addition (centre) and 0.8% AX addition (right) (**a**). Cell structure of the control (left), 1.2% AX addition (centre) and 0.8% AX addition (right). Both 0.8% and 1.2% AX addition in combination with 2.5% flour removal (**b**).

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Gas cells are formed during the mixing stage of bread making. After the mixing stage no new gas cells can be formed but during fermentation leavening agents expand the existing cells. The leavening agent generates carbon dioxide within the liquid phase. The gas diffuses from the liquid phase to the cells due to a concentration gradient (Scanlon & Zghal, 2001).

When evaluating the bread crumb texture, using the C Cell imaging system, the most important measurements considered were; number of cells, area of cells, cell volume and cell diameter. These cell properties are defined by specific parameters. Area of a cell refers to the total number of pixels within the cell whereas the volume combines information on the cell area and depth based on the degree of shadow within it. A difference in these cell properties would lead to an either finer or coarser crumb texture. A higher number of cells may be due to a finer structure or larger slice area. Larger area of cells indicate a more open texture whereas cell diameter and volume are indicators of the coarseness of the texture. The cell properties are a reflection of a number of factors including the gluten quality, mixing efficacy and fermentation process (moulding and sheeting).

For the final baking trial, the effect of extracted AX was compared to highly pure AX. Only one of the four cell properties measured for evaluation of crumb texture was influenced by AX addition. The cell volume was significantly increased in all samples with AX addition (E1, E2 and AX). The larger cell volume translates to a coarser crumb structure which is seen as a negative property in white bread (**Table 4.7**).

Although all samples with AX addition had the same initial water absorption, the moisture content of the loaf containing E1 was significantly (ANOVA $p<0.05$) higher than the other samples indicating that this sample was able to retain more moisture. The same trend was not observed for slice firmness, as E1 had the same softness as the other samples (control and E2). However, the sample containing highly pure AX led to a final product with a less firm texture. This indicates that the moisture content cannot always be directly correlated to the softness.

Table 4.7 Baking trial 4 crumb texture and slice properties

	Number of cells[ab]	Area of cells[ab]	Cell diameter[ab]	Cell volume[ab]	Moisture (%)[a]	Firmness[ac]
Control[d]	4768 a	48.85 a	10.8 a	4.1 a	38.60 a	169.89 a
AX	4765 a	49.65 a	11.2 a	4.6 b	39.70 a	147.75 b
E1	4623 a	49.45 a	11.0 a	4.6 b	40.44 b	163.41 a
E2	4583 a	49.5 a	11.3 a	4.6 b	39.69 a	165.60 a

[a]Different letter next to values represent significant differences between samples (column) as determined using a test for least significant differences (Statistica 64)
[b]Crumb structure properties as determined by the C Cell imaging system – cells are measured in terms of pixels
[c]Firmness measured as the grams of force required to compress the slice by 40%
[d] Control refers to samples with no flour removal or AX addition

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The texture of a breadcrumb is highly affected by the amount of water present after baking (Biliaderis et al., 1995). In this study moisture content decreases over time with significantly higher moisture contents in loaves containing AX. This reflects the higher water absorption values for the AX containing doughs (**Table 4.4**). Similar trends were observed by Biliaderis et al. (1995) using highly pure AX.

Not only does the moisture content decrease over time but it also gets redistributed within the bread structure contributing to the staling process (Goesaert et al., 2005). Moisture may also affect the perception of freshness as one study demonstrated two aged loaves having the same firmness but one with 2% higher moisture content was judged by a panel to be significantly fresher (Hebeda & Zobel, 1996).

Firmness is a good indicator of bread staling as a correlation factor of 0.98 was found between firmness level and taste panel assessment of staleness (Hebeda & Zobel, 1996). Staling is a complex process governed my more than one factor. As starch is the major component in bread it is also plays a significant role in staling. Starch retrogradation is mainly responsible for loaf firming over time which is also supported by loss of moisture over time (Hebeda & Zobel, 1996).

Biliaderis et al. (1995) and Denli & Ercan (2001) reported significantly reduced firmness for loaves supplemented with pure AX and extracted wheat flour pentosans, respectively. Similarly, Gómez et al. (2003) demonstrated a delay in bread staling over time caused by addition of various fibres. In contrast to this, the current study demonstrated no significant effect of AX addition on firmness. This may be due to the purity of AX used or the specific characteristics of the supplementation material, which is influenced by extraction method and origin. This is supported by the findings that only highly pure AX significantly improved slice firmness.

