MECHANICAL AND PHYSICAL PROPERTIES OF BIODEGRADABLE WHEAT BRAN, MAIZE BRAN, AND DRIED DISTILLERS GRAIN ARABINOXYLAN FILMS

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The Supervisory Committee certifies that this ***disquisition*** complies with North Dakota State University's regulations and meets the accepted standards for the degree of

MASTER OF SCIENCE

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ABSTRACT

Arabinoxylans are non-starch polysaccharides in the cell walls of cereal crops including maize (*Zea mays* L.) and wheat (*Triticum aestivum* L.). Arabinoxylans are produced when maize bran, dried distillers grain, and wheat bran are processed. The objective of this research was to extract arabinoxylan from cereal processing byproducts for use in biodegradable films. The arabinoxylan was extracted with dilute sodium hydroxide and purified using α-amylase and protease. In addition to arabinoxylan, these films were made with either glycerol or sorbitol as a plasticizer at levels of 100, 250 or 500 g kg^{-1}. These films had tensile strengths as high as 29.3 MPa and puncture resistances as high as 10.1 N. The water solubility of these films ranged from 305 to 956 g kg^{-1}, and the water vapor permeability ranged from 44.8 to 90.9 g h^{-1} m^{-2}. The characteristics of these films show promise for biodegradable food packaging materials.

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A:X	Arabinose to Xylose Ratio
AACCI	American Association of Cereal Chemists International
AOCS	American Oil Chemists' Society
ASTM	American Society of Testing and Materials
AX	Arabinoxylan
DDG	Dried Distillers Grain
FTIR	Fourier Transmission Infrared Spectroscopy
GC-FID	Gas Chromatography Flame Ionization Detector
HPLC	High Performance Liquid Chromatography
HPSEC	High Performance Size Exclusion Chromatography
MALS	Multi-Angle Light Scattering Detector
MB	Maize Bran
Mw	Weight Average Molecular Weight
NMR	Nuclear Resonance Spectroscopy
PI	Polydispersity Index
Ra	Roughness Average
RH	Relative humidity
RI	Refractive Index Detector
RMS	Root Mean Square
WB	Wheat Bran
WEAX	Water Extractable Arabinoxylan
WUAX	Water Unextractable Arabinoxylan
WVTR	Water Vapor Transmission Rate

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GENERAL INTRODUCTION

Byproducts of the cereal industry including wheat (*Triticum aestivum* L.) bran (WB) and maize (*Zea mays* L.) bran (MB) from the milling industry and dried distillers grain (DDG) from the ethanol industry are abundant. To increase the sustainability of the food industry, the amount of waste that is not re-used or re-purposed must be kept to a minimum. One of the ways to reuse these byproducts includes using arabinoxylans (AX) from WB, MB, and DDG in the production of food packaging. Since films made from AX do not have all the desirable properties of films used for food packaging, plasticizers must be added to increase the stability, flexibility, and mechanical strength of the films (Antoniou et al. 2014; Vieira et al. 2011). The mechanical properties of food packaging depend upon the type of food being packaged, but include water content, water solubility, water vapor permeability, hydrophilicity, puncture resistance, tear resistance, tensile strength, color, surface topography, and biodegradability (Tharanathan 2003). Two of the plasticizers commonly used in food packaging films are glycerol and sorbitol. These plasticizers are vital to modifying food packaging film properties for the type of food being stored because all of the mechanical properties previously listed will vary depending upon what is being stored and for how long it will be stored (Antoniou et al. 2014).

The bran of cereal crops is the outer layer of the cereal grain. In wheat, this layer is made up of the outer pericarp, inner pericarp, testa, hyaline cells, and aleurone layer (Apprich et al. 2014; Anson et al. 2012). The WB is about 165 g kg $^{-1}$ of the wheat kernel (Maes and Delcour 2002), and the AX content of WB is up to 690 g kg^{-1} (Saeed et al. 2011). In maize, the bran is made up of the pericarp, epidermis, and seed coat, and accounts for 55 g kg^{-1} of the maize kernel (Delcour and Hoseney 2010). Maize bran contains about 350 g kg^{-1} of AX (Saeed et al. 2011; Zhang and Whistler 2004). Dried distillers grain is one of the byproducts of the ethanol industry produced from the fermentation and distillation of the maize processing (Zarrinbakhsh et al. 2013). The AX content in DDG is usually around 90 g kg $^{-1}$.

Arabinoxylan is one of the most common polysaccharides, second only to cellulose (Zhang et al. 2011). It is composed of a xylose backbone linked via β-1,4-glycosidic bonds, and it has arabinose substituents that are O-2, O-3, or O-2 and O-3 linked to xylose (Reis et al. 2015; Aguedo et al. 2014; Kiszonas et al. 2013). Arabinoxylan can vary in substitution pattern, molecular weight, polydispersity index, and arabinose to xylose ratio. These variables influence the chemical characteristics of the food packaging films made from the AX.

Food packaging has traditionally been made from synthetic materials including polyoefins, polyamids, and polyesters because they have desirable barrier properties (Tharanathan 2003). However, these polymers are not biodegradable. Biodegradable food packaging materials are slowly being developed and used commercially. Use of AX as the basis for food packaging would be one type of biodegradable material that could be commercialized. Food packaging must be appropriate for the food being stored. This is determined by matching the barrier and mechanical properties of the packaging material with the requirements of the food. Some of these properties include water vapor permeability, tensile strength, and tear resistance (Arrieta et al. 2015).

Overall Objectives

- To develop an extraction and purification method that produces AX of purity higher than that of previously published research for WB, MB, and DDG
- To create films from WB AX, MB AX, or DDG AX in combination with a plasticizer (sorbitol or glycerol) at one of the following three levels: 100, 250, or 500 g kg^{-1}, and analyze their mechanical, physical, and biodegradability properties
- To determine if there are significant ($P \leq 0.05$) correlations between the chemical properties of the films and their mechanical, physical, and biodegradability properties

Overall Hypotheses

- Alkaline extraction coupled with numerous purification techniques including enzymatic treatment with amylase and protease, ethanol fractionation, and dialysis will produce WB AX, MB AX, and DDG AX that is purer than that in previously published research
- Films made from WB AX, MB AX, or DDG AX with a polyol plasticizer (sorbitol or glycerol) will have varying mechanical, physical, and biodegradation properties depending upon their composition
- There will be significant ($P \leq 0.05$) correlations between the chemical properties (molecular weight, polydispersity index, linkage profile, etc.) of the films and their mechanical, physical, and biodegradability properties

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CHAPTER 1. LITERATURE REVIEW

1.1. Cereals

Cereals are in the grass (*Gramineae*) family, which includes wheat, maize, barley, rye, rice, oats, sorghum, triticale, and millet (Delcour and Hoseney 2010a). Wheat and maize are two of the three most commonly produced cereal crops in the world (the third being rice) (Heikkinen et al. 2013). One of the defining characteristics of this type of grain is that it produces a fruit called a caryopsis (Delcour and Hoseney 2010a). This fruit is known to many as a kernel or grain. The main components of the caryopsis include starch (600 to 700 g kg^{-1}), protein (150 g kg^{-1}), and non-starch polysaccharides (30 to 80 g kg^{-1}) (Saulnier et al. 2007).

1.1.1. Wheat

1.1.1.1. Structure

Wheat (*Triticum aestivum* L.) can be divided into soft and hard classes (Delcour and Hoseney 2010a), which depends upon the puroindalin content (Giroux et al. 2000). The six classes of wheat in the U.S. are hard red winter, hard red spring, soft red winter, soft white, hard white, and durum (Delcour and Hoseney 2010a). The characteristics of each wheat class vary with genotypic and environmental factors such as temperature and precipitation. The average wheat kernel in North America is 8 mm long and 35 mg, but exact size depends greatly upon cultivar and the location of the kernel on the wheat spike. Wheat kernels are rounded on the dorsal side and contain a crease on the ventral side that runs the entire length of the kernel. Wheat kernels exist in different colors depending upon the pigment(s) present in the seed coat, but are usually white or red.

Regardless of class, all wheat kernels have the same four main parts, which are also known as layers due to their spatial arrangement. The main layers of a wheat kernel include the pericarp, seed coat, endosperm, and germ (Delcour and Hoseney 2010a). Each of these layers contain many tissues made of numerous cells that together make up the composition of the wheat kernel. Together all these tissues determine the quality of the wheat. Figure 1.1 shows an illustration of a wheat kernel and clearly labels all layers present (GoodMills Innovation GmbH 2015a).

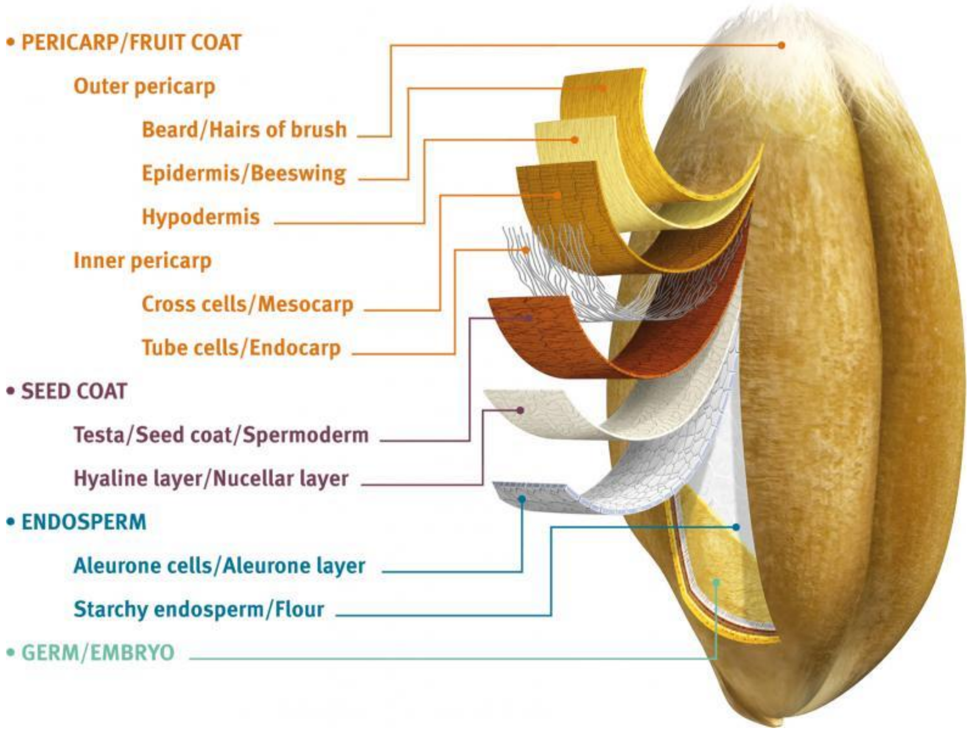

Figure 1.1. Illustration of the longitudinal cross section of a wheat kernel (GoodMills Innovation GmbH 2015a).

Wheat bran (WB), the outer portion of the wheat kernel that surrounds the endosperm, is a by-product of commercial wheat processing as it is often not desired for patent flours (Apprich et al. 2014; Swennen et al. 2006). As such, it is currently used as an ingredient in animal feed. However, it is of great nutritional value for humans as it is high in dietary fiber (Swennen et al. 2006). One of the main types of dietary fiber is arabinoxylan (AX). WB contains many valuable components including starch, lactic acid, poly lactic acid, succinic acid, AX, β-glucan, ferulic acid, protein, amino acids, and oil (Apprich et al. 2014).

The innermost layer of a kernel of wheat is the endosperm. The tissue in this layer is the portion of the wheat kernel most commonly utilized for baking and cooking due to its high starch content (Delcour and Hoseney 2010a). In addition, it can be made into a refined flour that is highly palatable. The endosperm is made up of peripheral, prismatic, and central cells (named after their location, size, and shape). The peripheral endosperm cells are the first row of cells on the inside of the aleurone layer, and are usually small with the same diameter in all directions of the cell. The next type of cell (from the outside of the endosperm to the inside) is the prismatic endosperm cell. This type of cell is about 150 x 50 x 50 µm and is present in multiple layers. The final type of cell in the starchy endosperm is the central cell, which is much more irregular in both shape and size than the other two types of endosperm cells. The cell walls of endosperm cells are made of AX, and in smaller amounts (1,3)(1,4)-β-D-glucans and other hemicelluloses (Ying et al. 2015; Ying et al. 2013; Ying et al. 2011). About 850 g kg^{-1} of all non-starch polysaccharides present in wheat are AX (Kiszonas et al. 2013). Despite the presence of many non-starch polysaccharides in this portion of the kernel, there is no cellulose present.

At one end of the wheat kernel is the germ, which is about 25 to 35 g kg^{-1} of the entire kernel (Delcour and Hoseney 2010a). It is made up of two main components: the scutellum and the embryonic axis. The composition of the germ is approximately 250 g kg^{-1} protein, 180 g kg^{-1} carbohydrate (mainly sucrose and raffinose), 160 g kg^{-1} oil (in the embryonic axis) or 320 g kg^{-1} oil (in the scutellum), and 50 g kg^{-1} ash. There is no starch present, but there are many enzymes as well as B vitamins and tocopherol.

1.1.1.2. Wheat Bran

Wheat bran is made up of many layers including the outer pericarp, inner pericarp (cross cells and epidermis cells), testa (seed coat), hyaline cells (nuclear epidermis), and the aleurone layer (Apprich et al. 2014; Anson et al. 2012). The outer and inner pericarp are made up of cells that are mainly open space due to the thickening and lignification of the cell walls of these cells after the cytoplasm of both the cross cells and tube cells degenerate (Anson et al. 2012). The walls of these cells have high levels of heteroxylans, lignin, ferulic acids, and cellulose. The testa, or seed coat, is rich in lignin and exceedingly hydrophobic. Hyaline cells have high levels of AX and ferulic acid, however there are few cross-links because the ferulic acid present is mainly in the monomeric form without the presence of ferulic acid dimers. The aleurone layer is the main layer of the bran (about 500 g kg^{-1} of the bran). The aleurone cells are not lignified and have high levels of phytate, niacin, and protein. Wheat bran contains many bioactive compounds including phenolic acids (mainly ferulic acid), alkylresorcinols, vitamin E, lignin, lignan, carotenoids, methyl donors, B-vitamins, glutathione, phytates, and minerals (iron, magnesium, zinc, selenium, and manganese).

The outermost layer of the wheat kernel is the pericarp. In wheat, this layer is about 50 g kg^{-1} of the kernel and consists of 715 g kg^{-1} non-starch polysaccharide, 200 g kg^{-1} cellulose, 60 g kg^{-1} protein, 20 g kg^{-1} ash, and 5 g kg^{-1} fat (Delcour and Hoseney 2010a). The outer pericarp is often lost before milling due to the thin-walled nature of the epidermis cells. In the inner pericarp, the cross cells and tube cells are present. The cross cells are long (125 x 20 µm), cylindrical cells that are tightly packed with their long axis perpendicular to the long axis of the wheat kernel. The tube cells are the same shape and size as cross cells, but oriented parallel to the long axis of the kernel with ample intercellular space.

The second outermost layer is the seed coat (Delcour and Hoseney 2010a). This layer is made up of cells that are firmly joined to the tube cells on the distal side of the tube cells. The seed coat is typically 5 to 8 µm thick, and the hyaline layer is about 7 µm thick. The hyaline layer is bound tightly to both the seed coat and the aleurone cells. This layer provides wheat with its red or white color.

The innermost layer of the bran, the aleurone layer, is typically one cell thick and surrounds the wheat kernel endosperm in its entirety (Delcour and Hoseney 2010a). When wheat is milled to produce refined flour, the aleurone layer is removed along with the pericarp and seed coat as a byproduct called bran. Aleurone cells are cuboidal cells (50 µm across) with thick walls (3 to 4 µm) that do not contain any starch when they are fully mature. The main component of aleurone cells is cellulose. Vitamins including niacin, riboflavin, and thiamin are present in high concentrations in this portion of the endosperm. The aleurone cells that cover the germ are only about 13 µm across and 1 to 2 µm thick.

The bran of a wheat kernel is about 140 to 190 g kg^{-1} of the total weight of the kernel (Maes and Delcour 2002). Amongst all layers, WB is primarily non-starch polysaccharides (460 g kg^{-1}), followed by starch (100 to 200 g kg^{-1}) (Zhang et al. 2011). The non-starch polysaccharides present include AX (700 g kg^{-1}), cellulose (240 g kg^{-1}), and β-glucan (60 g kg^{-1}) (Maes and Delcour 2002). Glucomannan and arabinogalactan are also present in very low levels in aleurone and endosperm cells. The other constituents of WB include proteins (150 to 220 g kg^{-1}) and lignin (40 to 80 g kg^{-1}).

1.1.2. Maize

1.1.2.1. Structure

Maize (*Zea mays* L.) is a cereal also known as corn (Delcour and Hoseney 2010a). Mature maize, also known as dented maize, has large kernels that are flattened, and the average kernel is around 350 mg. There are four main parts of a maize kernel including the bran (also known as the hull), germ, endosperm, and the tip cap. The bran, or hull, is composed of the pericarp, epidermis, and seed coat. The tip cap is the portion of the maize kernel that inserts into the cob and does not always remain attached to the kernel during processing. The hull is about 50 to 60 g kg^{-1} of the kernel, the germ is 100 to 140 g kg^{-1} of the kernel, and the endosperm makes up the remainder of the kernel. Figure 1.2 provides a graphical representation of all layers of a maize kernel (GoodMills Innovation GmbH 2015b). Maize kernels come in a variety of colors including purple, blue, dark brown, white, yellow, and red.