The functional role of AX on staling involves the interaction between starch and AX which plays an important role in retarding starch retrogradation (Denli & Ercan, 2001; Sidhu, Al-hooti, & Al-saqer, 1999). AX are able to disrupt the starch network and redistribute water. The water-holding capacity of AX also contribute to the higher moisture content of loaves (Denli & Ercan, 2001). Solubility of the extract, molecular weight and degree of substitution have also been linked to the variation in the effect of extract addition on both dough and bread properties (Biliaderis et al., 1995; Courtin & Delcour, 1998; Courtin & Delcour, 2002; Denli & Ercan, 2001; Hilhorst et al., 1999, 2002). In addition to the interaction between starch and AX, the higher water absorption of the AX supplemented breads may also contribute to a softer crumb texture due to the plasticizing effect of water on the gluten-starch network (Denli & Ercan, 2001).

The crumb structure of the final product is mainly affected by the networks formed during the proofing stage. The gas cells formed during mixing are fixed in a gluten-starch matrix and enclosed in a liquid film layer. The stability of this liquid layer is essential during the proofing stage where expansion of the gas cells occur. AX dissolved in the dough liquid may contribute to gas retention by stabilising the film layer so it can expand

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without rupturing. This may initiate the formation of holes (large cells) due to the increased expansion of gas cells without coalescence (Gan et al., 1995; Scanlon & Zghal, 2001).

This explains the crumb structure properties observed in the current study where AX addition resulted in increased cell volumes which in turn caused a coarser crumb structure. Unusual bubble formation on the outside of doughs with AX addition was observed during the proofing stages which supports the hypothesis of hole-formation. The coarser crumb structure could also be attributed to the effect of AX on the cell wall structure. The increased viscosity of the AX results stabilising of the cell walls by thickening of the wall structures which may translate to the final crumb structure (Gan et al., 1995; Scanlon & Zghal, 2001).

4.4.2.3 Effect of arabinoxylan addition on slice colour

Slice colour of the final product was significantly influenced by AX addition as a result of the initial AX extract colour.

As mentioned in the methods section, the AX extracts were solubilised before addition to the dough mixture. At this stage it was observed that the extract darkened the colour of the water, and it could be expected to affect the colour of both the dough and bread. This was confirmed using spectrometric measurements which indicated that AX addition significantly reduced both the whiteness index (WI) (**Figure 4.17**) and lightness (**Table 4.8**) of the final product. AX addition changed the slice colour towards a darker, more red and yellow colour (**Table 4.8**). This change in colour was already prevalent in the dough stage and is translated into the final crumb and crust colour. The more flour omitted from the formulation, the darker the slice appeared due to the relatively higher AX concentration.

Supplementation of the bread formula with a small amount of soy flour was tested as a means of reducing the colour changes observed with AX addition (**Figure 4.18**). Soy flour addition at 0.2% (flour basis) resulted in a significant increase in the whiteness index (WI) of the control sample indicating that the soy flour indeed had a bleaching effect on the wheat flour which translated to the final product. In the samples with AX addition a significant increase in WI was also observed with soy flour addition, but was not sufficient to produce a final product of the same whiteness as the control. Even at increased soy flour levels of 1%, the AX supplemented samples was still significantly darker than the control (results not shown). This indicates that the bleaching effect of the soy flour on the wheat flour alone was not sufficient to compensate for the dark extract colour.

Table 4.8 Baking trial 1 slice colour properties

Sample	Colour components[a]		
	*L**	*a**	*b**
Control[b]	78.29 a	0.17 a	18.22 a
0%	73.91 b	3.33 b	19.30 b
1.5%	73.20 b	3.59 b	20.02 b
2%	73.00 b	3.66 b	19.83 b
2.5%	71.23 c	3.64 b	19.91 b
3%	70.91 c	3.56 b	19.64 b
4%	70.46 c	3.52 b	19.76 b

[a] Different letter next to values represent significant differences between samples (columns) as determined using a test for least significant differences (Statistica 64)
[b] Control refers to samples with no flour removal or AX addition

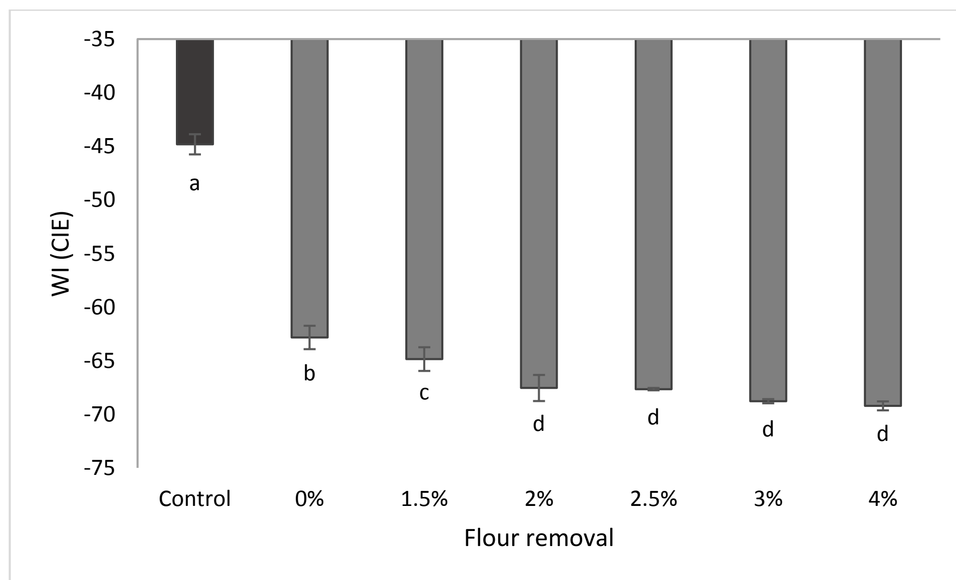

Figure 4.17 Whiteness index (WI) for samples with 0.8% AX addition and varying flour removal levels. Control refers to a sample with no flour removal or AX addition. The error bars represent the standard deviation of duplicate results. Different letter above each bar represent significant differences between samples as determined using a test for least significant differences (Statistica 64).

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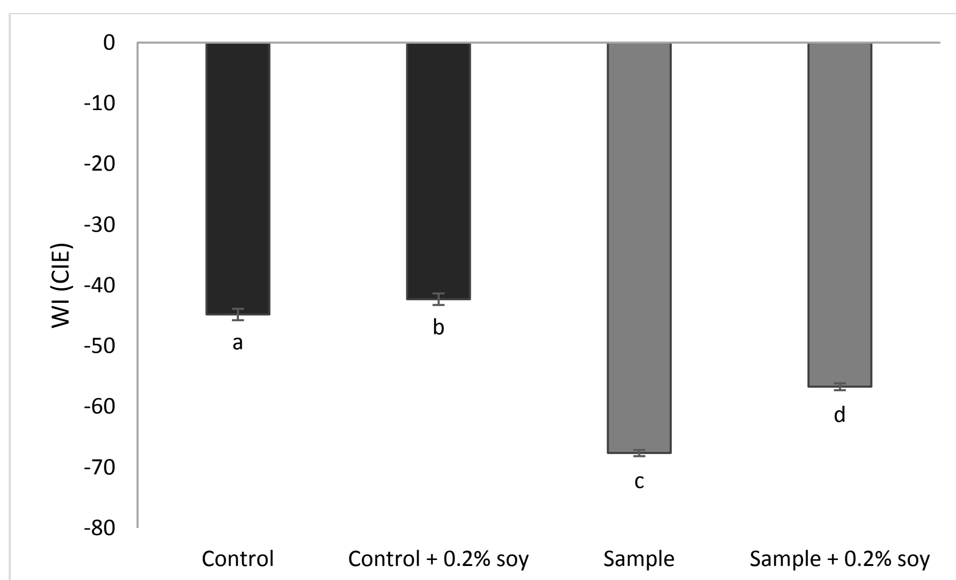

Figure 4.18 Whiteness index (WI) for the control (no flour removal or AX addition) and sample (0.8% AX addition and 2.5% flour removal) with and without soy flour supplementation. The error bars represent the standard deviation of duplicate results. Different letter above each bar represent significant differences ($p<0.05$) between samples as determined using a test for least significant differences (Statistica 64).

Similar to Baking trial 1, the lightness and whiteness of the slice was affected by AX addition in Baking trial 2. Both AX addition and flour removal level had a significant effect on slice colour (ANOVA $p < 0.05$). At the same flour removal levels, AX addition of 1.2% resulted in a significantly darker slice colour than 0.8% AX addition and both had a much darker slice colour than with no AX addition (**Table 4.9**). This would be expected as a higher extract addition level means more of the extract is added to the formulation. This causes a darker solution (water phase) which leads to darker crumb colour. At the higher AX addition level (1.2%), the WI was significantly decreased with an increase in flour removal level. This indicates that the higher AX addition level had a more pronounced effect on the whiteness.