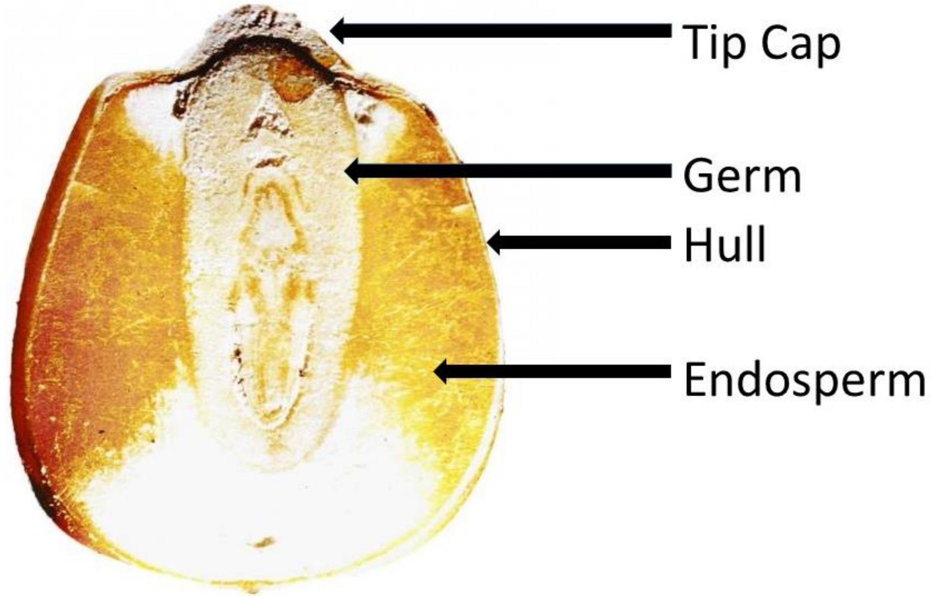

Figure 1.2. Longitudinal cross section of maize kernel (GoodMills Innovation GmbH 2015b).

1.1.2.2. Maize Bran

In 2007, it was reported by the Food and Agricultural Organization that 330•10^{6} tons of maize were produced, which corresponds to large quantity of maize bran (MB) being produced as a byproduct of this maize processing (Agger et al. 2010). Maize, like wheat, is a gramineaceous plant that has primary cell walls mainly composed of heteroxylans (Lucini and Panizzi 2016; Agger et al. 2010). The composition of MB is approximately 200 g kg^{-1} cellulose, 100 to 130 g kg^{-1} protein, 90 to 230 g kg^{-1} starch, 40 g kg^{-1}

phenolic acids, 20 to 30 g kg^{-1} lipid, and 20 g kg^{-1} ash (Carvajal-Millan et al. 2007). The remaining 500 g kg^{-1} (dry weight basis) of the MB is heteroxylans that are mainly found in the cell walls (Agger et al. 2010; Carvajal-Millan et al. 2007). AX is the main type of heteroxylan present in MB.

1.1.2.3. Dried Distillers Grain

Dried distillers grain (DDG) is a byproduct of the fermentation and distillation processes during ethanol production (Zarrinbakhsh et al. 2013). Figure 1.3 provides the step by step production of DDG from maize (U.S. Grains Council 2012). The exact composition of DDG depends upon the cultivars of maize processed and the processing conditions, but includes soluble material, protein, fiber, and lipids. Dried distillers grain has a high hemicellulose content and a low lignin content (Xiang et al. 2014). One example of the composition of DDG is 300 g kg^{-1} protein, 200 g kg^{-1} hemicellulose, 150 g kg^{-1} cellulose, 100 g kg^{-1} lipids, 50 g kg^{-1} starch, and 15 g kg^{-1} lignin. The AX content in DDG varies with each sample due to differences in sample preparation and sample source but is typically around 90 g kg^{-1} (Zarrinbakhsh et al. 2013). DDG is commonly used as animal feed because it has a high protein content, but it can be used in other applications including food-packaging films (Zarrinbakhsh et al. 2013; U.S. Grains Council 2012).

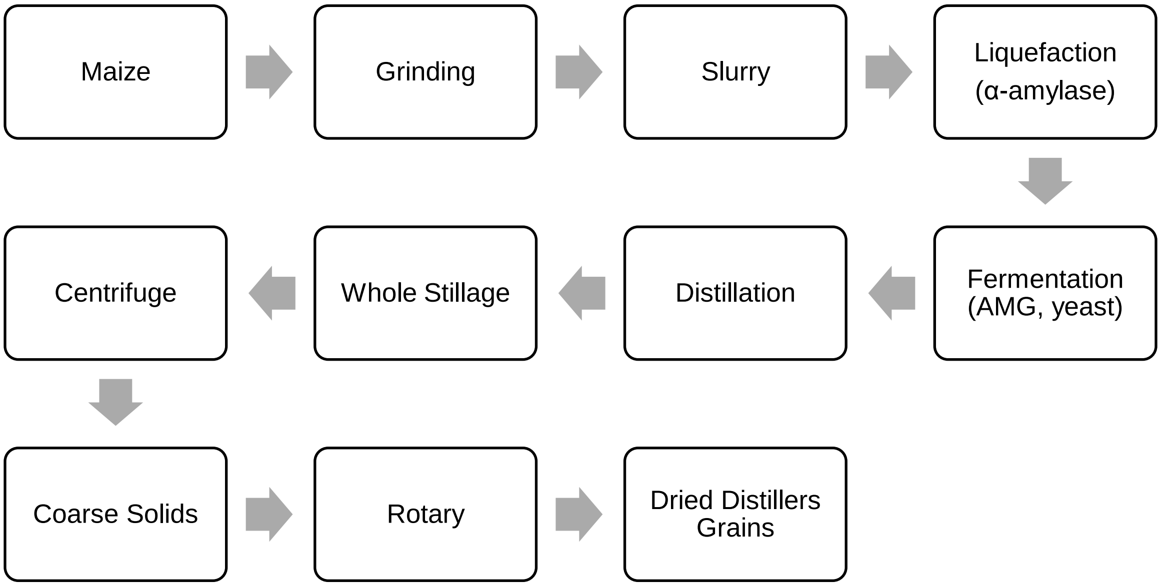

Figure 1.3. Production of dried distillers grain from maize adapted from U.S. Grains Council (2012).

1.2. Arabinoxylan

1.2.1. General Structure

Arabinoxylan is the one of the most common polysaccharides on earth, second only to cellulose (Zhang et al. 2011). Arabinoxylan is a type of non-starch polysaccharide classified as dietary fiber and found in the cell walls of the bran and aleurone layer that surround the endosperm, as well as the endosperm cell walls of cereal crops (Zhang et al. 2014; Saeed et al. 2011; BeMiller 2007b). It is a complex molecule composed of a linear xylose backbone of D-xylanopyranosyl residues (also known as xylans) connected by β-1,4-glycosidic bonds that has arabinose substituents (Reis et al. 2015; Aguedo et al. 2014; Kiszonas et al. 2013; Saeed et al. 2011).

Arabinose and xylose are the two main monosaccharides that make up AX, but glucose is also found in AX (Zhang et al. 2014; BeMiller 2007c). All three can be found as aldose or ketose sugars in the L- or D- configuration. However, D-xylose and L-arabinose are the predominate forms of these two sugars in AX. These monosaccharides can form both furanose and pyranose rings when in solution, but the pyranose ring structure is more common due to the decreased steric interactions between the hydroxyl groups present, which results in a more stable configuration. When in a ring configuration, the monosaccharides can have an α or β form depending upon if the hydroxyl group located at the C-1 position is below or above the plane. The linkages and arabinose to xylose ratio (A:X) varies between types of cereal grain, location of AX within the grain, and extraction method. However, arabinose and xylose are usually both present in AX as pentose sugars, and glucose is present as a hexose sugar. Figure 1.4 provides a simplified graphical representation of one possible configuration of arabinoxylan (Agger et al. 2010).

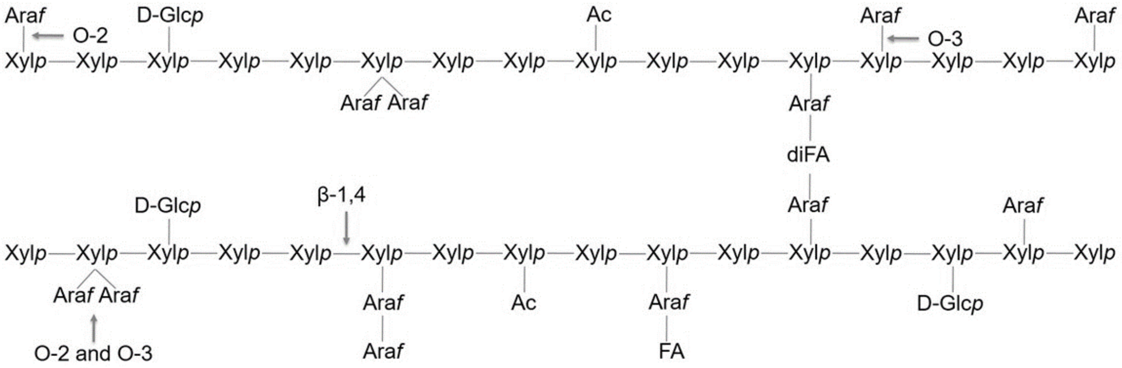

Figure 1.4. Representation of substitution patterns in arabinoxylan adapted from Agger et al. (2010).

One way to classify AX is as either water extractable arabinoxylan (WEAX) or water unextractable arabinoxylan (WUAX) (Aguedo et al. 2014; Kiszonas et al. 2013; Saeed et al. 2011). The water extractability of an AX fraction is determined by its molecular weight and the substitution on the xylose backbone. Firstly, as the molecular weight increases, water extractability decreases (Kiszonas et al. 2013). The molecular weight of WEAX is typically between 200,000 and 300,000 Da, and the molecular weight of WUAX is even higher (Kiszonas et al. 2013; Saulnier et al. 2007). Increased molecular weight also leads to increased water-holding capacity and potential for cross-linking (Zhang et al. 2014; Saeed et al. 2011). Secondly, as the substitution of arabinose on the xylose backbone increases (A:X increases), the water extractability decreases because there is an increase in cross-linking through ferulic acid moieties (Kiszonas et al. 2013; Saulnier et al. 2007). These features impact the water extractability of AX because as molecular weight and A:X increase, so do the number of entanglements, which decrease solubility in water. The amount of WEAX and WUAX present in a cereal is both under genetic and environmental control (Kiszonas et al. 2013; Saulnier et al. 2007).

Water extractable AX has a polydispersity index (PI) of about 1.7, whereas the PI of WUAX is 2.0 (Kiszonas et al. 2013; Saulnier et al. 2007). These values are relatively high, which is indicative of a high level of heterogeneity in structural compositions for both types of AX. It is also possible that the unsubstituted regions of the xylose backbone aggregate together and hydrogen bond, which results in increased stability and decreased solubility. The proportion of WEAX to WUAX impacts the end use quality of the cereal. For example, WEAX is beneficial to products typically made with hard wheat such as bread, but WEAX is detrimental to products typically made with soft wheat products such as cookies.

The substitution of arabinose along the xylose backbone is vital to the functional characteristics of the AX (Kiszonas et al. 2013). Saulnier et al. also support the idea that the heterogeneity of AX is not random, but is regulated during the biosynthesis of AX (2007). This theory is supported by the fact that when a random distribution is predicted for a given A:X, there are more monosubstituted xylose than experimentally observed. Through experimental observation, it was discovered that disubstitution is favored over monosubstition.

Acetic acid, ferulic acid, hydroxycinnamic acids, and *p*-coumaric acids are often linked to the xylose backbone, and can be removed when the AX is extracted (Kiszonas et al. 2013; Saeed et al. 2011). The substitutions on the xylose backbone of AX include non-terminal arabinofuranosyl substitutions (400 g kg^{-1}) and ß-L-arabinofuranosyl residues that are linked on the O-2 or O-3 positions on the xylanopyranosyls that are monosubstituted (400 g kg^{-1}) or doubly substituted (200 g kg^{-1}) (Agger et al. 2010). Further substitutions are possible including (1,3) linkages between xylose and xylopyranosyls, D-glucuronyl and D-galactopyranosyl residues on xylans, and arabinofuranosyls substituted with xylopyranosyls or L-galactopyranosyls. Acetic acid substitution can be found esterified directly to the xylose backbone in the O-2 or O-3 position. Hydrocinnamic acids (*p*-coumaric acid, ferulic acid, and dehydrodimers of ferulic acid) are also esterified to AX, but they are located at the O-5 position of arabinofuranosyls, xylopyranose, α-D-glucuronic acid, galactopyranose, and 4-O-methyl-α-D-glucuronic acid (Saeed et al. 2011).

Ferulic acid is the most common phenolic acid found in the bran of cereal kernels (Anson et al. 2012). The systematic name for ferulic acid is 3-(4-hydroxy-3-methoxyphenyl) propionic acid. Ferulic acid is commonly involved in cross-linkages between polysaccharides including AX and AX or AX and protein. Ferulic acid can be substituted on the xylose backbone at the O-3 and/or O-2 locations as α-L-arabinofuranose (Kiszonas et al. 2013; Saeed et al. 2011; Delcour and Hoseney 2010b). It is also possible for ferulic acid to be coupled at the O-5 location through an ester linkage. When ferulic acid residues form cross-linkages, the extractability of the AX decreases unless saponification is used. Under oxidizing conditions, WEAX cross-links via covalent coupling between ferulic acid residues, and this can result in the formation of a gel due to an increase in viscosity. Ferulic acid also makes it possible for AX to cross-link to one another and to the tyrosine that is found in proteins (Kiszonas et al. 2013).This type of cross-linking is through oxidation.

Ferulate is only present in AX in small amounts, but can have a large impact on the molecular weight, viscosity, solubility, and gelation properties of the AX (Kiszonas et al. 2013). The amount of ferulate in AX is usually only about 5 g kg^{-1} of the total mass of AX. Ferulate is linked to the O-5 of the arabinose moiety in the form of an ester. Ferulate can form cross-linkages, which results in the formation of diferulates and oligoferulates. However, the most common form is monoferulate. It is also possible for ferulate to cross-link with lignin and proteins. For these cross-linkages to form, hydrogen peroxide and peroxidase must be present.

1.2.2. Wheat Bran Arabinoxylan

The distribution of AX throughout the WB is as follows: 380 g kg^{-1} in the pericarp, 250 g kg^{-1} in the epidermis, 250 g kg^{-1} in the aleurone layer, and 20 g kg^{-1} in the seed coat (Swennen et al. 2006). The bran contains up to 690 g kg^{-1} AX, with the aleurone layer containing 600 to 700 g kg^{-1} (Saeed et al. 2011; BeMiller 2007b). The A:X in the bran on average is 0.57 to 1.07, and the main type of AX contains disubstituted xylose (Zhang et al. 2014; Zhang et al. 2011). The A:X in the aleurone layer is 0.3 to 0.4, and mainly composed of WUAX (Ying et al. 2015; Kiszonas et al. 2013; Saulnier et al. 2007). In addition, these AX have a high level of esterification with about 32 g kg^{-1} ferulic acid and 4.5 g kg^{-1} dehydrodiferulic acid (Saulnier et al. 2007). There is also a small amount of *p*-coumaric acid in the aleurone AX. The inner pericarp has an A:X of about 1.06, whereas the testa has an A:X of 0.13, which goes to show that there is a high level of heterogeneity in the AX found in WB AX. In the pericarp, about 9 g kg^{-1} of the AX is ferulic acids, which is about 30 ferulic acid residues for every 100 xylose moieties. There is also acetic acid present in WB AX.

Substitution in WB AX is not random, and there are typically regions that are highly substituted that are 20 to 25 xylose residues long followed by five unsubstituted xylose residues (Kiszonas et al. 2013). Monosubstituted xylose residues are 210 g kg^{-1} of all xylose, disubstituted xylose make up about 130 g kg^{-1} of all xylose residues, and unsubstituted xylose make up the other 660 g kg^{-1} of all the xylose residues. Out of all the monosubstituted xylose, most have the arabinose substitution present on the third carbon. Only a small amount of the monosubstituted xylose are substituted on the second carbon. These substitution patterns impact the solubility and chemical characteristics of WB AX.

1.2.3. Maize Bran Arabinoxylan

Maize bran contains about 300 to 400 g kg^{-1} AX (Saeed et al. 2011; Zhang and Whistler 2004), composed of equal amounts of WEAX and WUAX (Agger et al. 2010). In addition to AX, MB contains 200 g kg^{-1} cellulose, 100 to 140 g kg^{-1} lignin, and 50 g kg^{-1} structural proteins (Agger et al. 2010; Kale et al. 2010). According to Agger et al. (2010), the WEAX fraction has an A:X of 0.66 and the WUAX ratio has an A:X of 0.40. However, another study found that the A:X ratio for all fractions was found to be around 0.5 (Kale et al. 2010). The A:X in MB is 0.75 to 1.82 (Zhang et al. 2014). The seed coat of MB is about 500 to 600 g kg^{-1} AX, and about 300 to 400 g kg^{-1} of this can be easily extracted using alkaline extraction (Zhang and Whistler 2004).

In MB, at most 850 g kg^{-1} of the xylopyranosyl residues have substituents (Agger et al. 2010). The main substituent is arabinose, which is O-2 or O-3 linked to monosubstituted xylose or both O-2 and O-3 linked on doubly substituted xylose. About 400 g kg^{-1} of the xylose is monosubstituted, 200 g kg^{-1} are disubstituted, and 400 g kg^{-1} are unsubstituted. Other substituents that can be present on MB AX via glycosidic linkages include xylopyranosyls (linked to other xylose moieties), arabinofuranosyls (linked to xylose), L-galactopyranosyls (linked to xylose), D-galactopyranosyl (linked to arabinose), and D-glucuronyl (linked to arabinose). Each of these substituents that are linked via glycosidic linkages account for 30 to 50 g kg^{-1} of the dry mass of MB each. Other substituents can be linked via esterification including acetic acid (30 to 50 g kg^{-1}) linked via O-2 or O-3 linkages, hydrocinnamic acids (30 to 60 g kg^{-1}) including *p*-coumaric acid, ferulic acid, and ferulic acid dehydrodimers.

1.2.4. Arabinoxylan Functionality

1.2.4.1. Health and Nutrition

Arabinoxylan is known to have many health benefits including limitation or prevention of the following: type two diabetes, cancers of the digestive system, and cardiovascular disease (Doring et al. 2016; Mendis and Simsek 2014). These benefits of consuming AX are largely due to AX being dietary fiber, and are outlined in Figure 1.5 (Mendis and Simsek 2014). AX is a type of dietary fiber because it is not broken down in the small intestine, but instead it is fermented in the large intestine (BeMiller 2007b).

Carbohydrates are mainly digested and absorbed in the small intestine. For this to take place, they must be hydrolyzed into monosaccharides such as glucose. However, the only carbohydrases active in the small intestine cannot break down AX, but they include maltase, lactase, α-amylase, and isomaltase. When AX reaches the large intestine, the microorganisms present ferment it anaerobically, which produces lactic acid and volatile fatty acids. Dietary fiber is known as non-nutritive, but vital for health and well-being (Saeed et al. 2011). The molecular weight, molecular size, molecular shape (degree and locations of branch points), and substituents affect how AX acts as dietary fiber in the body (Kale et al. 2010). AX can also serve as prebiotics, which aid in the production and growth of beneficial bacteria in the intestines (Mendis and Simsek 2014; Neyrinck et al. 2011). This prebiotic behavior of AX can help prevent inflammatory bowel disease, Type I diabetes, and rheumatoid arthritis (Mendis and Simsek 2014).