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Table 4.9 Baking trial 2 slice lightness and whiteness index (WI)

Arabinoxylan(%)	Flour removal (%)	Lightness (L^*)	WI
0	0	78.29 a	-44.82 a
	2	78.83 a	-45.09 a
	2.5	77.97 a	-44.79 a
	3	78.10 a	-45.57 a
0.8	0	73.91 b	-62.84 c
	2	73.00 b	-67.55 b
	2.5	71.23 c	-67.66 b
	3	70.91 c	-68.78 b
1.2	0	70.26 cd	-72.97 d
	2	69.17 d	-78.43 e
	2.5	67.44 e	-83.52 f
	3	67.14 e	-85.45 g

As for the previous trials, the slice colour was directly affected by the colour of the extract added in the final baking trial (**Table 4.10**). Highly pure AX consists of a white powder which is responsible for the light final product obtained. E2 has a lighter colour than E1, due to the discolouration step included in the purification of E2 (**Figure 4.19**). Samples containing E1 and E2 result in significant differences in slice lightness and WI compared to the control. AX results in the whitest final product followed by the control, E2 and finally E1. **Figure 4.19** illustrates the differences in slice colour for the samples containing the three different AX sources. It is clear that the sample containing the highly pure AX is the lightest followed by E2 and then E1. Between E1 and E2, E2 appears lighter and more comparable to the control, this is confirmed by die spectrophotometric results illustrated in **Table 4.10**.

In the current study, it was observed that extraction and purification methods used for AX extraction affects the final extract colour. Jacquemin et al. (2012) obtained an AX extract of dark brown colour using similar alkaline extraction methods. They suggested that the molecules responsible for colour were large or bound to larger structures because they were retained by ultrafiltration. Further purification by anion exchange chromatography and ethanol precipitation was successfully implemented for discolouration of the extract. Lightness (L^*) was increased from 58.9 to 61.9. This concurs with the results from Zeitoun et al. (2010) using twin-screw extrusion for AX extraction with ethanol precipitation and anion exchange chromatography purification steps.

The extract colour obtained by the two extraction methods used in this study (with purification and without purification) directly affected the final bread colour.

Figure 4.19 Extract colour and slice colour of commercial AX (left), extract 1 (E1) (centre) and extract 2 (E2) (right).

Table 4.10 Baking trial 4 slice colour properties

	L*	***a****	***b****	**WI**
Control	81.63 a	0.03 a	17.57 a	-30.81 a
AX	80.64 a	-0.09 b	16.52 b	-28.11 a
E1	73.42 b	3.34 c	19.05 c	-59.93 b
E2	77.54 c	1.02 d	18.68 c	-47.50 c

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4.4.3 Determination of the optimum arabinoxylan addition level for maximum flour removal

From the results obtained in Baking trial 2 it was observed that at a maximum flour removal level of 2.5% the physical properties of the loaves (i.e. weight and volume) were comparable to that of the control at both 0.8% and 1.2% AX addition levels. With a larger change in colour at the higher AX addition level but with no significant advantages, the optimum of 0.8% was chosen. Not only can the same physical properties be obtained using the lower addition level but there is an added economic advantage due to the lower cost with application of the lower addition level. **Figure 4.20** illustrates the physical appearance of the control (right) compared to the optimum condition (left), once again demonstrating that the only major difference is loaf colour. Even though this difference in colour is substantial, it's not necessarily a negative property. Further market research and sensory testing is required to determine whether the difference in colour and crumb structure is acceptable or even preferable to the consumer.

Figure 4.20 The final product obtained at optimum conditions, 0.8% crude AX addition with 2.5% flour removal (left) and control (right).

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4.4.4 Effect of laccase addition on bread properties

Laccase addition (single dosage) did not affect bread physical properties but did significantly decrease slice firmness.

After optimisation of AX addition, potential improvement of the final product was investigated. Enzyme additions are commonly used in commercial bread making and was therefore included in this study. The inclusion of laccase has the potential to improve crumb softness and loaf volume (Selinheimo et al., 2007). Laccase acts as an oxidising agent which allows for further, non-enzymatic, cross-linking of monomers. In the case of bread-making, this linking can occur between ferulic acid bound to AX which may result in the formation of larger linked polymeric structures or linking of AX polymers to gluten proteins.

Table 4.11 indicates that laccase addition did not significantly affect any of the physical bread properties except for bread weight (**Appendix 1**). AX addition and flour removal did however affect all the physical properties, which corresponds to the results obtained in Baking trial 2. Although laccase addition did not significantly affect specific loaf volume, condition 4 (**Table 4.11**) shows higher specific loaf volumes for samples with both AX and laccase addition. This result therefore supports the hypothesis that the effect of laccase is mainly based on the cross-linking of ferulic acid bound to AX as AX addition allows for more available substrate (FA) for the laccase enzyme resulting in the observed improvement in specific volume.

Table 4.11 Outline of baking trial 3 bread loaf physical properties

A[a]	**L**[b]	**F**[c]		**Dough weight (g)**	**Bread weight (g)**	**Proof height (mm)**	**Bread height (mm)**	**Loaf volume (cm^3)**	**Specific Volume (cm^3/g flour)**
+	-	-	1	176.62±0.12	152.93±0.23	84.5±0.7	98.5±0.7	1070±7	10.8±0.1
-	-	+	2	169.77±0.33	146.74±0.11	79.5±0.7	93.5±0.7	1035±7	10.7±0.1
-	+	-	3	173.75±0.21	152.60±0.14	82.0±1.4	96.5±0.7	1060±4	10.7±0.1
+	+	+	4	173.15±0.49	150.50±0.57	81.5±0.7	96.5±0.7	1070±4	11.0±0.1

[a]AX addition (0.8% based on flour weight) (-) no AX addition, (+) AX addition
[b]Laccace addition (1U per gram flour) (-) no flour removal, (+) flour removal
[c]Flour removal (2.5% based on flour weight) (-) no laccase addition, (+) laccase addition

Crumb structure (**Table 4.12**) was not affected by laccase addition. Final moisture content was significantly affected by both flour removal and AX addition which concurs with the results from Baking trial 2, but laccase addition did not alter the moisture content as similar water absorption values were used for sample with and without laccase addition (**Appendix 2**). The slice firmness however was significantly decreased in samples containing laccase (**Appendix 2**).

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Table 4.12 Baking trial 3 crumb texture and slice properties

A[a]	L[b]	F[c]		Number of cells	Area of cells	Cell diameter	Cell volume	Moisture (%)	Firmness (grams of force)
+	-	-	1	4565±144	49.4±0.4	11.7±0.4	4.4±0.1	40.90±0.09	194.62±13.20
-	-	+	2	4638±148	48.9±0.8	11.1±0.5	4.3±0.2	37.45±0.20	199.31±6.82
-	+	-	3	4558±31	50.1±0.1	11.2±0.1	4.5±0.2	39.74±0.10	179.59±7.82
+	+	+	4	4623±134	49.6±0.4	11.0±0.0	4.8±0.0	39.49±0.45	158.78±1.26

[a]AX addition (0.8% based on flour weight) (-) no AX addition, (+) AX addition
[b]Laccace addition (1U per gram flour) (-) no flour removal, (+) flour removal
[c]Flour removal (2.5% based on flour weight) (-) no laccase addition, (+) laccase addition

All slice colour properties (**Table 4.13**) were significantly (ANOVA p<0.05) affected by both AX and laccase addition but not by flour removal (**Appendix 3**). Because the AX extract and the laccase enzyme were both a darker coloured powder, compared to the flour, they resulted in darker slice colours. Condition 2, with only laccase addition and no AX resulted in the lightest colour with the highest whiteness index (WI). The lighter product could be more acceptable for consumers if the product is marketed as a white bread product.

Table 4.13 Baking trial 3 slice colour properties

A[a]	L[b]	F[c]		L*	a*	b*	WI
+	-	-	1	73.31±0.26	3.19±0.08	18.35±0.34	-59.34±0.38
-	-	+	2	81.58±0.56	0.04±0.01	17.54±0.13	-30.59±1.01
-	+	-	3	79.63±0.84	0.37±0.13	18.42±0.03	-40.61±2.14
+	+	+	4	72.27±1.07	3.66±0.02	19.52±0.06	-65.48±3.27

[a]AX addition (0.8% based on flour weight) (-) no AX addition, (+) AX addition
[b]Laccace addition (1U per gram flour) (-) no flour removal, (+) flour removal
[c]Flour removal (2.5% based on flour weight) (-) no laccase addition, (+) laccase addition

Laccase oxidation can proceed in gluten proteins and AX polymer through certain amino acid residues of gluten or via phenolic moieties (specifically ferulic acid) present in the polymers. The mechanism of laccase in bread making is complicated as the enzyme may cause the formation of linkages in or between AX polymers, in or between gluten proteins, and between AX polymer and gluten.

Selinheimo et al. (2007) demonstrated that the effects of laccase on bread properties is mainly due to the interaction within the AX polymers rather that the gluten network. With combined dosage of laccase and xylanase the dough hardening effect of laccase was decreased, indicating that the depolymerisation of AX diminished the effects of laccase. Labat et al. (2001) observed no covalent complexes between AX and gluten with laccase addition, which resulted in no significant differences in the viscoelastic properties of dough compared to the control (Caballero et al., 2007). Furthermore, no differences in cell wall properties were

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observed (Selinheimo et al., 2007) indicating that laccase addition did not affect crumb structure which is in agreement with the results of the current study.

The mechanism behind the functional effect of laccase on slice firmness is not yet fully understood. A decrease in firmness with laccase addition was observed in the current study. The same result have been reported in literature (Primo-Martín et al., 2003; Selinheimo et al., 2007). Other authors however, observed an increase in firmness with laccase addition but this effect was attributed to the β-glucanase side activity of the purified laccase (Flander et al., 2008).

Finally, the observation that laccase addition has no significant effect on bread physical properties also correlates with what has been found in literature (Selinheimo et al., 2007).