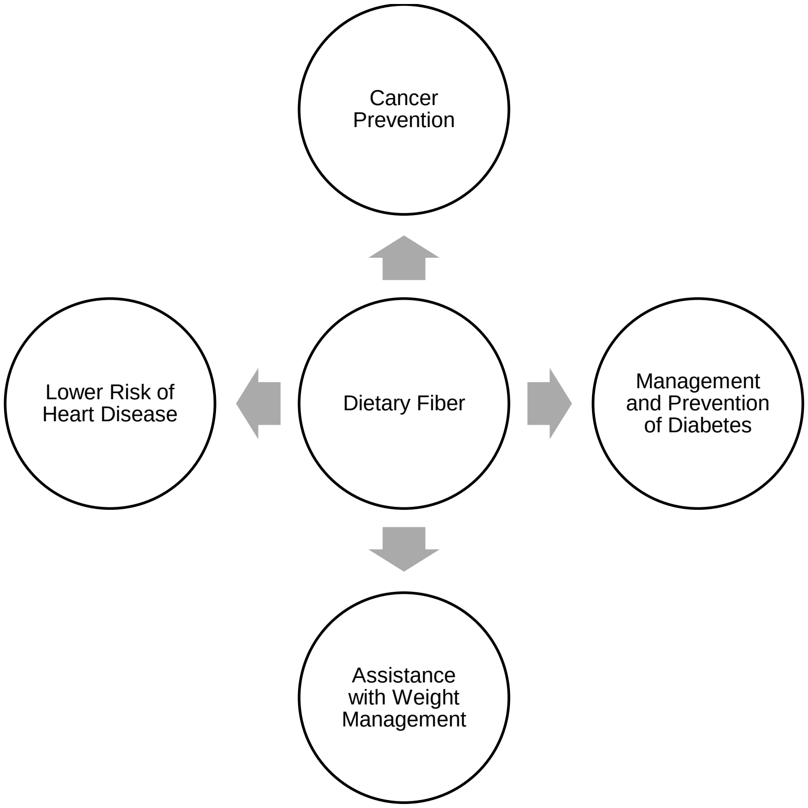

Figure 1.5. Relationship between dietary fiber and prevention of health risks adapted from Mendis and Simsek (2014).

1.2.4.2. Gel Rheology

For a proper gel to form from polysaccharides such as AX, they must cross-link via covalent bonding (Ayala-Soto et al. 2014). In AX, this takes place via a dimerization reaction that forms dehydrodimers (5-5', 8-O-4', 8-5', and 8-8') and/or isomers that are dehydrotrimers (8-O-4/8-O-4, 8-8'/8-O-4). These covalent bonds are interactions between AX and AX or AX and another chemical species such as ferulic acid that provide the AX gels with their physical and chemical properties. These interactions occur under oxidative coupled cross-linking, which can be catalyzed by laccase or a hydrogen peroxide/peroxidase system. Gels made from AX have a neutral taste, little to no odor, are stable under heat, not susceptible to pH changes or electrolytes, have a high water absorption capacity, and do not exhibit syneresis during storage. The rheological properties of AX gels can differ depending upon the solvent used, temperature of testing, frequency utilized, strain rate employed, AX concentration of the gel, AX mesh size, and the pH of the gel during testing.

1.2.4.3. Baking Quality

Arabinoxylan also plays an important role in many properties of foods including textural characteristics, shelf life, water binding capacity, and the stability of foams (Doring et al. 2016). In addition, WEAX can act as a cryostabalizing agent by preventing the growth of ice crystals (Skendi and Biliaderis 2016). When in bread dough, AX increases the viscosity of the dough and increases the interactions between proteins and starch (Buksa 2016). The result of these things is an increase in the size of the gas cells formed during fermentation due to their increased stability. High molecular weight WEAX improves the loaf volume and texture when bread is baked (Wang et al. 2016). On the other hand, WUAX can have a negative effect on the qualities of bread because it disrupts the gluten matrix.

1.2.5. Extraction and Purification of Arabinoxylan

There are numerous ways to extract AX from cereal grain including alkaline extraction, acid extraction, water extraction, enzyme hydrolysis, microwave extraction, ultrasound extraction, twin-screw extrusion, steam explosion, and hot compressed water (Zhang et al. 2014; Zhang et al. 2011). However, the characteristics of the AX changes depending upon the extraction method utilized. Some of these features include degree of branching, spatial arrangement of the AX and its substituents, and molecular weight. Only about 25 to 33 g kg^{-1} of AX can be extracted at room temperature in water, and to extract the remaining AX, an enhanced extraction method such as alkaline solution must be utilized (Kiszonas et al. 2013). Figure 1.6 shows a general outline for extracting and purifying arabinoxylan from wheat bran using the most common method for extracting AX (Zhang et al. 2014; Zhang et al. 2011).

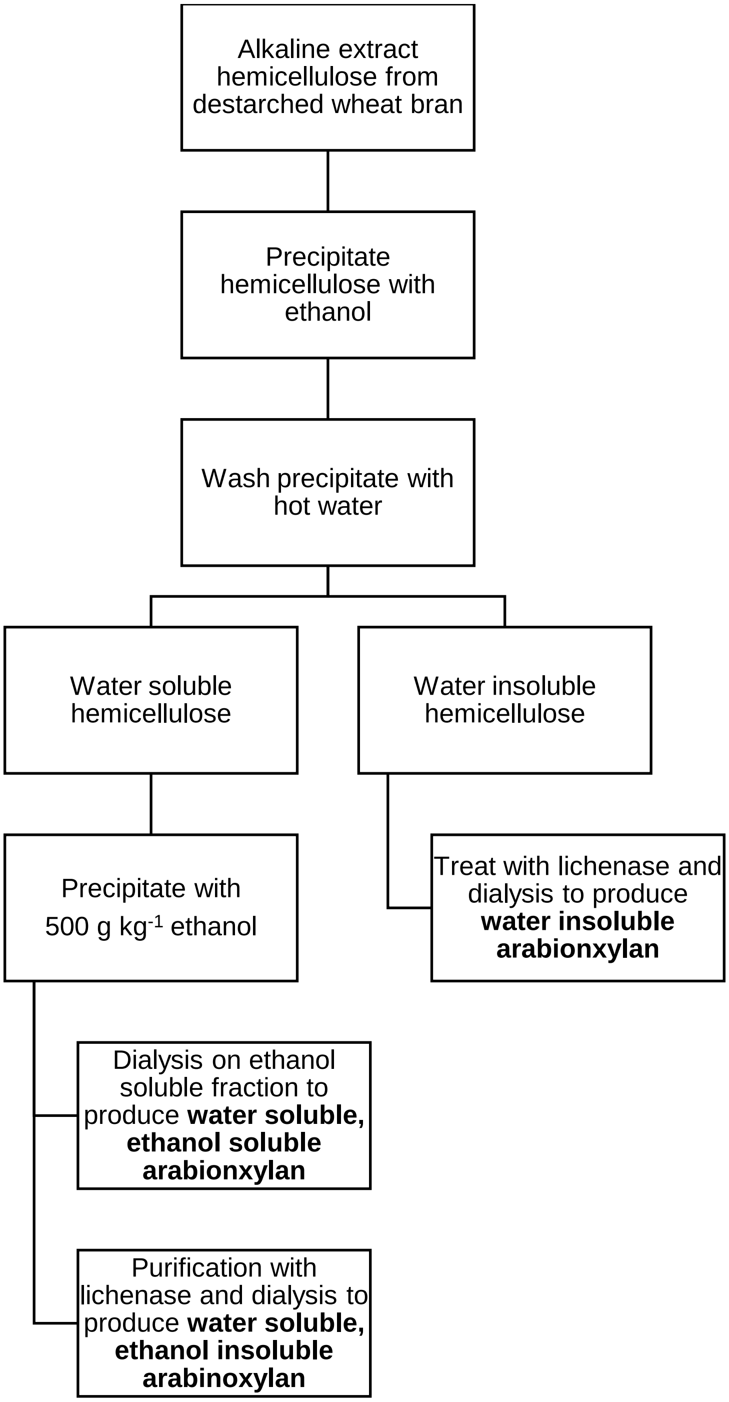

Figure 1.6. Outline of alkaline extraction of arabinoxylan and purification using ethanol precipitation and dialysis adapted from Zhang et al. (2013).

1.2.5.1. Water Extraction

Utilization of water to extract AX is one of the most common methods (Zhang et al. 2014; Ebringerová and Heinze 2000). To use water to extract AX from cereals, such as those in WB, the material is stirred for 90 minutes at room temperature with a 1:10 w v^{-1} mixture of AX and water. When this is complete, starch can be removed using an enzyme such as amyloglucosidase. Lastly, 650 g kg^{-1} ethanol can be used to fractionate and precipitate AX. When this method is used, the extraction yield is around 4.3 g kg^{-1} of wheat flour WEAX. The extraction efficiency of water extraction is lower than for other methods such as enzymatic extraction and chemical extraction due to the inability of water to break the cross-linkages present in AX.

1.2.5.2. Alkaline Extraction

The extraction of AX under alkaline conditions is most commonly done using dilute sodium hydroxide solution, after which the AX fractions can be separated based upon solubility in ethanol (Zhang et al. 2014). This method is similar to water extraction in that the material the AX is being extracted from is mixed with an alkaline solution. When this method is used, 640 to 680 g kg^{-1} AX can be extracted from WB (Zhang et al. 2011), 400 g kg^{-1} AX from MB, and 660 g kg^{-1} AX from DDG (Reis et al. 2015; Saeed et al. 2011). Alkaline extraction is effective because the alkaline solution (e.g. sodium hydroxide) breaks both hydrogen and covalent bonds present in the cell walls of cereals (Zhang et al. 2014). Once these bonds are disrupted, the AX (and other polysaccharides) are released from the cell wall. The hydroxyl ions present in solutions of dilute sodium hydroxide break the hydrogen bonds present between cellulose and hemicellulose as well as ester linkages, which makes the AX soluble in dilute sodium hydroxide. In addition, when uronic acids experience alkaline conditions, they revert to their negative forms, which results in repulsions and increased extractability of AX.

In addition to using dilute sodium hydroxide for alkaline extracting AX, the WUAX from cereals can also be extracted using only hydrogen peroxide (Zhang et al. 2014; Maes and Delcour 2002). When this method is used, 690 g kg^{-1} of the AX present in WB can be extracted (Zhang et al. 2014). As the concentration of hydrogen peroxide and extraction temperature increase, so does the yield of WUAX from WB. Destarching the WB prior to extraction also increases yield.

Instead of using only one solvent for AX extraction, sodium hydroxide and hydrogen peroxide can be used together to extract WUAX (Zhang et al. 2014; Sun et al. 2000). When this system is used to extract AX (0.15 M sodium hydroxide with 5 g kg^{-1} hydrogen peroxide), 185 g kg^{-1} yield of AX with a molecular weight of $3.5•10^5$ to $7.2•10^6$ Da and an A:X of 0.8 is extracted (Zhang et al. 2014). However, when 0.16 mol mL^{-1} sodium hydroxide with 5 g kg^{-1} hydrogen peroxide is used, the extraction yield is 212 g kg^{-1}.

Regardless of the solvent used for alkaline extraction, it is likely that other polysaccharides will be extracted along with the AX including lignin and cellulose (Zhang et al. 2014). The presence of these species decreases the purity of the extracted AX. Utilizing hydrogen peroxide can remove lignin from AX, which increases the whiteness of the extracted AX (Zhang et al. 2014; Gould 1984). This occurs because when in alkaline solutions, hydrogen peroxide forms hydroperoxide anions, and these species oxidize lignin by reacting with carboxyl groups present (Zhang et al. 2014).

1.2.5.3. Acid Extraction

When acid is used to extract AX, there are many options including ethanol and 1 g kg^{-1} hydrochloric acid, methanol, acetic acid, formic acid, and combinations of these reagents (Zhang et al. 2014). These solutions are then used to extract AX (85 °C for four hours). The combination that has proved the most effective is formic acid, acetic acid, and water (30/60/10 by volume). This solvent combination yielded 296 g kg^{-1} AX extracted on a dry weigh basis from wheat straw. The purity of this extract was 781 g kg^{-1} and the A:X was less than 0.1. The very low A:X is due to the high rate of hydrolysis cleaving the xylans from the AX backbone. When methanol and 1 g kg^{-1} hydrochloric acid or ethanol and 1 g kg^{-1} hydrochloric acid, the extraction yields of AX from wheat straw are 55 g kg^{-1} and 86 g kg^{-1} respectively. One downside of acid extraction is that if there is excessive hydrolysis, the AX may have a very low molecular weight (BeMiller 2007c).

1.2.5.4. Enzymatic Extraction

Enzymes can be utilized to extract the AX from cereal flours and brans (Zhang et al. 2014). When this method is done, xylanases, such as endo-β-1,4, xylanases, are the most common because they attack the AX backbone cleaving the internal β-1,4-linkages in a manner that is random which serves to penetrate through the cell wall network present in cereals. The result of this is a mixture of unsubstituted and substituted AX. Using this method will also make a portion of the WUAX extractable. The majority of xylanases are classified as either GH10 or GH11 (Zhang et al. 2014; Beaugrand et al. 2004). The first group includes xylanases from plants, fungi, or bacteria. The second group includes xylanases produced by bacteria and fungi. GH10 xylanases typically have an extraction rate of 180 to 260 g kg^{-1}, whereas GH11 extract 410 to 490 g kg^{-1} of AX (Zhang et al. 2014). This discrepancy is due to GH11 being more highly selective for insoluble species and a greater ability to penetrate the cell walls of cereals. The resulting A:X when GH10 is used is typically 0.38 to 0.43, whereas the resulting A:X when GH11 is used is 0.23 to 0.28, indicating distinct differences in the actions of each class of xylanases. In addition to xylanase, other enzymes have also been utilized to purify AX.

In addition to xylanase, three other enzymes are commonly used to purify AX including α-amylase, amyloglucosidase, and protease (Zhang et al. 2014; Maes and Delcour 2002). When α-amylase (pH = 6, 80 °C, 45 minutes), protease, and amyloglucosidase (60 °C and 45 minutes) are used to purify barley husk AX, a 251 g kg^{-1} yield of AX with a molecular weight of $3.43 \cdot 10^4$ Da and an A:X or 0.37 can be obtained (Zhang et al. 2014). In this case, the α-amylase and amyloglucosidase are used to remove starch, while the protease is utilized to remove the protein.

1.2.5.5. Ultrasound Assisted Extraction

Ultrasound assisted extraction has also been used in the extraction of AX from cereal processing byproducts including WB and DDG (Reis et al. 2015; Wang et al. 2014). Using ultrasound as a portion of the extraction method for extracting AX can improve the yield, however, ultrasound extraction must always be coupled with another extraction method such as alkaline extraction (Zhang et al. 2014). When ultrasound is used, it is a mechanical pretreatment that can increase extraction yield and decrease the extraction time needed. However, ultrasound can also modify the molecular weights of the extracted AX. When enzymatic extraction is coupled with ultrasound extraction of WB AX, the yield is around 430 g kg^{-1} (Wang et al. 2014). When ultrasound and alkaline extraction are paired to extract AX from DDG, the yield is about 450 g kg^{-1} (Reis et al. 2015). One downside to ultrasound aided extraction is that it can promote AX degradation, which generates polymers that are short and often degrade during AX analysis.

1.2.5.6. Microwave Assisted Extraction

Microwave assisted extractions of AX utilize microwave treatment during the extraction (Zhang et al. 2014). Utilizing microwave treatment on MB AX results in feruloylated AX that are soluble. This method is used because it can reduce the time required for extraction. As the treatment temperature increases from 180 °C to 200 °C, the extraction yield decreases for MB AX. Similarly, as extraction time increases from 10 to 20 minutes, extraction yield for MB AX decreases. The optimal extraction time and temperature for MB AX is 180 °C and 10 minutes, which yields 258 g kg^{-1} AX.

1.2.5.7. Steam and Hydrothermal Extractions

Using steam to extract AX requires exposing the starting material to high-pressure steam followed by rapidly releasing the pressure (Zhang et al. 2014). This rapid release of pressure results in a rapid depolymerization of the material. The result of this process is a breakdown of the lignin and cellulose, which allows the hemicellulose to be extracted. This can be done in two stages with the steam extraction being a precursor to alkaline treatment. The specifications for this are 200 °C and 15 bar for 10 to 33 minutes or 220 °C and 22 bar for 3 to 8 minutes, and then subsequent treatment with alkaline peroxide (20 g kg^{-1} hydrogen peroxide, 50 °C, five hours, pH = 11.5) and precipitation with ethanol (three volumes). The steam treatment yielded 205 to 285 g kg^{-1} AX from wheat straw and after the alkaline extraction and ethanol fractionation, 770 to 876 g kg^{-1} of the total AX was extracted.

Hydrothermal techniques can be utilized to extract hemicelluloses such as AX from cereals (Aguedo et al. 2014). This is done by heating the starting material inside a reactor that uses pressurized water and wood. Two other hydrothermal techniques include microwave heating and using steam explosions in aqueous systems. While these methods can be used to extract AX, two downsides include the formation of furfural and a decrease in extraction yield.

1.2.5.8. Arabinoxylan Purification

Arabinoxylan fractions contain many impurities including β-glucan, lignin, starch, and proteins. Firstly, β-glucan can be removed using lichenase and β-glucosidase (Zhang et al. 2011). Secondly, lignin can be removed by enzymatic hydrolysis with phenoloxidase followed by peroxidase (Dashtban et al. 2010). When ethanol is utilized to fractionate the AX extracted from cereals, it serves as a delignification agent (Zhang et al. 2014; Saulnier et al. 2007). Thirdly, Termamyl is a heat stable amylase from a bacterial source that can be used to remove starch, and amyloglucosidase can be used to remove dextrin (Swennen et al. 2006; Maes and Delcour 2002). Fourthly, the proteins in cereals can be removed by treatment with Neutrase or filtration through silica gel. Termamyl and Neutrase hydrolyze the starch and protein so it can be separated from the AX by dialysis. In order to determine the best purification technique, the AX sample must be analyzed to determine the impurities present. After analysis, a method can be determined that will provide the purest AX. The optimal pH and temperature conditions for each enzyme should be used to obtain the best results. Purification often modifies the structure of AX, which directly impacts the molecular weight of the extract. In Table 1.1, the molecular weights of various types of AX and their extraction and purification methods are given (Zhang et al. 2014).

Table 1.1. Extraction and purification methods of wheat bran arabinoxylan and maize bran arabinoxylan and corresponding the molecular weights adapted from Zhang et al. (2014).

Arabinoxylan source	Extraction method	Molecular weight of arabinoxylan (Da)
WB[a]	Ba(OH)2	$3.8 \cdot 10^5$
WB	0.1 M Trifluoroacetic Acid	$8.0 \cdot 10^4$
WB	*B. subtillis* endoxylanase	0.2 to $3.1 \cdot 10^4$
WB	*A. aculeatus* endoxylanase	1.0 to $1.2 \cdot 10^3$
MB[b]	NaOH, pH = 11.1	$3.9 \cdot 10^5$
MB	Ca(OH)2, pH = 9.8	$2.8 \cdot 10^5$
MB	NaOH + Ca(OH)2 (1:1 v v^{-1})	$3.0 \cdot 10^5$

[a] Wheat bran
[b] Maize bran

1.2.6. Chemical Characterization of Arabinoxylan

1.2.6.1. Proximate Composition

The proximate analysis of extracted AX involves the analysis of the total contents of AX, moisture, ash, protein, starch, and lipid. In addition to the determination of AX content, the other sugars present can be, and often are, classified. The analysis of sugar composition involves reducing the sugars to alditol acetates so that they can be analyzed using gas chromatography (Blankeney et al. 1983). See Table 1.2 provides the standard methods used to determine proximate composition of AX. Tables 1.3 and 1.4 contain information about the proximate composition and sugar compositions of AX extracted from WB, MB, and DDG extracted using multiple different methods.

Table 1.2. Standard methods for analysis of proximate composition of materials such as arabinoxylan.

Parameter	Method title	Method number
Moisture Content	Moisture-Air Oven Method	AACCI[a] Method 44-15.02
Total Ash	Basic Ash Method	AACCI[a] Method 08-01.01
Total Protein	Crude Protein-Combustion (Leco)	AACCI[a] Method 46-30.01
Total Starch	Total Starch Assay Procedure (Megazyme)	AACCI[a] Method 76-13.01
Total Lipid	Oil Content	AOCS[b] Method Ba 3-38

[a] American Association of Cereal Chemists International

[b] American Oil Chemists' Society

Table 1.3. Proximate compositions of wheat bran, maize bran, and dried distillers grain arabinoxylan extracted using a variety of methods.

	Composition (g kg^{-1})						
Material	Moisture	Ash	Protein	Starch	Lipid	Extraction method	Source
WB AX[a]	-	-	11.0	-	-	240 g kg^{-1} potassium hydroxide and 10 g kg^{-1} NaBH$_4$ (w v^{-1})	Zhang et al. 2011
WB AX	-	5.7	7.1	-	3.4	0.44M NaOH	Aguedo et al. 2014
WB AX	-	4.4	19.0	0.3	-	4.0M NaOH	Maes and Delcour 2001
MB AX[b]	-	-	5.6	-	-	50 g kg^{-1} sodium hydroxide + 200 to 400 g kg^{-1} Ethanol	Kale et al. 2010
MB AX	-	-	6.5	-	-	50 g kg^{-1} sodium hydroxide + 400 to 600 g kg^{-1} Ethanol	Kale et al. 2010
MB AX	-	-	14.0	-	-	Hot Water Soluble Fraction	Agger et al. 2010
MB AX	-	-	57.0	-	-	Hot Water Insoluble Fraction	Agger et al. 2010
DDG AX[c]	5.3	2.8	36.0	5.2	10.2	Hot Water	Zarrinbakhsh et al. 2011
DDG AX	-	5.4	19.0	-	1.1	30 g kg^{-1} Sodium Hydroxide	Xiang et al. 2014
DDG AX	-	-	25.0	0.4	-	Ultrasound Assisted Alkaline	Reis et al. 2015
DDG AX	-	-	17.0	7.0	-	Alkaline Solution	Reis et al. 2015

[a] Wheat bran arabinoxylan

[b] Maize bran arabinoxylan

[c] Dried distillers grain arabinoxylan

Table 1.4. Sugar profiles and arabinose to xylose ratio of wheat bran, maize bran, and dried distillers grain arabinoxylan extracted using a variety of methods.

	Sugar profile (g kg^{-1})							
Material	Arabinoxylan	Arabinose	Xylose	Glucose	Galactose	A:X[d]	Extraction method	Source
WB AX[a]	-	14	66	2.0	1.0	0.2	240 g kg^{-1} potassium hydroxide and 10 g kg^{-1} NaBH$_4$ (w v^{-1})	Zhang et al. 2011
WB AX	-	28	30	2.9	2.0	0.9	0.44M NaOH	Aguedo et al. 2014
WB AX	45	21	35	8.5	6.5	-	4.0M NaOH	Maes and Delcour 2001
MB AX[b]	-	19	35	-	-	-	50 g kg^{-1} sodium hydroxide + 200 to 400 g kg^{-1} Ethanol	Kale et al. 2010
MB AX	-	22	41	-	-	-	50 g kg^{-1} sodium hydroxide + 400 to 600 g kg^{-1} Ethanol	Kale et al. 2010
MB AX	-	47	37	-	-	-	Hot Water Soluble Fraction	Agger et al. 2010
MB AX	-	18	30	-	-	-	Hot Water Insoluble Fraction	Agger et al. 2010
DDG AX[c]	21	-	-	-	-	-	Hot Water	Zarrinbakhsh et al. 2013
DDG AX	-	18	24	-	-	-	30 g kg^{-1} Sodium Hydroxide	Xiang et al. 2014
DDG AX	45	25	65	-	2.0	-	Ultrasound Assisted Alkaline	Reis et al. 2015
DDG AX	66	26	49	9.0	2.0	-	Alkaline Solution	Reis et al. 2015

[a] Wheat bran arabinoxylan
[b] Maize bran arabinoxylan
[c] Dried distillers grain arabinoxylan
[d] Arabinose to xylose ratio

1.2.6.2. High Performance Size Exclusion Chromatography-Multi Angle Light Scattering-Refractive Index

High performance size exclusion chromatography (HPSEC) is used in conjunction with a multi-angle light scattering detector (MALS) and a detector that determines concentration such as a refractive index detector (RI) to determine the molecular weight of a chemical species such as AX (Mendis and Simsek 2015). In this method, a known concentration of AX is put into a column to be separated by hydrodynamic size. The differences in particle size is determined using the multi-angle light scattering detector. This type of detector is used because it provides more valuable information about the radius of gyration of the AX being analyzed. The RI detector provides information about the concentration of each chemical species. Table 1.5 shows the differences in molecular weights and polydispersity indices of AX from WB and MB that have been extracted using different methods.

Table 1.5. Molecular weights and polydispersity indices for wheat bran arabinoxylan and maize bran arabinoxylan determined using high performance size exclusion chromatography with a multi-angle light scattering detector.

Arabinoxylan type	Molecular weight (kDa)	Polydispersity index	Extraction method	Source
WB[a]	200 to 300	1.7 to 2.0	Hot Water	Saulnier et al. 2007
WB	152 to 218	0.3 to 1.7	24 g kg^{-1} KOH and 1 g kg^{-1} $NaBH_4$	Zhang et al. 2011
WB	670	-	Alkaline Solution	Aguedo et al. 2014
WB	5 to 12.5 and 140 to 160	-	Enzymatic and Thermal Extraction	Aguedo et al. 2014
WB	50	-	Hot Water and 0-40 g kg^{-1} Ethanol	Maes and Delcour 2002
WB	177 to 233	-	Hot Water	Ying et al. 2011
WB	191	-	5 g kg^{-1} Sodium Hydroxide and 20-40 g kg^{-1} Ethanol	Kale et al. 2010
WB	128	-	5 g kg^{-1} Sodium Hydroxide and 40-60 g kg^{-1} Ethanol	Kale et al. 2010
MB[b]	293	3.0	Alkaline Solution	Zhang et al. 2011
MB	506	-	Film, not AX	Heikkinen et al. 2013

[a] Wheat bran

[b] Maize bran

1.2.6.3. Nuclear Magnetic Resonance Spectroscopy

Nuclear magnetic resonance spectroscopy (NMR) is used to determine the linkages present in a chemical species such as AX. It can be used to analyze either anomeric carbon or anomeric hydrogen linkages present in the sample. In addition, the solvent that the sample is dissolved in prior to analysis can be varied. The temperature and type of NMR can also vary by facility. These things will determine the location of the resonance peak of the anomeric carbon or anomeric hydrogen and can account for some variation amongst NMR results. Table 1.6 shows some of the common peaks found in AX from WB, MB, or DDG using proton NMR.

Table 1.6. Proton nuclear magnetic resonance spectroscopy peak identities for arabinoxylan.

Peak location (ppm)	Peak identity	Source
5.47	Arabinose C-(O)-3 Linked to Anomeric Xylose	Swennen et al.2006
5.37	Anomeric Proton of Arabinose C-(O)-3 to Disubstituted Xylose	Swennen et al.2006
5.30	Anomeric Proton of Arabinose C-(O)-2 to Disubstituted Xylose	Swennen et al.2006
4.66	Anomeric Proton of Xylopyranosyl Unit	Swennen et al.2006
5.98	Arabinose C-(O)-3 Linked to Anomeric Xylose	Hoffman et al.1992
5.86	Anomeric Proton of Arabinose C-(O)-3 to Disubstituted Xylose	Hoffman et al.1992
5.76	Anomeric Proton of Arabinose C-(O)-2 to Disubstituted Xylose	Hoffman et al.1992
5.02	Anomeric Proton of Xylopyranosyl Unit	Hoffman et al.1992

1.3. Plasticizers and Plasticization

Plasticization is a process in which the thermal and mechanical properties of a system or material such as a polymer are changed (Immergut and Mark 1965). These changes involve the polymer becoming more flexible at room temperature, increasing the amount of elongation possible before breakage occurs (at room temperature), and increasing the mechanical strengths (tensile and tear) of the polymer (Vieira et al. 2011; Bergo and Sobral 2007). Plasticizers are defined by the desired properties they impart to a polymer system. For example, in a food packaging biopolymer, polyols are used as plasticizers to increase flexibility.

1.3.1. Types of Plasticizers

There are two main types of plasticizers: external and internal. The first type, external plasticizers, are plasticizers that interact with the polymer without chemically reacting (Vieira et al. 2011; Immergut and Mark 1965). They typically have low vapor pressures and interact with polymers at increased temperatures by way of their swelling (solvent) power. When external plasticizers do not completely enter both the amorphous and crystalline regions of the polymer (often only the amorphous region is penetrated), they are considered secondary plasticizers because they are non-solvent plasticizers. The second type of plasticizers, internal plasticizers, are chemically bonded to the polymer. This is due to the plasticizer being a second monomer that is copolymerized as an integral part of the polymer system. The result of this copolymerization is that the polymer is more disordered, which reduces the glass transition temperature of the material.

1.3.2. Opposition to Plasticization

Intermolecular forces including dispersion forces, induction forces, dipole-dipole interactions, and hydrogen bonding must be overcome by a plasticizer in order for it to enter the intermolecular spaces serve as a plasticizer (Vieira et al. 2011; Immergut and Mark 1965). All intermolecular forces must be of the same magnitude in order for plasticization to take place: plasticizer-plasticizer, polymer-polymer, and plasticizer-polymer. Crystallinity must be interrupted for a substance to be properly plasticized. This is important because if the substrate is completely crystalline, the plasticizer cannot penetrate the ordered polymer chains.

1.3.3. Requirements for Plasticization

The four main requirements for plasticization include solvent power, compatibility, efficiency, and permanence (Immergut and Mark 1965). Firstly, plasticizers must be able to act as solvents in order to penetrate the crystalline regions of polymers. When plasticizers are non-solvent, they act as softeners and only enter the amorphous regions. The downside of disrupting the crystallinity of a polymer with a plasticizer is that the mechanical properties of said polymer that depend upon its crystallinity, including tensile strength and tear resistance, will change. Secondly, the optimal temperature range for the plasticizer must match that of the polymer. This ensures that processing and use temperature ranges will be optimal for all chemical species involved. In addition, the polarity, molecular weight, and shape of the plasticizer should be complimentary to those of the polymer. Thirdly, when efficiency is used to describe a plasticizer-polymer relationship, it refers to the balance between the levels of plasticizer and polymer required to produce a desired polymeric system. This is determined by plasticizer size, shape, molecular weight, and rate of diffusion into the polymer’s amorphous and crystalline regions. For example, smaller molecules will diffuse more quickly than larger molecules which makes them more efficient. Fourthly, plasticizers must be permanently ingrained within the polymeric matrix in order for an effective plasticizer-polymer relationship to have taken place. One of the main factors in permanence is volatility of the plasticizer. The larger the molecule, the lower the volatility and longer the permanence. The ability of the plasticizer to diffuse into the polymer matrix also impacts permanence, so the greater the diffusion, the greater the permanence. However, if a plasticizer can diffuse quickly into a polymeric matrix it can also diffuse out of the matrix quickly. Thus molecular size must be well thought out when choosing a plasticizer.

1.3.4. Polyols as Plasticizers

Films made from only AX can be brittle, causing them to crack when handled and have low extensibility and flexibility. Plasticizers are small, non-volatile compounds added to reduce the brittleness and increase the amount of flexibility of a film (Antoniou et al. 2014; Vieira et al. 2011). Polyols such as sorbitol are commonly used as plasticizers in polysaccharide-based films (Antoniou et al. 2014). Sorbitol is the common name for D-Glucitol, which is the fully reduced form of glucose (BeMiller 2007a). It provides desirable barrier properties, low hydrophobicity, and a low water permeability (Antoniou et al.

2014). Sorbitol is a highly soluble compound that is available in both crystalline and syrup forms (BeMiller 2007a). Sorbitol is a compound that is generally recognized as safe that is non-cariogenic and a humectant. Polyols are a form of dietary fiber, but they also have a sweet taste (the degree of sweetness depends upon the polyol). Glycerol has a lower molecular weight than sorbitol, and as a result, has a higher ratio of hydroxyl groups per unit mass than sorbitol (Antoniou et al. 2014).

The type of plasticizer used greatly influences the characteristics of a material. For example, films that utilize glycerol as a plasticizer tend to have good mechanical properties including elongation and resistance (Antoniou et al. 2014). However, films made with sorbitol have lower hydrophobicity and water vapor transmission rates than those made with glycerol. Regardless of which polyol is chosen, both increase the mobility present in the polymer chains and reduce the glass transition temperature.

1.4. Arabinoxylan Film Casting and Mechanical Characterization

Biopolymeric food packaging and films cannot be extrusion blown in the same way that synthetic polymers can be due to their heterogeneity and decomposition when exposed to high temperatures (Tharanathan 2003). Wet casting is the most common method used for casting biodegradable films. This process involves pouring an aqueous film solution onto a suitable surface and allowing it to dry either at or above room temperature. Using infrared chambers can speed the curing process. Casting and curing conditions will directly influence the properties of the product due to their influence on the molecular structure of the film being made.

Food packaging must have a high tensile strength, high shear strength, and low oxygen permeability to extend the shelf life of the food. A small A:X also produces desirable properties including decreased water uptake and increased crystallinity (Zhang et al. 2011). When the xylose backbone of AX is highly substituted, the local chain motion decreases, which increases the stability of the chain. AX is an excellent barrier to nonpolar migrants including oxygen and aromatics because it is a polar compound. Little research has been published on the mechanical properties of MB or DDG AX films, but there has been research published on utilization of polyols in films. One of the main purposes for AX films is for determination of mechanical properties to see if it is feasible to develop food packaging material from the films. All films used for food packaging are classified as food contact substances and must adhere to all standards set forth by the Food and Drug Administration.

1.4.1. Moisture Content

The moisture content of a film will impact many of its other properties, therefore it should be carefully measured. One method to determine the moisture content of a film is to take the mass of the film, place the film in an oven for 24 hours at 110 °C, and determine the mass of the film after drying (Garcia et al. 2004). One notable trend in moisture content is that films made with glycerol have higher moisture contents than those made with sorbitol (Antoniou et al. 2014). This occurs because glycerol is more hydrophilic than sorbitol. The impact of this is that films created with glycerol can pull more moisture out of the material(s) around them than films made with sorbitol.

1.4.2. Film Water Solubility

The amount of water soluble material present in a film will determine what its end uses can be. For example, food with a high moisture content such as fruit cannot be stored in a package that has water soluble material touching the food because it would dissolve. One method to determine the amount of water soluble material in a film is to follow the method of Garcia et al. (2004). The amount of water soluble material present is dependent upon many chemical characteristics. One of these characteristics is that as the A:X decreases, the AX structure becomes more crystalline and the films become less water soluble (Heikkinen et al. 2013). This is due to increased crystallinity leading to fewer arabinose present to interact with water and the xylose polymers align more closely together, which decreases the surface area of the AX available to interact with water.

1.4.3. Puncture Resistance

Puncture resistance is the resistance of a material to a large force over a small area. This is a crucial parameter for food packaging due to the stress placed upon food packaging during the transport of food from production facility to consumer. The puncture resistance of a food packaging material can be measured with the American Society for Testing and Materials (ASTM) method D7192 (ASTM International 2010). When choosing a plasticizer to use in food packaging, the desired puncture resistance must be taken into consideration. For example, when glycerol is used instead of sorbitol, the puncture force that the film can withstand is higher but the deformation is smaller (Thomazine et al. 2005). In addition, as the level of plasticizer is increased, the maximum force before puncture decreases, but the maximum deformation increases.

1.4.4. Tensile Strength and Modulus of Elasticity

The tensile strength of a material is a measure of the material's resistance to deformation when under tension. The modulus of elasticity, also known as Young's modulus or *E*, is the tendency of a material to undergo deformation when under tension. The modulus is also defined as the ratio of stress to strain when under tension. To determine the tensile strength and modulus of elasticity for films made from AX, the ASTM method D882 can be utilized (ASTM International 2002). Food packaging is often designed to be opened when under tension, so the packaging must be strong enough to withstand these forces. Typically, an adhesive is chosen so that the adhesive will break before the packaging.