4.4.5 Evaluation of the cost/savings of flour replacement

At the optimum flour replacement level (2.5% flour removal and 0.8% AX addition) the final bread production cost is approximately R457 (9.2%) more per ton compared to the control. To maintain production profits, loaves with AX addition need to be sold for R1.00 more than commercial white bread (**Table 4.17**).

Table 4.14 and 4.15 indicates that the production cost of AX influences bread production cost at the optimum condition resulting in higher costs compared to the control. To make this application viable either higher selling prices should be implemented for the AX supplemented loaves or the profit margin for bread producers have to decrease. Alternatively, wheat bran can be fractionated to include the extraction of other bran constituents such as starch and protein. This co-production may improve the viability of this process by increasing the value obtained from the source.

Table 4.14 Production cost of bread based on 100 g baking test formula for the control

Component	Cost per kg (R)	Baker's %	Baker's to Formula %	Unit Content (kg)	Cost per unit (R)	Cost per ton (R)
Flour	5.60	100.00	56.66	0.08	0.48	3 172.80
Arabinoxylan		-	-	-	-	-
Salt	2.78	1.50	0.85	0.00	0.00	23.63
Sugar	9.02	6.00	3.40	0.01	0.05	306.63
Yeast	23.84	5.30	3.00	0.00	0.11	715.88
Ascorbic acid	65.04	0.00	0.00	0.00	0.00	0.01
Shortening	43.92	3.00	1.70	0.00	0.11	746.52
Water	-	60.70	34.39	0.05	-	-
Total BOM	-	176.50	100.00	0.15	0.74	**4 965.46**

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Table 4.15 Production cost of bread based on 100 g baking test formula with AX addition

Component	Cost per kg (R)	Baker's %	Baker's to Formula %	Unit Content (kg)	Cost per unit (R)	Cost per ton (R)
Flour	5.60	97.50	55.78	0.08	0.47	3 123.57
Arabinoxylan	110.66	0.80	0.46	0.00	0.08	506.44
Salt	2.78	1.50	0.85	0.00	0.00	23.63
Sugar	9.02	6.00	3.40	0.01	0.05	306.63
Yeast	23.84	5.30	3.00	0.00	0.11	715.88
Ascorbic acid	65.04	0.00	0.00	0.00	0.00	0.01
Shortening	43.92	3.00	1.70	0.00	0.11	746.52
Water	-	62.6	34.73	0.05	-	-
Total BOM	-	174.80	100.00	0.15	0.82	**5 422.68**

Table 4.16 outlines the production cost of bread with AX and laccase addition. The results indicate that inclusion of laccase results in higher production costs compared to both the control and loaves with only AX addition. With the enzyme additions comes the added advantage of a softer texture which could be a characteristic that consumers are willing to pay more for. The selling price for loaves with laccase and AX addition will be 10.9% more, when only taking into account the effect of production cost.

Table 4.16 Production cost of bread based on 100 g baking test formula with AX and laccase addition

Component	Cost per kg (R)	Baker's %	Baker's to Formula %	Unit content (kg)	Cost per unit (R)	Cost per ton (R)
Flour	5.60	97.50	55.77	0.08	0.47	3 123.39
Arabinoxylan	110.66	0.80	0.46	0.00	0.08	506.41
Laccase	1 200.00	0.01	0.01	0.00	0.01	68.65
Salt	2.78	1.50	0.86	0.00	0.00	23.85
Sugar	9.02	6.00	3.43	0.01	0.05	309.59
Yeast	23.84	5.30	3.03	0.00	0.11	722.80
Ascorbic acid	65.04	0.00	0.00	0.00	0.00	0.01
Shortening	43.92	3.00	1.72	0.00	0.11	753.73
Water	-	60.70	34.72	0.05	-	-
Total BOM	-	174.81	100.00	0.15	0.83	**5 508.44**

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The selling price of commercial white bread was estimated at R10.99 which obtained from the commercial selling price available.

Table 4.17 Adjustment of bread selling price (per 700 g) for loaves with AX addition compared to commercial white bread

	Production cost (R/ton)	**Selling price (R/ton)**	**Profit (%)**	**Adapted price**	**Selling price per loaf (R/700 g)**
Control	4965	15700	316	-	10.99
AX addition	5422	15700	290	17133	11.99
Difference	457	-	26	-	**1.00**

The values obtained in this costing section is only an estimation of the potential for AX application. The aim was to present a broad overview in which loaves containing AX can be compared to commercial white bread in term of production and selling price.