The tensile strength of an AX film depends upon many of the chemical characteristics of the film including the A:X, plasticizer type, and plasticizer amount. Wheat AX films with an A:X of 0.33 have an ultimate stress of 131 ± 25 N mm^{-2} and an ultimate strain of 46 ± 6 % (Ying et al. 2015). As the A:X increases to 0.53, the ultimate stress decreases to 98 ± 16 N mm^{-2} and the ultimate stress decreases to 39 ± 9 %. As the A:X increases even further to 0.73, the ultimate stress decreases to 61 ± 8 N mm^{-2} and the ultimate stress decreases to 22 ± 9 %. The tensile strength of films plasticized by glycerol and sorbitol is inversely related to the amount of plasticizer used (Antoniou et al. 2014). In addition, the modulus and elongation at break are positively correlated to the amount of plasticizer present in the film. Table 1.7 provides tensile properties of WB, MB, and DDG AX films created from various formulas.

Table 1.7. Tensile strength profiles for films made from wheat bran, maize bran, and dried distillers grain arabinoxylan.

Arabinoxylan type	Tensile strength (MPa)	Modulus of elasticity (MPa)	Elongation at break (%)	Film composition	Source
WB[a]	40	-	2	-	Hansen and Plackett 2008
WB	40	990	7	-	Heikkinen et al. 2013
WB	5 to 10	-	30 to 40	Chitosan + WB AX	Costa et al. 2015
MB[b]	10	-	6	0 g kg^{-1} Plasticizer	Hansen and Plackett 2008
MB	50	-	10	220 g kg^{-1} Glycerol	Hansen and Plackett 2008
MB	50	-	9	220 g kg^{-1} Sorbitol	Hansen and Plackett 2008
MB	50	1300	6	-	Heikkinen et al. 2013
MB	10	370	10	200 g kg^{-1} Glycerol	Zhang and Whistler 2004
MB	50	1100	6	27 g kg^{-1} Glycerol	Zhang and Whistler 2004
MB	20	460	9	198 g kg^{-1} Sorbitol	Zhang and Whistler 2004
MB	50	1200	6	51 g kg^{-1} Sorbitol	Zhang and Whistler 2004
DDG[c]	20	1000	-	-	Zarrinbakhsh et al. 2013

[a] Wheat bran
[b] Maize bran
[c] Dried distillers grain

1.4.5. Tear Resistance

Tear resistance is a measure of how resistant a material is to propagation of a tear, which is determined by measuring the force required to initiate tearing (ASTM International 2013b). This is measured by cutting a right angle notch into a film and measuring the resistance to tension forces using ASTM method D1004-13, this can be tested (ASTM International 2013b). In food packaging, tear resistance is important because the packaging must protect the food even when small imperfections are present. When it comes to polyols as plasticizers, using glycerol instead of sorbitol will impart lower tear resistance but higher elongation (Hong-rui et al. 2014). This is because glycerol is smaller than sorbitol, which allows it to insert into the polymer chains more easily. Disruption of polymer chains decreases strength but increases flexibility.

1.4.6. Color

The color of a film can be determined using a variety of machines including a MacBeth Color Eye. When the color of a material is determined there are two main possibilities for analyzing the data: CIELab and Hunter Lab. The standard method for using this equipment has been developed by the ASTM (ASTM International 2015b). Even when food packaging is biodegradable and of mechanical strength suitable for the purpose, it must also be aesthetically pleasing. Traditionally, this is done with printing put onto the packaging after it is made, but some packaging is engineered in a way that is does not have to be printed due to desirable native coloring. Pure AX does not impart any color into the films it is the basis for, so when AX films are colored it is from another source. The yellow color in alkali extracted WB AX films is due to lignin (Zhang et al. 2011). The WB AX extracted using alkaline extraction had a brownish tint to it (Aguedo et al. 2014). WB AX extracted by hydrothermal processes was much darker than the alkaline extracted WB AX, and the enzymatically extracted WB AX is lighter than the others.

1.4.7. Contact Angle

Contact angle is a measure of the angle formed between a drop of a liquid and the surface of a material. Most often this is determined using a highly polar substance such as water or a highly non-polar substance such as mineral oil. Depending upon the type of liquid chosen the contact angle will either give direct information about the hydrophilicity or hydrophobicity of the surface of the substance being analyzed. Due to glycerol being more hydrophilic than sorbitol, the contact angle of water on a glycerol film will be less than that of a film plasticized with sorbitol (Antoniou et al. 2014). On the other hand, the contact angle between a film plasticized with sorbitol and mineral oil will be less than that of a film plasticized with glycerol and mineral oil. One explanation for this is that the structure of films utilizing sorbitol as a plasticizer have a more compact structure than those plasticized with glycerol, and this results in less interaction with water. The ASTM method D7490-13 can be used to determine the surface properties of a film using one polar and one non-polar liquid (ASTM International 2013a).

1.4.8. Atomic Force Microscopy

An atomic force microscope utilizes the tapping of a small tip, known as a cantilever, on the surface of a material such as an AX film (Alvarez and Siqueiros 2014). This can be done using the ASTM E2382-04(2012) as a guide (ASTM International 2012b). Using this type of microscopy, the microscopic characteristics of a local area of an AX film can be determined. Atomic force microscopy can provide information about the height differences over a small area of a film as well as information about the density of the film (Alvarez and Siqueiros 2014). These things can provide information about the film including homogeneity of plasticization and density due to plasticization. In theory, as the level of plasticization increases, the crystallinity of the film will decrease and this will be clear in the density and topographical information provided while scanning with an atomic force microscope.

1.4.9. Water Vapor Permeability

When storing food, the water vapor permeability of the packaging material is very important. It is important because most foods contain water that is vital to shelf life. The water vapor permeability of food packaging can be determined using the ASTM method E96/E96M (ASTM International 2015a). As the amount of glycerol increases, so does the WVP of the films due to the hygroscopic nature of glycerol (Hansen and Plackett 2008). Glycerol is very hydrophilic, and due to this characteristic imparts a high level of water retention into the films it plasticizes (Antoniou et al. 2014). One of the main results of this is a higher water vapor transmission rate than films made with sorbitol. Table 1.8 provides data showing the trends produced by changing the plasticizer and plasticizer level for WB and MB AX films.

Table 1.8. Water vapor permeability for wheat bran arabinoxylan films and maize bran arabinoxylan films made with a variety of plasticizers.

Arabinoxylan type	Water vapor permeability	Relative humidity (%)	Film composition	Source
WB[a]	7.9 g mm k^{-1} Pa^{-1} m^{-2} d^{-1}	0/54	No Plasticizer	Heikkinen et al. 2013
WB	3.3-5.2 g m^{-1} s^{-1} Pa^{-1}	054	WB AX + Chitosan	Costa et al. 2015
MB[b]	4.1 g mm k^{-1} Pa^{-1} m^{-2} d^{-1}	0/54	No Plasticizer	Heikkinen et al. 2013
MB	2.0 g mm k^{-1} Pa^{-1} m^{-2} d^{-1}	0/54	130 g kg^{-1} Sorbitol	Heikkinen et al. 2013
MB	$0.23 \cdot 10^{-10}$ g m^{-1} Pa^{-1} s^{-1}	-	130 g kg^{-1} Sorbitol	Hansen and Plackett 2008
MB	$0.36 \cdot 10^{-10}$ g m^{-1} Pa^{-1} s^{-1}	-	50 g kg^{-1} Glycerol	Hansen and Plackett 2008
MB	$0.23 \cdot 10^{-10}$ g m^{-1} Pa^{-1} s^{-1}	0/54	128 g kg^{-1} Sorbitol	Zhang and Whistler 2004
MB	$0.31 \cdot 10^{-10}$ g m^{-1} Pa^{-1} s^{-1}	0/54	51 g kg^{-1} Glycerol	Zhang and Whistler 2004

[a] Wheat bran
[b] Maize bran

1.4.10. Biodegradability

The use of biodegradable materials is becoming increasingly popular, and as such the biodegradability of food packaging is also increasing. To definitively say that a material is biodegradable, it must be tested. Testing for biodegradability can be done using the standard for determining aerobic biodegradability of plastic materials in soil (ASTM International 2012a). When AX films are created, typically their intended purpose is either in the food or pharmaceutical industry. In either case, the shelf life of the material contained by the AX material must be maintained (Tharanathan 2003). This can be difficult due to the hydrophilic nature of AX, which can result in poor moisture barrier properties and structural integrity after a short amount of time. Research on biodegradable AX films made from WB, MB, or DDG have seldom been done for these reasons.

1.5. Food Packaging

Food packing must be both aesthetically pleasing and mechanically appropriate for storing the food it is specified for. Some of the required qualities include protecting food from oxidation, microbial spoilage, physical damage, and also increasing shelf life (Arrieta et al. 2015; Tharanathan 2003). It is also very important to prevent the movement of volatile compounds through food packaging (Casariego et al. 2009; Tharanathan 2003). Unfortunately, synthetic food packaging including polyoefins, polyamids, and polyesters has become popular due to its barrier properties (low oxygen and water vapor transmission rates), but this has resulted in ecological problems because often synthetic compounds are not biodegradable or recyclable and require large quantities of resources for production (as well as large quantities of harmful byproducts).

In most cases, food packaging must be inexpensive to produce so that the cost to the consumer is also low. It must also have low oxygen transmission rates, water vapor permeability, and aroma compound transmission, high tensile strength, and high tear resistance (Hansen and Plackett 2008; Tharanathan 2003). It is also desirable for food packaging to be transparent so that consumers can directly see what they are purchasing (Arrieta et al. 2015). With the increased research focused on biodegradable food packaging, it is becoming clear that plant-based food packaging, such as that made from hydrocolloids including AX, is one option for transparent food packaging. Food often has water present, and depending upon the water activity can lead to a high rate of perishability (Tharanathan 2003). Food packaging can also serve as a carrier for food additives including flavors, colors, antimicrobial agents, and antioxidants. To be more ecologically mindful, it would be beneficial to incorporate biopolymeric food packaging materials into the food packaging sector because they can be used and thrown away without detriment to the environment. Hydrocolloids and lipids are generally used to create biodegradable packaging. These two biomolecules are often combined to increase mechanical strength and create an optimal barrier for oxygen and water vapor. Plasticizers such as polyols are often added to biopolymeric packaging to increase flexibility and pliability. In addition, lignin, cellulose, and hemicelluloses (xyloglycans (xylans), β-glucans, mannoglycans (mannans), and xyloglucans) can also be utilized for food packaging (Hansen and Plackett 2008).

The presence of arabinose side chains improves the water solubility of AX, which is desired in some applications involving WB AX and MB AX, but not for food packaging (Saeed et al. 2011). Food packaging films must remain hydrophobic in order to lengthen the shelf life of a product. The A:X of these side chains impacts the end-use qualities of AX films because this ratio determines the stability and characteristics of the AX molecule (Kiszonas et al. 2013).

Carbohydrates including AX can be utilized as the basis for films that have high mechanical strengths and pliability when combined with the appropriate plasticizers (Zhang and Whistler 2004; The Phan et al. 2002). It has also been demonstrated that AX films have the same barrier and mechanical properties as starch and protein films (The Phan et al. 2002). Someday it would be ecologically advantageous to replace synthetic food packaging with AX packaging due to the ecological advantages (Peroval et al. 2004).

1.6. Need Statement

A change in the food packaging industry must be made should we desire to provide our children and grandchildren with a healthy environment to live in. The change that is necessary is the switch from unsustainable food packaging made mainly from plastics including polyoefins, polyamids, and polyesters to food packaging made from the byproducts of the cereal processing industry. When the AX is extracted from WB, MB, and DDG, it can be used as the basis of food packaging that is not only biodegradable but also edible (depending upon manufacturing processes used). This type of food packaging would improve the sustainability of both the food and packaging industries. In short, we need to recycle the AX from WB, MB, and DDG to utilize it in food packaging to create a sustainable tomorrow.

This research will address the discrepancy of data for the materials properties of films made from MB AX and DDG AX. In addition it will present an alternative method for extracting and purifying the AX from WB, MB, and DDG. Some of the materials properties that will be addressed in this research that have not been previously addressed include the surface topography, color, and biodegradability of all three types of AX films.

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CHAPTER 2. EXTRACTION AND CHEMICAL CHARACTERIZATIONS OF WHEAT BRAN, MAIZE BRAN, AND DRIED DISTILLERS GRAIN ARABINOXYLAN

2.1. Abstract

Chemical characterization of arabinoxylan (AX) from cereals is vital to evaluating the method of extraction. Using a combination of different extraction and purification methods provided insight into the overall effectiveness of the novel extraction method developed in this research. The chemical characteristics of AX extracted from wheat (*Triticum aestivum* L.) bran (WB), maize (*Zea mays* L.) bran (MB), and dried distillers grain (DDG) were determined. These three starting materials were obtained from commercial facilities. Subsequently, the AX was extracted from all three using an alkaline treatment. The chemical characteristics of each type of AX extracted were assessed including proximate composition, sugar profile using gas chromatography, molecular weight determination using high performance size exclusion chromatography with multi-angle light scattering and refractive index detectors (HPSEC-MALS-RI), and linkage assessment using nuclear magnetic resonance spectroscopy (NMR). The AX from WB that originally had a total AX content of 410 g kg^{-1} was extracted and purified to 730 g kg^{-1} AX. Similar purities were obtained for MB AX (850 g kg^{-1} purity) and DDG AX (580 g kg^{-1} purity) after extraction and purification. The arabinose to xylose ratio (A:X) for all AX extracts was 0.51. The order of the weight average molecular weights (Mw) of the extracted AX were from largest to smallest: MB AX, WB AX, and DDG AX. Linkage analysis of the extracted AX demonstrated that unsubstituted xylose was the most abundant in WB AX, monosubstituted xylose was the most abundant in MB AX, and disubstituted xylose was the most abundant in DDG AX. The effectiveness of the extraction and purification method developed in this experiment is clearly shown in the level purity of AX obtained in this experiment.

2.2. Introduction

Cereals including wheat and maize are two of the top three most commonly produced crops in the world (Heikkinen et al. 2013). Both wheat and maize are in the grass (*Gramineae*) family, which also includes barley, rice, rye, oats, triticale, sorghum, and millet (Delcour and Hoseney 2010). These cereal crops are defined by their ability to produce a fruit known as a caryopsis. The composition of the caryopsis is typically 600 to 700 g kg^{-1} starch, 100 to 150 g kg^{-1} protein, and 30 to 80g kg^{-1} non-starch polysaccharides. (Saulnier et al. 2007). One of the most common non-starch polysaccharides present in cereals is AX.

There are six classes of wheat in the U.S. (Delcour and Hoseney 2010). These classes include hard red winter, soft red winter, hard red spring, hard white, and durum wheat. The caryopsis (also known as the kernel) of wheat is found on the head, and is about 8 mm long and has a mass of 35 mg. One defining characteristic of the kernel of wheat is the presence of a crease on the ventral side. The main parts of a wheat kernel are the pericarp, seed coat, endosperm, and germ. WB, the pericarp and seed coat of the wheat kernel, is one of the main byproducts of the wheat milling industry because it is not desirable for refined flours (Apprich et al. 2014; Swennen et al. 2006). Currently WB is typically mixed back into whole wheat flours or sold as feed. The bran makes up 140 to 190 g kg^{-1} of the total wheat kernel mass (Maes and Delcour 2002). One of the major components of WB is non-starch polysaccharides, which make up about 460 g kg^{-1} of the WB (Zhang et al. 2011). Out of all the non-starch polysaccharides present, about 700 g kg^{-1} are AX (Maes and Delcour 2002).

Similar to wheat, maize is a cereal grain that produces a caryopsis (Delcour and Hoseney 2010). Maize kernels are large and flattened, and have a mass of around 350 mg. The four main parts of a maize kernel include the endosperm, germ, bran (hull), and tip cap. Maize kernels can come in a variety of colors including red, yellow, white, dark brown, blue, and purple. Maize bran is one of the main byproducts of the maize milling industry because it is seldom used in the products made from maize (Agger et al. 2010). The hull is 50 to 60 g kg^{-1} of the total mass of the maize kernel (Delcour and Hoseney 2010). The major components of maize bran include about 500 g kg^{-1} heteroxylans, 90 to 230 g kg^{-1} starch, 200 g kg^{-1} cellulose, 100 to 130 g kg^{-1} protein, 40 g kg^{-1} phenolic acids, 20 to 30 g kg^{-1} lipid, and 20 g kg^{-1} ash (Agger et al. 2010; Carvajal-Millan et al. 2007). One of the main types of heteroxylan present is AX, and this chemical species is present in the cell walls of the MB.

Dried distillers grain is one of the byproducts of the ethanol industry produced during the fermentation and distillation processes (Zarrinbakhsh et al. 2013). This byproduct of producing the ethanol industry is typically used for animal feed (Zarrinbakhsh et al. 2013; U.S. Grains Council 2012). The exact composition of DDG produced depends upon the exact processing and what type of maize was used as a starting material for the ethanol. However, it is known that DDG has a high hemicellulose content and a low lignin content (Xiang et al. 2014). The amount of AX that is present in DDG is on average around 90 g kg^{-1} (Zarrinbakhsh et al. 2013).

Arabinoxylan is the second most common polysaccharide found on earth after cellulose (Zhang et al. 2011). It is one type of dietary fiber present in the cell walls of the bran and endosperm cells of cereal grain (Zhang et al. 2014; Saeed et al. 2011; BeMiller 2007b). Arabinoxylan is composed of a linear xylose backbone made up of D-xylanopyranosyl residues, which are also known as xylans (Reis et al. 2015; Aguedo et al. 2014; Kiszonas et al. 2013). This xylose backbone is connected via β-1,4-glycosidic linkages and has arabinose substituents. The arabinose can be linked to the xylose backbone via O-2 or O-3 linkages, or it can be disubstituted at O-2 and O-3 (Agger et al. 2010). In addition to xylose and arabinose, glucose, ferulic acid, and acetic acid are also found in AX (Kiszonas et al. 2013; Saeed et al. 2011). AX can be classified by numerous chemical and physical characteristics, but the most commonly used description is extractability. There are many ways to extract AX, but one of the most common method is with the use of an alkaline solution such as sodium hydroxide (Zhang et al. 2014).

The objective of this research was to extract AX from WB, MB, and DDG using a combination of alkaline extraction and multiple purification methods to obtain AX with high purity; and then characterize the structural properties of the AX.

2.3. Materials and Methods

2.3.1. Procurement and Milling

The WB used was a commercial sample from the North Dakota State Mill in Grand Forks, North Dakota. The MB used was a commercial sample from Agricor, Incorporated. This sample was dent MB from a mill in Marion, Indiana. The DDG was from Tharaldson Ethanol in Casselton, North Dakota. Once WB and DDG were procured, they were milled on a Falling Number Hammer Mill (Type KT-12; Numer 10071)[1]. A Perten Instrument Mill Feeder 3170 (Perten Instruments North America, Incorporated) was used in conjunction with the mill. The MB was milled by Agricor, Incorporated before procurement.

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[1] Mention of trade names, proprietary products, or vendors does not constitute a guarantee or warranty for the product by North Dakota State University and does not imply its approval to the exclusion of other products or vendors that may be suitable.

2.3.2. Arabinoxylan Extraction and Purification

2.3.2.1. Defatting

Once milling of the WB and DDG was complete, all three starting materials were defatted using hexane. To begin this procedure, milled WB, MB, or DDG was combined with hexane (1:10 w v^{-1}). The hexane used was analytical grade from Sigma Aldrich Incorporated (Saint Louis, MO). The solution was stirred at 300 rpm for one hour at 23 °C. Next, the hexane was decanted into waste and new hexane was added and the solution allowed to stir for another hour. This process was repeated for a third time before the WB, MB, and DDG was rinsed for five minutes with hexane (1:5 w v^{-1}). After rinsing, the defatted material was dried overnight at 23 °C in a fume hood.

2.3.2.2. Alkaline Extraction

To extract the AX from the WB, MB, and DDG, an alkaline extraction based off the extraction method of Xiang et al. (2014) was used. The alkaline solution used was a 30 g kg^{-1} sodium hydroxide solution. The sodium hydroxide used was reagent grade from Sigma-Aldrich Incorporated (Saint Louis, MO; Lot No. SLBL7294V; ≥ 980 g kg^{-1} anhydrous pellets). The WB, MB, or DDG was added to the sodium hydroxide to form a 1:20 w v^{-1} solution. This solution was then stirred for three hours at 50 °C in a Lab-Line Mistral Multi-Stirrer (Model No. 1286; Serial No. 1657091026420) fitted with a Polyscience Temperature Controller (Model No. 712; Serial No. 10660810). After stirring for three hours, the samples were centrifuged at 3,000 rpm for 20 minutes to form a pellet out of the alkaline insoluble material. The centrifuge used was a Beckman Coulter Allegra X-12. After centrifugation, the supernatant was collected and kept for further purification.

2.3.2.3. Enzymatic Purification

Enzymatic purification was performed according to a modified method of Swennen et al, (2006). To begin purification, the pH of the AX was adjusted to seven using concentrated hydrochloric acid. The hydrochloric acid used was from EMD Millipore Corporation (GRACS; HX0603-3). A Symphony SB70P snD05434 pH Meter was used to read the pH of the solutions. To destarch the AX samples, α-amylase from *Bacillus licheniformis* was used (Sigma-Aldrich Incorporated; Saint Louis, MO; Termamyl 120; 1186 Units mg^{-1} protein; 19.8 mg protein mL^{-1}; A-3403-1MU). The amount of α-amylase used was 0.250 mL per liter AX solution. The AX solutions were destarched for three hours at 90 °C on a Lindberg Blue M Hot Plate (Model No. HP53014C; Serial No. P30K479076RK).

After destarching, the AX was deproteined using protease from *Bacillus amyloliquefaciens* (Sigma-Aldrich Incorporated; Saint Louis, MO; P1236-50 mL; Lot No. SLBG5002V; PC Code: 1001778189; ≥0.8 units g^{-1}). To begin this process, the solutions were allowed to cool to 50 °C and the pH of the AX solutions were adjusted to six using the same hydrochloric acid and pH meter as was utilized during destarching. Next, 2 mL of protease was added to each one liter of AX solution. These solutions were deproteined for three hours at 50 °C in a Lab-Line Mistral Multi-Stirrer (Model No. 1286, Serial No. 1657091026420) fitted with a Polyscience Temperature Controller (Model No. 712, Serial No. 10660810). After three hours, the solutions were heated to 100 °C for ten minutes to inactivate all enzymes.

2.3.2.4. Ethanol Fractionation

Fractionation with ethanol was performed in a modified method of Xiang et al. (2014). After enzymatic purification, the AX solutions were fractionated using analytical grade 950 g kg^{-1} ethanol. To do this, the ethanol was added to the AX solutions in a 2:1 v v^{-1} ratio and stirred for one hour in a Lab-Line Mistral Multi-Stirrer (Model No. 1286; Serial No. 1657091026420) fitted with a Polyscience Temperature Controller (Model No. 712; Serial No. 10660810). After fractionation was complete, the solutions were filtered using a Büchner funnel and Whatman No. 4 filter paper (150 mm diameter circles; Cat No. 1004 150). The solids were washed with 950 g kg^{-1} ethanol and allowed to dry overnight at 23 °C in a fume hood.

2.3.2.5. Dialysis and Freeze Drying

Dialysis was performed to remove species with molecular weights below 12 kDa. To do this, each type of AX was individually homogenized along with at least two liters of distilled water using a Polytron Homogenizer with a Polytron 93, PTA 205 tip and a PCU 13 power control unit. The dialysis membranes used were Spectra Por 2 Dialysis Membranes from Spectrum Laboratories Incorporated (12 to 14 kDa; Lot No. 9200228). Dialysis was performed at 23 °C in 10 gallon buckets of distilled water for 72 hours. The water was changed at least twice daily to maintain a favorable state of osmotic pressure. Sodium azide was used to keep the growth of microorganisms to a minimum (0.5 g per 10 gallons of distilled water). After 72 hours of dialysis, all samples were freeze-dried in a VirTis Freeze Dryer (Model No. 10-MRSM; Serial No. SM-1028).

2.3.3. Chemical Characterization

2.3.3.1. Composition

The particle sizes of the milled and defatted WB, MB, and DDG were determined in accordance with American Association of Cereal Chemists International (AACCI) Method 55-60.01: Guideline for Determination of Particle Size Distribution (AACC International 2011). The moisture content of each of the starting materials, defatted materials, and AX was determined in duplicate by following the AACCI Method 44-15.02: Moisture Air-Oven Method (AACC International 1999d). The ash content of all materials was determined in duplicate following the AACCI Method 08-01.01: Ash-Basic Method (AACC International 1999c). To determine the N content of these materials, AACCI Method 46-30.01: Crude Protein-Combustion Method. Protein content (estimated by N content) was determined in at least duplicate (AACC International 1999b). The lipid content in each milled material (WB, MB, and DDG) was analyzed in duplicate according to the American Oil Chemists Society (AOCS) Method Ba 3-38: Oil (AOCS 2009).

Total Starch analysis was carried out in at least quadruplicate using a Megazyme Enzyme Assay Kit K-TSTA (Megazyme International Ireland, Wicklow, Ireland) (AACC International 1999a). To begin, 100 mg of each sample was added to a clean, empty test tube (16 X 20 mm). Next, 0.2 mL of aqueous ethanol (800 g kg^{-1}) was added to each test tube and stirred using a VWR Analog Vortex Mixer (VM-3000 Mini Vortexer; Serial No. 061220025). After vortexing, 3 mL of α-amalyse solution (1 mL α-amalyse to 29 mL 3-(N-morpholino)propanesulfonic acid buffer) was added to each test tube. The test tubes were then vortexed again before being incubated at 100 °C in a VWR Type 89032-226 Water Bath for 10 minutes with vortexing after two and four minutes. Next, 4 mL of sodium acetate buffer (200 mM; 0.2 g kg^{-1} sodium azide; pH 4.5) and 0.1 mL of amyloglucosidase were added to each test tube. All samples were then incubated at 50 °C for 30 minutes. After incubation, the WB, MB, and DDG samples were transferred to 100 mL volumetric flasks and diluted to volume. All samples were then filtered into test tubes and 0.1 mL aliquots were transferred (in duplicate) to small glass test tubes (16 X 100 mm). Once in the small test tubes, 3 mL of glucose oxidase peroxidase was added to each test tube including the blanks and glucose controls. The blanks were 0.1 mL deionized water, and the glucose controls were 0.1 mL glucose standard solution. All tubes were then incubated at 50 °C for 20 minutes. The absorbance of all solutions were then read at 510 nm by a Thermo Electron Corporation Multiskan Ascent Type 354 microplate reader (Ref No. 51118407) that utilized Ascent Software for Multiskan Ascent (ThermoFisher Scientific 2010).

To determine sugar profile and A:X, acid hydrolysis followed by derivatization to alditol acetates was completed (Blankeney et al. 1983). After derivatization, the samples were analyzed using a gas chromatograph with a flame ionization detector (GC-FID). To begin this process, a standard curve was developed for each sugar. To do this, 250 mg of each sugar standard was placed into individual 25 mL volumetric flasks. Myo-inositol was used as an internal standard, so 250 mg of this material were also placed into a 25-mL volumetric flask. For each sugar, four different concentrations were used to make the standard curve: 250 ng mL^{-1}, 500 ng mL^{-1}, 750 ng mL^{-1}, and 1000 ng mL^{-1}. Next, 250 µL of 2 M TFA were added to each tube. The samples were then dried under nitrogen in a Thermo Scientific Reacti-Therm III (Model No. TC-18823).

The next step in this method was reduction, which began with the addition of 100 µL 1 M ammonium hydroxide. The contents of each standard were then swirled and 0.5 mL of 20 mg mL^{-1} sodium borohydride in dimethyl sulfoxide were added to each tube. The standards were then vortexed on a VWR Analog Vortex Mixer (Serial No. 061220025) and heated for 90 minutes at 40 °C vortexing every 30 minutes. After heating, 6 drops of glacial acetic acid were added to each standard.

After reducing, the standards were acetylated. The first step was to add 100 µL of 1-methylimidazol and then each sample was swirled. Next, 0.5 mL acetic anhydride were added to each standard before vortexing on a VWR Analog Vortex Mixer (Serial No. 061220025). The standards were then allowed to sit for 10 minutes at 23 °C before 4 mL of deionized water were added. The standards were vortexed once again and 1 mL methylene chloride was added. Each standard was vortexed and the bottom layer was removed using a long pasture pipette and transferred to a clean test tube. The addition of 1 mL methylene chloride and removal of the bottom layer was repeated once again. This bottom layer of each standard was dried under nitrogen at 45 °C in a Thermo Scientific Reacti-Therm III (Model No. TS-18823). Once dried, 1 mL acetone was added to each test tube and the dissolved contents were then transferred to the auto sampler vials for analysis by GC-FID.

After the standard curve was created, 2 to 4 mg of each sample was analyzed. The hydrolysis of this process began with adding 250 µL 2M TFA to each test tube with sample and sealing tightly with a cap. Next, the tubes were heated at 121 °C for one hour. After heating, 75 µL of inositol were added to each test tube and they were dried under nitrogen at 55 °C in a Thermo Scientific Reacti-Therm III (Model No. TS-18823). After hydrolyzing the samples, they were reduced and acetylated using the same method described for the control samples.

After sample preparation, the analysis of the sugar composition was performed using GC-FID. The gas chromatograph used was a Hewlett Packard 5890 Series II GC system with a flame ionization detector (Agilent Technologies, Incorporated Santa Clara, CA). The column used was a SupelcoSP-2380 fused silica capillary column (30 m x 0.25 mm x 2 µm) (Supelco Bellefonte, PA). The parameters under which testing was completed were as follows: 827372 Pa flow pressure, 100 °C oven temperature, 0.8 mL min^{-1} flow rate, detector temperature of 250 °C, and injector temperature of 230 °C. Helium was used as the carrier gas.

2.3.3.2. High Performance Size Exclusion Chromatography-Multi Angle Light Scattering-Refractive Index

To determine the Mw of each AX extract, a HPSEC-MALS-RI system was used. The Mw and polydispersity index (PI), which is defined as the ratio Mw Mn^{-1}, where Mw is the weight average molecular weight and Mn is the number average molecular mass, of AX hydrolyzates was determined using a modified method of Menids and Simsek (2015). A small amount of sample (4 mg) was dissolved in deionized water that had been filtered using a 0.1 µm filter by heating at 40 °C with continuous stirring for 60 minutes. Each sample solution was then filtered through a 5 µm filter and analyzed using an HPSEC-MALS-RI system. The high performance liquid chromatograph (HPLC) used was an Agilent 1200 with a Wyatt Dawn Helios-II multi-angle light scattering detector (MALS) and a refractive index detector (RI). The columns used were a Shodex OHpak guard column and a SB 806-HQ column. To normalize the MALS detector, 300 kDa pullulan was used. The mobile phase used was water with a 0.5 mL min^{-1} flow rate. To calculate the Mw and PI, Astra 6.0.5 software (Wyatt Technology 2116) based on a 3rd order Debye plot with second-order polynomial fit was used. The values of dn dc^{-1}, which is defined as the proportional change in the refractive index as the polymer concentration changes were assumed to be 0.146 for AX (Dervilly et al. 2000).

2.3.3.3. ^{1}H Nuclear Magnetic Resonance Spectroscopy

The NMR spectra for each type of AX (WB, MB, and DDG) was determined using duplicate samples (Mendis and Simsek 2015). To begin the process, 5 mg of each sample were dissolved in 600 µL of deuterium oxide by heating at 40 °C using continuous stirring with a Reacti-therm III No. 18823 (Thermo Scientific, USA). After dissolving the samples, they were freeze-dried on a Labconco Freeze Zone 4.5 Freeze Dryer (Labconco Corporation Kansas City, MO; Cat No. 7751070; Serial No. 070975444 Rev. S.). The samples were redissolved and freeze-dried two more times. For the NMR analysis, the samples were dissolved in 650 µL deuterium oxide and analyzed at 80 °C on a 400 MHz spectrometer (Bruker AV3 HD 400 MHZ NMR that had a 5 mm PABBO BB/19F-!H/D Z-GRD Z probe). Data was analyzed using TopSpin 3.2 software (Bruker BioSpin Corporation 2015).

2.3.4. Statistical Analysis

This experiment utilized completely random design with a factorial arrangement. The AX source was the first factor, the type of plasticizer was the second factor, and the plasticizer level was the third factor in the arrangement. The analysis of all data was completed using Statistical Analysis Software (SAS) version 9.3 (SAS Institute Inc. 2011). SAS was used to produce the analysis of variance for each data set. The data was then further analyzed using Fischer's protected least significant difference to analyze the separation of the means.

2.4. Results and Discussion

2.4.1. Extraction and Purification Method

The extraction and purification method developed in this research is a combination of multiple previously published extraction and purification methods. An outline of the method used is shown in figure 2.1. Firstly, the alkaline extraction method was used from the research of Xiang et al. (2014) with some modifications. The centrifugation speed and time were modified to be 3,000 rpm for 20 minutes instead of 3,900 rpm for 10 minutes. Secondly, the enzymatic purification method proposed by Swennen et al. (2006) was utilized to remove starch and N from the extracted AX. Thirdly, ethanol fractionation is a method commonly used for fractionating polysaccharides such as AX. The method for ethanol fractionation of AX proposed by Xiang et al. (2014) was used with one modification. The ratio of AX solution to ethanol used was 1:2 (v v^{-1}) instead of 1:3 (v v^{-1}). Fourthly, dialysis was used as a final purification method to remove all material with a molecular weight lower than 12 kDa. Lastly, the AX was dialyzed to remove excess water so the AX could be chemically characterized.

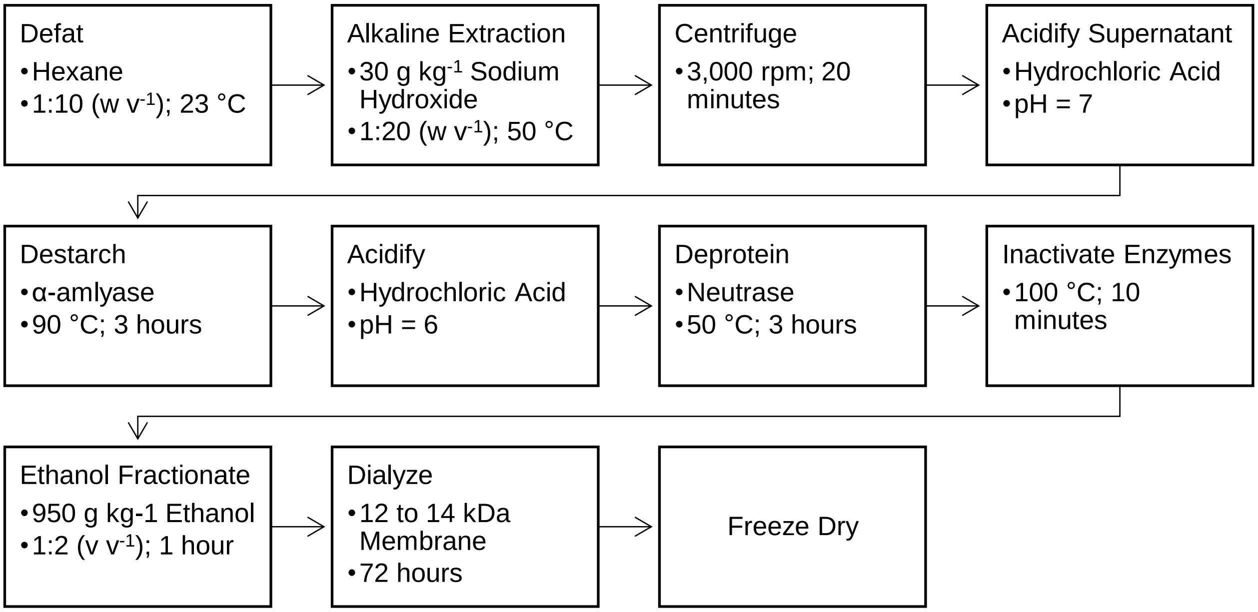

Figure 2.1. Extraction and purification method outline.

This method allowed for the extraction of AX from WB that was of higher purity than previously published research about alkaline extraction of AX. The WB AX extracted was higher in purity than the WB AX extracted by Maes and Delcour (2002). In addition, the WB AX extracted in this research had much less residual galactose after extraction than the WB AX extracted by multiple groups including Zhang et al. (2011), Aguedo et al. (2014), and Maes and Delcour (2002). The residual glucose remaining in the WB AX extracted using this method was also lower than in the WB AX extracted by Aguedo et al. (2014) and Maes and Delcour (2002). This demonstrates more effective removal of starch using this methodology. The A:X was between the A:X of 0.20 obtained by Zhang et al. (2011), and the A:X of 0.9 obtained by Aguedo et al. (2014).