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4.5 Conclusion

From Baking trial 1 the optimum range for flour removal was determined to be between 2 and 3%. This optimum was determined by taking into account various product properties. One important observation was the increase in water absorption required with AX addition, due to the high water holding capacity of AX. The water addition was required to maintain the dough consistency and to ensure that dough development is not adversely affected by the AX addition. The optimum flour removal range resulted in products which were comparable to the control in terms of bread weight and loaf volume with the beneficial effect of increased moisture content. The only adverse effect observed with AX addition, is the darkened final product colour. However, this change in colour may have a marketing advantage in the industry. Due to the increase in consumer health awareness it is possible that consumers may prefer the darker loaf as an indication of a healthier option due to the increased fibre content.

Baking trial 2 continued with the optimum flour removal range and investigated different AX addition levels. From the two levels tested (0.8 and 1.2%), both performed similar in most of the responses evaluated. However, the higher addition level resulted in higher specific volumes and increased bread height but also had a darker crumb and crust colour.

With 0.8% AX addition and nearly 2% increased water absorption, 2.5% flour could be removed resulting in a comparable product to the control. In all physical aspect the AX supplemented loaf was similar to the control with the only difference being the final product colour. After determining the optimum condition, further improvement was investigated using enzyme addition, specifically laccase. The inclusion of laccase did not significantly affect any physical loaf properties and the specific loaf volume and volume was similar to the results in baking trial 2 (without laccase addition). Loaves containing AX, and loaves containing both AX and laccase had a specific volume of 11 cm^3/g flour. Laccase addition did however significantly improve the softness of the loaf indicating that its inclusion may hold some benefit.

During the final baking trial it was concluded that both the extracts (E1 and E2) performed similar to pure AX in the application process. The extracts resulted in a final product with similar weight and volume compared to the control and pure AX. E2 also showed an improvement in final product colour compared to E1 due to addition of a discolouration step during the purification process.

In terms of the economics of this process, the cost of AX extraction makes the application of the product more costly compared to the traditional white bread-making process. To maintain profits loaves (700 g) with AX addition needs to be sold for R1.05 (9.6%) more than commercial white bread. The design of a more effective extraction process, including the co-production of other high value products from the low value source, could improve potential for commercialisation of the process making it a viable option.

In conclusion, this study proved that AX, extracted from wheat bran, can be used as a partial flour replacer while maintaining the product properties and increasing water and fibre contents of the final product.

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FINAL CONCLUSIONS AND RECOMMENDATIONS

Arabinoxylans are of interest as they are known to be present in wheat bran in large quantities, and due to their functional properties including viscosity, water solubility, water holding capacity, oxidative cross linking, gel formation and foam stability are ideal candidates to provide high value products from a low value source. The range of functional properties displayed by AX enables its use in a wide variety of industrial applications for both food and non-food uses.

The bread making industry is an ideal target market for AX, being the second largest food manufacturing industry in South Africa. The benefits of AX include the relatively higher fibre content in loaves with AX addition due to flour removal. To date, however, no commercial supply of AX is available. Therefore the aim of the current work was to study the feasibility of extracting AX from the animal feed co-product produced during wheat flour production, and its application in the bread making process as a partial flour replacer.

The progress achieved in this study was the production of sufficient quantities of AX to give new insights into the functionality of a crude AX fraction extracted from wheat bran in the bread making process. This feedstock is mainly used as low value animal feeds produced as a co-product in a wheat milling. This study establishment AX as a novel food ingredient for the bread making industry as a flour replacer.

This work forms the basis from which further work can be undertaken and opens up a variety of areas where further investigation is recommended. The extraction process can be further refined to improve the yields and purity. The expense of purification on a commercial scale in a biorefinery should however have to be considered to determine the most cost effective option. For the application purpose presented in this study, it was observed that the purity of AX was sufficient to produce the response desired. Extraction scale-up should also be optimised so that the production costs can be defined in greater detail. Research into the economic feasibility of process integration into a biorefinery is also essential. Biorefinery of wheat bran could include fractionation into more than one value added product to increase feasibility of the process. The expectations and requirements of the bread making industry also needs to be investigated to develop a target market. Water should be included as cost input for bread production cost determination.

The arabinoxylans extracted in this study was only partly characterised. Monosaccharide analysis using HPLC gave information on the purity (AX content) of the extracts and average degree of substitution (A/X ratio). However, information on the substitution pattern of AX molecules through the use of techniques such as Nuclear Magnetic Resonance (NMR) would give additional information on the mechanisms of AX functionality in bread making.

Owing to the amounts of AX available for bread making trials, only a small scale trial could be conducted to give initial insight into AX functionality. A more holistic investigation of AX functionality in the dough and proofing phase is required as well as up-scaled baking trials on commercial size loaves. Finally, the effect of

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enzyme addition needs to be evaluated using a full factorial design and various enzyme dosages to enable robust conclusions to be drawn.

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APPENDIX 1

Factor	ANOVA; Var.:Dough weight; R-sqr=.99141; Adj:.98497 2**(3-1) design; MS Residual=.1025625				
	SS	df	MS	F	p
(1)Flour removal	27.71401	1	27.71401	270.2158	0.000080
(2)Hemi's	19.50001	1	19.50001	190.1281	0.000160
(3)Lac	0.13261	1	0.13261	1.2930	0.318993
Error	0.41025	4	0.10256		
Total SS	47.75689	7			

Factor	ANOVA; Var.:Bread weight; R-sqr=.99173; Adj:.98553 2**(3-1) design; MS Residual=.101425				
	SS	df	MS	F	p
(1)Flour removal	34.36205	1	34.36205	338.7927	0.000051
(2)Hemi's	8.36405	1	8.36405	82.4654	0.000815
(3)Lac	5.91680	1	5.91680	58.3367	0.001578
Error	0.40570	4	0.10142		
Total SS	49.04860	7			

Factor	ANOVA; Var.:Proof height; R-sqr=.87879; Adj:.78788 2**(3-1) design; MS Residual=.875				
	SS	df	MS	F	p
(1)Flour removal	15.12500	1	15.12500	17.28571	0.014173
(2)Hemi's	10.12500	1	10.12500	11.57143	0.027235
(3)Lac	0.12500	1	0.12500	0.14286	0.724659
Error	3.50000	4	0.87500		
Total SS	28.87500	7			

Factor	ANOVA; Var.:Bread height; R-sqr=.92727; Adj:.87273 2**(3-1) design; MS Residual=.5				
	SS	df	MS	F	p
(1)Flour removal	12.50000	1	12.50000	25.00000	0.007490
(2)Hemi's	12.50000	1	12.50000	25.00000	0.007490
(3)Lac	0.50000	1	0.50000	1.00000	0.373901
Error	2.00000	4	0.50000		
Total SS	27.50000	7			

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APPENDIX 2

Factor	ANOVA; Var.:Slice firmness; R-sqr=.87576; Adj:.78257 2**(3-1) design; MS Residual=70.87381				
	SS	df	MS	F	p
(1)Flour removal	129.847	1	129.847	1.83208	0.247313
(2)Hemi's	325.253	1	325.253	4.58918	0.098839
(3)Lac	1543.179	1	1543.179	21.77361	0.009545
Error	283.495	4	70.874		
Total SS	2281.773	7			

Factor	ANOVA; Var.:Moisture; R-sqr=.97923; Adj:.96366 2**(3-1) design; MS Residual=.0652402				
	SS	df	MS	F	p
(1)Flour removal	6.81324	1	6.813238	104.4331	0.000517
(2)Hemi's	5.10895	1	5.108950	78.3098	0.000900
(3)Lac	0.38363	1	0.383632	5.8803	0.072376
Error	0.26096	4	0.065240		
Total SS	12.56678	7			

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APPENDIX 3

Factor	ANOVA; Var.:L*; R-sqr=.98263; Adj:.96961 2**(3-1) design; MS Residual=.5609375				
	SS	df	MS	F	p
(1)Flour removal	0.4186	1	0.4186	0.7463	0.436383
(2)Hemi's	122.0703	1	122.0703	217.6184	0.000123
(3)Lac	4.4551	1	4.4551	7.9423	0.047916
Error	2.2437	4	0.5609		
Total SS	129.1878	7			

Factor	ANOVA; Var.:b*; R-sqr=.96662; Adj:.94159 2**(3-1) design; MS Residual=.0340625				
	SS	df	MS	F	p
(1)Flour removal	0.040612	1	0.040612	1.19229	0.336226
(2)Hemi's	1.814513	1	1.814513	53.27009	0.001874
(3)Lac	2.091013	1	2.091013	61.38752	0.001433
Error	0.136250	4	0.034062		
Total SS	4.082388	7			

Factor	ANOVA; Var.:a*; R-sqr=.99892; Adj:.99811 2**(3-1) design; MS Residual=.0056875				
	SS	df	MS	F	p
(1)Flour removal	0.00911	1	0.00911	1.602	0.274296
(2)Hemi's	20.70461	1	20.70461	3640.371	0.000000
(3)Lac	0.32401	1	0.32401	56.969	0.001651
Error	0.02275	4	0.00569		
Total SS	21.06049	7			

Factor	ANOVA; Var.:WI; R-sqr=.98965; Adj:.98189 2**(3-1) design; MS Residual=4.119288				
	SS	df	MS	F	p
(1)Flour removal	7.547	1	7.547	1.8320	0.247320
(2)Hemi's	1437.820	1	1437.820	349.0459	0.000048
(3)Lac	130.492	1	130.492	31.6783	0.004902
Error	16.477	4	4.119		
Total SS	1592.336	7			

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