The purity of MB AX obtained by Zhang and Whistler was only 330 g kg^{-1} arabinose and 490 g kg^{-1} xylose (2004). Similarly, Kale et al. (2010) extracted AX from MB using sodium hydroxide and ethanol fractionation resulting in an arabinose content of 190 to 220 g kg^{-1} and a xylose content of 350 to 410 g kg^{-1} depending upon the concentration of the ethanol. The AX extracted by Agger et al. (2010) using hot water extraction had an arabinose content of 470 g kg^{-1} and a xylose content of 370 g kg^{-1}. In addition, the residual N in the MB AX extracted using this procedure was much lower than in the MB AX extracted by these two groups using multiple extraction procedures (Agger et al. 2010; Kale et al. 2010).

Similarly, the DDG AX extracted using this method was purer than DDG AX obtained using multiple methods. Firstly, Zarrinbakhsh et al. (2013) obtained DDG AX by hot water extraction with a purity of only 210 g kg^{-1}, while this method produced DDG AX with a purity of 580 g kg^{-1}. In addition, Reis et al. (2015) DDG AX using ultrasound assisted alkaline extraction that had a purity of 450 g kg^{-1}, which is much lower than the purity obtained in this research. The residual glucose and galactose remaining in the DDG AX after utilization of the extraction and purification method developed in this research is also much lower than in the DDG AX extracted using ultrasound assisted alkaline extraction and straight alkaline extraction done by Reis et al. (2015).

2.4.2. Particle Size Range and Composition

Particle size range was the first analysis completed after receiving all starting materials. This was done because the MB was milled before procurement, but the WB and DDG were not. The WB and DDG were milled on a Perten Hammer Mill, whereas the MB was milled on a commercial maize mill. The full particle size ranges for all three materials are shown in Table 2.1. The particle size range for the WB falls mainly in the first two screens: screen 60 (459 g kg^{-1}) and screen 80 (469 g kg^{-1}). The range for the DDG is very similar and falls mainly in the same two screens: screen 60 (396 g kg^{-1}) and screen 80 (443 g kg^{-1}). The MB particle size range is smaller, which is expected for a product that is from a commercial mill. Most of the MB fell on screen 60 (971 g kg^{-1}). The actual difference between screen 60 and screen 80 is 73 microns (screen 60 is 250 microns and screen 80 is 177 microns).

The milling process plays a major role in the particle size range and can impact qualities including water absorption. In addition, reducing particle size increases extractability of AX and decreases water absorption (Liu et al. 2016). Because WB, MB, and DDG are byproducts of the industries they come from, particle size is often larger than that of a refined flour. Common particle sizes of WB range from coarse (609 μm) to fine (278 μm) (Zhang and Moore 1999). It is also possible to mill WB to a particle size that is finer, which is seen in the particle size range of the WB used in this experiment. MB particle sizes often range from 1000 μm to 250 μm (Agger and Meyer 2012). The MB used in this research falls on end with the smaller particle size (on average). As for DDG, the particle size range will vary greatly depending upon processing. The DDG was milled using the same mill as the WB, so they have comparable particle sizes. Despite the MB being milled elsewhere, its particle size range is still very similar to the WB and DDG.

Table 2.1. Particle size range for wheat bran, maize bran, and dried distillers grain.

Material	Screen 60	Screen 80	Screen 100	Screen 120	Screen 200	Screen 325	Bottom pan
				($g\ kg^{-1}$)			
WB[a]	459	469	59	8	3	10	2
MB[b]	971	16	1	2	4	10	5
DDG[c]	396	443	107	29	2	4	2

[a] Wheat bran
[b] Maize bran
[c] Dried distillers grain

All materials used throughout this experiment including the milled materials (WB, MB, and DDG), defatted materials (WB, MB, and DDG), and extracted AX (from WB, MB, and DDG) were analyzed for proximate composition. The analyses performed to give the proximate composition of all materials included total moisture, total ash, total N, total lipid (all materials aside from AX), total starch, and sugar profile. This information obtained from these analyses provided information about how to properly extract and purify the AX in addition to the effectiveness of the extraction and purification methods utilized. The compositions for all materials are given in Table 2.2 (milled materials), Table 2.3 (defatted materials), and Table 2.4 (extracted AX). The overall compositions show great variation depending upon the material (WB, MB, or DDG). Despite these differences, the overall trends are shared amongst all materials throughout the processing and extraction of AX.

Table 2.2. Proximate composition of wheat bran, maize bran, and dried distillers grain in dry weight basis.

	Proximate composition ($g\ kg^{-1}$)										
Material	Moisture	Ash	N	Lipid	Total starch	Arabinoxylan	Arabinose	Xylose	Galactose	Glucose	A:X[e]
WB[a]	84.5	67.9	179.4	40.7	126.2	409.2	190.8	274.2	15.3	238.1	0.70
MB[b]	96.1	7.6	49.1	24.3	128.5	683.7	263.2	513.7	1.5	235.8	0.51
DDG[c]	93.3	68.4	286.8	85.6	39.8	179.2	84.7	118.9	8.0	111.5	0.71
LSD ($P \leq 0.05$)[d]	-	0.1	7.4	9.8	3.3	14.1	6.5	0.5	0.1	3.2	0.01
LSD ($P \leq 0.01$)	-	0.3	13.5	18.0	4.8	25.8	11.8	17.5	0.2	5.8	0.02

[a] Wheat bran
[b] Maize bran
[c] Dried distillers grain
[d] Least significant difference
[e] Arabinose to xylose ratio

Table 2.3. Proximate composition of defatted wheat bran, maize bran, and dried distillers grain in dry weight basis.

	Proximate composition (g kg^{-1})									
Material	Moisture	Ash	N	Lipid	Total starch	Arabinoxylan	Arabinose	Xylose	Galactose	Glucose	A:X[e]
WB[a]	72.1	62.7	174.0	18.6	102.4	376.2	169.4	258.0	9.4	170.1	0.52
MB[b]	76.9	5.9	65.7	12.8	95.8	533.7	207.9	398.5	8.1	143.0	0.69
DDG[c]	90.3	73.6	318.1	13.2	46.7	179.6	83.5	120.6	8.2	138.9	0.66
LSD (*P*≤0.05)[d]	15.2	5.5	4.2	6.3	5.8	18.9	8.6	12.9	0.2	5.9	0.01
LSD (*P*≤0.01)	35.1	10.1	6.3	11.6	8.4	34.8	15.9	23.7	0.3	10.9	0.02

[a] Wheat bran
[b] Maize bran
[c] Dried distillers grain
[d] Least significant difference
[e] Arabinose to xylose ratio

Table 2.4. Proximate composition of arabinoxylan extracted from wheat bran, maize bran, and dried distillers grain using alkaline extraction in dry weight basis.

	Proximate composition (g kg^{-1})									
Material	Moisture	Ash	N	Total starch	Arabinoxylan	Arabinose	Xylose	Galactose	Glucose	A:X[e]
WB[a]	62.2	85.5	136.2	15.0	729.4	280.0	548.9	5.9	21.1	0.51
MB[b]	111.1	12.2	38.9	3.3	847.1	325.1	637.5	6.4	22.0	0.51
DDG[c]	63.1	18.9	149.5	3.1	580.5	223.5	436.2	4.2	14.2	0.51
LSD (*P*≤0.05)[d]	13.9	0.6	13.1	1.1	29.7	10.2	23.7	0.2	1.7	-
LSD (*P*≤0.01)	25.6	1.1	24.0	1.5	54.5	18.8	43.6	0.3	3.2	-

[a] Wheat bran
[b] Maize bran
[c] Dried distillers grain
[d] Least significant difference
[e] Arabinose to xylose ratio

The moisture content of WB, MB, and DDG will vary greatly with storage conditions and processing methods used. Due to this, moisture content is used as a baseline for correcting other elements of the composition including ash, N, lipid, and starch (Haynes et al. 2010). The moisture contents of all materials decreased throughout processing (milling, defatting, and extraction). After milling, the MB had the highest moisture content (96.1 g kg^{-1}), followed by the DDG (93.3 g kg^{-1}) and WB (84.5 g kg^{-1}). The moisture contents of the milled materials were determined in singlet, as it was used as a baseline correction for all other elements of the composition. Following milling the materials were defatted with hexane, and their moistures decreased to DDG (90.3 g kg^{-1}), MB (76.9 g kg^{-1}), and WB (72.1 g kg^{-1}). The moisture contents of the milled materials were not significantly (*P*≤0.01) different from one another.

After all extraction and purification, the moisture contents of the AX decreased from the starting materials except for the MB AX, which increased: MB AX (111.1 g kg^{-1}), DDG (63.1 g kg^{-1}), and WB AX (62.2 g kg^{-1}). The moisture contents of the WB AX and DDG AX were not significantly ($P \leq 0.01$) different from one another, but significantly different from the moisture content of the MB AX ($P \leq 0.01$).

Ash in both wheat and maize is more concentrated in the bran, as this is the location of the inorganic material present in the kernel (Delcour and Hoseney 2010). Both WB and MB have ash contents around 20 g kg^{-1} (Delcour and Hoseney 2010; Carvajal-Millan et al. 2007). However, processing affects the ash content of the material, which can be seen in the variations of the ash contents of the materials processed in this experiment. After milling, all materials had significantly ($P \leq 0.01$) different ash contents: DDG (68.4 g kg^{-1}), WB (67.9 g kg^{-1}), and MB (7.6g kg^{-1}). These values were either much higher or much lower than the typical average (20 g kg^{-1}), which could be due to the varieties and growing locations of wheat and maize present in the samples. When treated with hexane to remove lipids and other non-polar compounds, the ash contents decreased for the most part. The relative ash content of the DDG increased to 73.6 g kg^{-1}, but the relative amounts of ash in WB and MB both decreased to 62.7 g kg^{-1} and 5.9 g kg^{-1}, respectively. This could be due to removal of small minerals that interact with the non-polar compounds present in the WB and MB. DDG is more processed than both WB and MB, so removal of impurities can be more difficult. After complete extraction and purification, the ash remaining in the AX fractions are as follows: WB AX at 85.5 g kg^{-1}, DDG AX at 18.9 g kg^{-1}, and MB AX at 12.2 g kg^{-1}. On average, the amount of ash in the extracted AX fraction was less than that of previously published values including an ash value of 54 g kg^{-1} in DDG extracted with 30 g kg^{-1} sodium hydroxide (Xiang et al. 2014).

N plays many roles in WB, MB, and DDG including providing structure and support for the cells and serving as storage location in proteins (Delcour and Hoseney 2010). The protein content of WB is usually around 150 to 220 g kg^{-1} (Maes and Delcour 2002), MB has a N content of around 100 to 130 g kg^{-1} (Carvajal-Millan et al. 2007), and DDG has the highest protein content at around 300 g kg^{-1} (Xiang et al. 2014). The N contents measured in the milled materials were very comparable to these values. The N in the WB after milling was about 180 g kg^{-1}, the MB was about 50 g kg^{-1} N which was lower than the expected N content, and the DDG was around 290 g kg^{-1} N. These values are all significantly ($P \leq 0.01$) different from one another. After defatting with hexane, the relative N contents increased for the most part due to removal of the lipids. The WB showed a decrease to 170 g kg^{-1}, but both the MB and DDG increased to N contents of 60 g kg^{-1} and 320 g kg^{-1} respectively. Once again, these values were significantly ($P≤0.01$) different from one another. Finally, after extracting and purifying the AX, the N contents all decreased to 140 g kg^{-1} for WB AX, 40 g kg^{-1} for MB AX, and 150 g kg^{-1} for DDG AX due to treatment with protease. These N contents were significantly ($P≤0.01$) different from one another. In addition, these N contents were less than those obtained in AX that has been alkaline extracted by other research groups in WB (190 g kg^{-1} protein in AX fraction) (Maes and Delcour 2002), MB (50 to 70 g kg^{-1} protein in AX fraction) (Kale et al. 2010), and DDG (170 g kg^{-1} protein in AX fraction) (Reis et al. 2015). To increase the effectiveness of the removal of N, dialysis could have been performed for longer or the water in the buckets could have been changed more. In addition, it may have been beneficial to utilize multiple proteases and a longer treatment with these enzyme to remove more N (in the form of protein).

In WB and MB, lipids are found in relatively low amounts unless there is contamination from the germ (Delcour and Hoseney 2010). Whereas in DDG, there is a much higher lipid content, typically around 100 g kg^{-1} (Xiang et al. 2014). The lipid contents of the milled starting materials followed this trend and had the following lipid contents that were significantly ($P≤0.05$) different from one another: 41 g kg^{-1} lipid in WB, 24 g kg^{-1} lipid in MB, and 86 g kg^{-1} lipid in DDG. After defatting with hexane (to remove the non-polar lipids), the total lipid content of all materials decreased to 19 g kg^{-1} for WB, and 13 g kg^{-1} for both MB and DDG. These values were not significantly ($P≤0.05$) different from one another. The extracted AX was not analyzed for total lipid due to the low lipid content of the defatted materials and utilization of a saponification reaction for the AX extraction, which would remove further defat the samples.

In WB, MB, and DDG, starch is a storage compound broken down when excess energy is needed such as during germination. The amounts of starch vary depending upon growing conditions, but on average are 100 to 200 g kg^{-1} for WB (Zhang et al. 2011), 90 to 230 g kg^{-1} in MB (Carvajal-Millan et al. 2007), and 50 g kg^{-1} in DDG (Xiang et al. 2014). The total starch in the milled WB, MB, and DDG were like these compositional trends. The total starch contents of the WB and MB were not significantly ($P≤0.01$) different, as both were about 130 g kg^{-1}. However, the total starch content in the DDG was significantly ($P≤0.01$) lower at about 40 g kg^{-1} due to the processing required to make DDG from maize (i.e. enzymatic treatments and fermentation). After defatting with hexane, the relative amount of total starch decreased to 102 g kg^{-1} and 96 g kg^{-1} in the WB and MB respectively. However, after defatting, the relative total starch content in the DDG increased to about 50 g kg^{-1}. The difference between the amount of total starch present in all three materials was significantly ($P \leq 0.05$) different from one another. After extraction and purification of the AX, the total starch present in all three compounds decreased. The WB AX contained 15 g kg^{-1} starch, and both the MB AX and DDG AX contained about 3 g kg^{-1} starch. The amounts of total starch were significantly ($P \leq 0.01$) different between the WB AX and MB AX, and the WB AX and DDG AX. When alkaline extraction was used to extract AX from DDG, Reis et al. (2015) obtained AX with a total starch content of about 70 g kg^{-1}, which is much higher than the starch content of the DDG AX extracted in this research. However, Maes and Delcour (2001) were able to obtain WB AX with a starch content of 3 g kg^{-1} using alkaline extraction, which is purer than the WB AX extracted in this research.

Arabinoxylan is a non-starch polysaccharide present in the cell walls of WB and MB (Zhang et al. 2014; Saeed et al. 2011). In WB, the AX content can be as high as 300 g kg^{-1}, and the A:X is usually 0.57 to 1.07 (Zhang et al. 2014; Zhang et al. 2011). In MB, the total AX content is typically 300 to 400 g kg^{-1} (Saeed et al. 2011; Zhang and Whistler 2004), and the A:X ranges from 0.40 to 0.66 (Agger et al. 2010). The AX content and A:X for DDG vary and are dependent upon the processing of the DDG. The total AX content present in the starting materials was higher than the values of previously published. The AX contents of the milled materials were as follows: WB (410 g kg^{-1}), MB (680 g kg^{-1}), and DDG (180 g kg^{-1}). These values are all significantly ($P \leq 0.01$) different from one another. The A:X for the milled materials are also significantly ($P \leq 0.05$) different from each other at 0.70 for WB, 0.51 for MB, and 0.71 for DDG. These A:X are the expected ratios when compared to previously published work. After treatment with hexane, the relative total AX decreased to 380 g kg^{-1} for WB and 530 g kg^{-1} for MB, but remained at 180 g kg^{-1} for DDG. Treatment with hexane also changed the A:X so they were the following significantly ($P \leq 0.01$) different values: 0.52 in WB, 0.69 in MB, and 0.66 in DDG. Extraction and purification of the AX from the three starting materials yielded WB AX with a purity of 730 g kg^{-1}, MB AX with a purity of 850 g kg^{-1}, and DDG AX with a purity of 580 g kg^{-1}. The purity of AX was significantly ($P \leq 0.01$) different amongst all three types of extracted AX, but they all had the same A:X of 0.51. The purities of these AX extracts are much higher than the purity of alkaline extracted AX in previously published work. For example, Maes and Delcour (2001) obtained WB AX with a purity of 450 g kg^{-1}.

Overall, after treatment with hexane during defatting there were relative decreases in moisture content, ash content, total lipid, total starch, and AX content. After defatting, there was an overall increase in relative N content. In addition, after further processing by alkaline extraction, enzymatic purification, ethanol fractionation, and dialysis, there were relative decreases in moisture content, ash content, N content, and total starch. However, the relative AX content increased after extraction and purification. In addition, the AX extracted in this experiment was lower in ash, N, and starch than previously published AX extracted from the same materials.

2.4.3. High Performance Size Exclusion Chromatography-Multi Angle Light Scattering-Refractive Index

The Mw of the extracted WB AX, MB AX, and DDG AX, as shown in Table 2.5, were determined using HPSEC-MALS-RI. Figure 2.2 provides a graphical representation of the retention times for each of the three types of AX. From largest to smallest, the Mw of the three types of AX were MB AX (7700333 Daltons), WB AX (7116667 Daltons), and DDG AX (5900000 Daltons). The Mw of all three of these materials are significantly ($P \leq 0.01$) different. The Mw of WB AX alkaline extracted by Aguedo et al. (2014) was 670,000 Daltons, which is much smaller than the WB AX extracted in this research. The Mw of MB AX alkaline extracted by Zhang et al. (2011) was 293,000 Daltons, which is also much smaller than the Mw obtained in this research. This is most likely due to differences in the purification and fractionation methods used in this research as compared to those used by Aguedo et al. (2014) and Zhang et al. (2011). These other research groups utilized a multi-angle light scattering detector for Mw determination.

Table 2.5. Molecular weights and polydispersity indexes for wheat bran arabinoxylan, maize bran arabinoxylan, and dried distillers grain arabinoxylan determined using high performance size exclusion chromatography with a multi-angle light scattering detector and a refractive index detector.

Material	Molecular weight (Da)	Polydispersity index
WB AX[a]	7116667	1.59
MB AX[b]	7700333	1.27
DDG AX[c]	5900000	1.04
LSD (*P*≤0.05)[d]	59535	0.23
LSD (*P*≤0.01)	78906	0.31

[a] Wheat bran arabinoxylan
[b] Maize bran arabinoxylan
[c] Dried distillers grain arabinoxylan
[d] Least significant difference

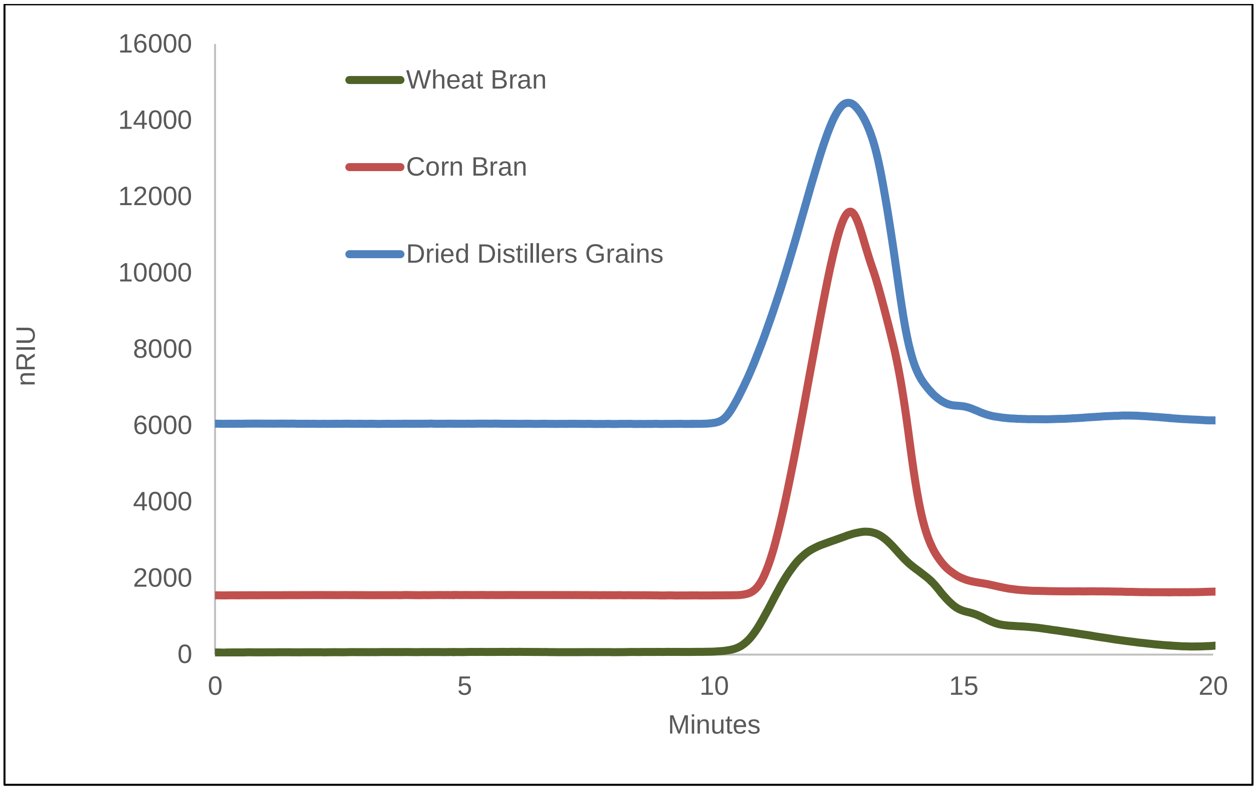

Figure 2.2. Elution times of wheat bran, maize bran, and dried distillers grain arabinoxylan determined using high performance size exclusion chromatography with a refractive index detector.

In addition to the Mw, the PI for these three AX extracts were calculated. The PI is a measure of the width of the variation in the Mw distribution (Rogosic et al. 1995). This is a ratio between the Mw and the Mn. The PI for all three types of AX is significantly (*P*≤0.01) different, and largest to smallest were WB AX (1.59), MB AX (1.27), and lastly DDG AX (1.05). These differences indicate that there is more heterogeneity in the Mw of the WB AX and MB AX than the DDG AX. The PI of 1.05 for DDG AX shows that almost all the AX had the same Mw. The PI obtained for MB AX by Zhang et al. (2011) was 3.0, which is much higher than the PI of the MB AX extracted in this research. Smaller PI, such as those obtained in this research, are indicative of a narrower band of Mw obtained for each type of AX.

2.4.4. ^1H Nuclear Magnetic Resonance Spectroscopy

Proton (^1H) NMR was utilized to obtain the spectra for the resonances of all anomeric protons present in all types of AX (WB, MB, and DDG). In these spectra, there are seven notable species with corresponding peaks, which are described in Table 2.6. The first species present is the arabinose C-(O)-3 linked to anomeric xylose with a resonance located at δ 5.89 ppm in WB AX, and two resonance peaks in MB and DDG at δ 6.05 and δ 6.03 ppm. The second species present is the anomeric proton of arabinose C-(O)-3 linked to disubstituted xylose with two resonance peaks in all three AX species at δ 5.80 and δ 5.78 ppm in WB AX, δ 5.91 and δ 5.90 ppm in MB AX, and δ 5.92 and δ 5.89 ppm in DDG AX. Thirdly, the anomeric proton of arabinose C-(O)-2 linked to disubstituted to xylose that has an adjoining disubstituted xylose has resonance peaks located at δ 5.73 and 5.71 ppm in WB AX, and δ 5.78 ppm in MB AX and DDG AX. Fourthly, the anomeric proton of arabinose C-(O)-2 linked to disubstituted xylose is has a resonance peak at δ 5.67 ppm in WB AX, and δ 5.27 ppm in both MB AX and DDG AX. Fifthly, the anomeric proton of the disubstituted xylose has a resonance peak located at δ 5.15 ppm in WB AX, δ 5.27 ppm in MB AX, and three locations in DDG AX including δ 5.27 ppm, δ 5.22 ppm, and δ 5.19 ppm. Sixthly, the anomeric proton of the monosubstituted xylose has a resonance peak present at δ 5.09 ppm in AB AX, δ 5.22 ppm and δ 5.19 ppm in MB AX, and δ 5.07 ppm in DDG AX. Finally, the peak for the anomeric proton of the unsubstituted xylose has a resonance peak located at δ 4.99 ppm in WB AX, δ 5.07 ppm in MB AX, and δ 4.96 ppm in DDG AX.

The abundances of each chemical species present in the three types of AX identified by Hoffman et al. (1992) were also determined relative to the abundance of unsubstituted xylose. In WB AX, the three most abundant species from most abundant to least were unsubstituted xylose, arabinose that is C-(O)-3 linked to disubstituted xylose, and arabinose C-(O)-2 linked to disubstituted xylose with adjoining disubstituted xylose. MB AX had the following three most abundant species (from most abundant to least) monosubstituted xylose, unsubstituted xylose, and arabinose that is C-(O)-3 linked to disubstituted xylose. Lastly, in DDG AX the top three most abundant chemical species from most abundant to least were disubstituted xylose, monosubstituted xylose, and arabinose that is C-(O)-2 linked to disubstituted xylose that has an adjoining disubstituted xylose.

Table 2.6. ^{1}H nuclear magnetic resonance peak identities and relative abundances (integrated with unsubstituted xylose equal to one) for wheat bran arabinoxylan, maize bran arabinoxylan, and dried distillers grain arabinoxylan.

	Wheat Bran		Maize Bran		Dried Distillers Grain	
Linkage Present	δ [ppm]	Integral [rel]	δ [ppm]	Integral [rel]	δ [ppm]	Integral [rel]
Arabinose C-(O)-3 Linked to Anomeric Xylose	5.89	0.18	6.05	0.15	6.05	0.29
Arabinose C-(O)-3 Linked to Anomeric Xylose	-	-	6.03	0.19	6.03	0.62
Anomeric Proton of Arabinose C-(O)-3 to Disubstituted Xylose	5.80	0.19	5.91	0.20	5.92	0.64
Anomeric Proton of Arabinose C-(O)-3 to Disubstituted Xylose	5.78	0.28	5.90	0.48	5.89	1.20
Anomeric Proton of Arabinose C-(O)-2 to Disubstituted Xylose with adjoining disubstituted xylose	5.73	0.11	5.78	0.59	5.78	2.16
Anomeric Proton of Arabinose C-(O)-2 to Disubstituted Xylose with adjoining disubstituted xylose	5.71	0.29	-	-	-	-
Anomeric Proton of Arabinose C-(O)-2 to Disubstituted Xylose	5.67	0.09	5.71	0.09	5.72	0.40
Anomeric Proton of Disubstituted Xylose	5.15	0.26	5.27	0.34	5.27	0.60
Anomeric Proton of Disubstituted Xylose	-	-	-	-	5.22	0.96
Anomeric Proton of Disubstituted Xylose	-	-	-	-	5.19	1.91
Anomeric Proton of Monosubstituted Xylose	5.09	0.33	5.22	0.46	5.07	2.63
Anomeric Proton of Monosubstituted Xylose	-	-	5.19	0.66	-	-
Anomeric Proton of Unsubstituted Xylose	4.99	1.00	5.07	1.00	4.96	1.00

2.5. Summary and Conclusions

Cereal processing byproducts including WB, MB, and DDG obtained from commercial sources had varying chemical compositions. DDG was the highest in moisture, ash, and N. Whereas MB was the highest in total starch and AX. WB and DDG had similar A:X at 0.70 and 0.71 respectively, while MB had a much lower A:X at 0.51. After extraction and purification, AX from these materials also had varying compositions. The MB AX was the highest in moisture and AX content, while the WB AX was the highest in ash and total starch, and the DDG AX was highest in N. The A:X for all extracted AX are the same (0.51). From largest to smallest, the Mw of the AX extracted were MB AX, WB AX, and DDG AX. The PI for these materials decreased moving from WB AX to MB AX to DDG AX. These three types of AX also had different relative abundances of linkages present. These characteristics demonstrate a wide variety in the physicochemical properties of AX present in cereals. The greatly increased purity in all three types of AX over the steps in the extraction and purification process demonstrated its effectiveness. In addition, there was also a gap between the purity of the AX extracted in this experiment over the AX extracted in previously published research. Alkaline extraction provided an effective method for removing the AX from WB, MB, and DDG. After extraction, purification with α-amylase and protease provided effective removal of both starch and N remaining in the AX extract. This enzymatic purification yielded AX that had less starch and N than AX extracted using other methods. Coupling these enzymatic purifications with dialysis allowed for the removal of low molecular weight compounds from the AX extract. Overall, the utilization of these methods allowed for production of AX from WB, MB, and DDG that was of higher purity than previously established extraction methods.

2.6. References

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CHAPTER 3. MECHANICAL PROPERTIES OF ARABINOXYLAN FILMS

3.1. Abstract

Arabinoxylan (AX) from wheat (*Triticum aestivum* L.) bran (WB), maize (*Zea mays* L.) bran (MB), and dried distillers grain (DDG) shows promising mechanical characteristics for use in food packaging material. This study determined the mechanical characteristics of films made from WB AX, MB AX, and DDG AX with the addition of either glycerol or sorbitol. The AX was extracted from these starting materials via alkaline extraction and purified using α-amylase, protease, and dialysis. The proximate composition, molecular weight, and linkages of all extracted AX were determined before analyzing the mechanical properties of the films they were used to make. The mechanical properties analyzed included moisture content, water solubility, tensile strength, tear resistance, puncture resistance, contact angle, and water vapor permeability. The moisture contents of the AX films ranged from 97 g kg^{-1} to 370 g kg^{-1}. The amount of water soluble material in the AX films ranged from 305 g kg^{-1} to 956 g kg^{-1}. The highest maximum tensile strength of the AX films was 29.3 MPa. The same set of AX films had tear resistances ranging from 0.32 N to 1.9 N and puncture resistances of 0.7 N to 10.1 N. The contact angles of the films varied with composition and the type of fluid used for contact angle determination. Water vapor permeability was highest for the MB AX films plasticized with 500 g kg^{-1} glycerol. Overall, this experiment utilized AX extracted from WB, MB, and DDG as the basis of films. The mechanical characteristics of all films were subsequently characterized to provide a comprehensive material profile for each type of AX film, which demonstrated unique materials properties for all films.

3.2. Introduction

Cereals including wheat and maize are two of the most commonly produced cereals in the world (Heikkinen et al. 2013; Linquist et al. 2012). Both of these crops produce a fruit called a caryopsis or kernel (Delcour and Hoseney 2010b) that is made up of starch (600 to 700 g kg^{-1}), protein (100 to 150 g kg^{-1}), and non-starch polysaccharides (30 to 80 g kg^{-1}) (Saulnier et al. 2007). Three of the major byproducts of processing wheat and maize include WB, MB, and DDG

Wheat bran, the pericarp and seed coat of the kernel, is undesirable for refined flours, so it is often sold as animal feed when it is not used in whole wheat flour (Apprich et al. 2014; Swennen et al. 2006). This portion of the wheat kernel is about 140 to 190 g kg^{-1} of the total kernel by mass (Maes and

Delcour 2002). The composition of WB is 460 g kg^{-1} non-starch polysaccharides, 150 to 220 g kg^{-1} protein, 100 to 200 g kg^{-1} starch, and 40 to 80 g kg^{-1} lignin (Zhang et al. 2011; Maes and Delcour 2002). Out of all the non-starch polysaccharides, about 700 g kg^{-1} are AX, 240 g kg^{-1}cellulose, and 60 g kg^{-1} β-glucan (Maes and Delcour 2002).

A maize kernel has a mass around 350 mg, and the bran is about 50 to 60 g kg^{-1} of the total mass of the kernel (Delcour and Hoseney 2010b). About $330 \cdot 10^6$ tons of maize are produced and processed annually in the U.S. (Agger et al. 2010). The result of this maize processing is large quantities of MB that are used mainly for feed due to the desirable composition of the MB for this purpose. MB is 200 g kg^{-1} cellulose, 100 to 130 g kg^{-1} protein, 90 to 230 g kg^{-1} starch, 40 g kg^{-1} phenolic acids, 20 to 30 g kg^{-1} lipid, and 20 g kg^{-1} ash (Carvajal-Millan et al. 2007). The remaining 500 g kg^{-1} of the total mass of the maize kernel is heteroxylans, the majority being AX (Agger et al. 2010; Carvajal-Millan et al. 2007).

DDG is another byproduct of the maize industry produced by the fermentation and distillation processes that occur when maize ethanol is produced (Zarrinbakhsh et al. 2013). The main operations that take place to produce DDG from Maize include grinding, treatment with α-amylase and amyloglucosidase, fermentation, distillation, centrifugation, and drying (U.S. Grains Council 2012). Due to the highly processed nature of DDG, its composition is highly variable, but typically contains highest levels of protein (around 30 g kg^{-1}), hemicellulose (around 20 g kg^{-1}), and cellulose (around 15 g kg^{-1}) (Xiang et al. 2014). Out of all the hemicellulose present in DDG, about half is AX (Zarrinbakhsh et al. 2013).

Arabinoxylan is a hemicellulose present in the cell walls of cereals including wheat and maize (Zhang et al. 2014; Saeed et al. 2011; BeMiller 2007a). Arabinoxylan is composed of a linear backbone of xylans connected to one another by β-1,4-glycosidic bonds with arabinose substituents (Reis et al. 2015; Aguedo et al. 2014; Kiszonas et al. 2013). In addition to xylose and arabinose, glucose is also often present in AX (Zhang et al. 2014; BeMiller 2007b). Ferulic acid is a substituent commonly present in AX that facilitates cross-linking (Kiszonas et al. 2013; Anson et al. 2012; Saeed et al. 2011). When ferulic acid cross-links with AX, it does so at the O-3 and/or O-2 positions on the xylose backbone (Kiszonas et al. 2013; Saeed et al. 2011; Delcour and Hoseney 2010a). This formation of cross-linkages impacts the mechanical properties of materials made from AX.

In addition to feed, WB, MB, and DDG can be used as the basis for food packaging materials. One way this is done is through the extraction and utilization of the AX present in these three materials. Development of the food packaging material begins with the manufacture of films to determine the mechanical properties of the materials. AX films can be quite brittle, so plasticizers must be added to increase flexibility and strength (Vieira et al. 2011; Bergo and Sobral 2007). Plasticizers commonly used in these types of films include glycerol and sorbitol due to their low molecular weight and the desirable properties they impart on the films (Antoniou et al. 2014). In addition, these two polyols are generally recognized as safe, which would make the films and food packaging materials edible (depending upon processing techniques utilized).

Mechanical characterization of AX films created from WB, MB, or DDG for use in the food packaging industry must be properly analyzed to determine the feasibility of use in industry. Food packaging must be suitable for the food being packaged in terms of barrier and mechanical properties. Some of these properties revolve around the material's interaction with water and include moisture content, water solubility, contact angle and water vapor permeability. While other properties are the mechanical strengths of the material including puncture resistance, tensile strength, modulus of elasticity, and tear resistance. All of these characteristics of the AX films demonstrate their usability as a food packaging material.

There were three main objectives guiding this research. The first objective was to extract and purify AX from three sources including WB, MB, and DDG and use it as the basis for films. The second objective was to determine the mechanical characteristics of these films. The third objective was to determine the relationships between and statistical significance of the mechanical properties of all films.

3.3. Materials and Methods

3.3.1. Procurement and Milling

All raw starting materials (WB, MB, and DDG) were provided by commercial producers. The materials provided are the same materials as those mentioned in chapter two page 47.The North Dakota State Mill (Grand Forks, ND) provided the WB. Agricor, Incorporated (Marion, Indiana) provided the MB. Tharaldson Ethanol (Casselton, ND) provided the DDG. The MB was milled at Agricor, Incorporated, but the WB and DDG were milled after they were received. Milling was performed on a Falling Number

Hammer Mill (Type KT-12; Number 10071) that was accompanied by a Perten Instrument Mill Feeder 3170 (Perten Instruments North America, Incorporated). The particle size of all starting materials was determined according to the American Association of Cereal Chemists International Method 55-60.01, which is the standard for determination of particle size range (AACC International 2011).

3.3.2. Extraction and Purification of Arabinoxylan

The extraction and purification of the AX from the starting materials is the same as that described in chapter two beginning on page 48. This method is described concisely here for reference. To begin the extraction, defatting with hexane was done. The ratio of starting material to hexane was 1:3 (w v^{-1}). Arabinoxylan was extracted from WB, MB, and DDG using a 30 g kg^{-1} sodium hydroxide solution. This is a method adapted from a combination of AX extraction and purification methods (Xiang et al. 2014; Swennen et al. 2006). This was done at 50 °C with stirring for three hours. The AX was added to the sodium hydroxide to make a 1:2 (w v^{-1}) AX to sodium hydroxide solution. After stirring for three hours, the solution was centrifuged for 20 minutes at 3,000 rpm. Next, the supernatant was collected and neutralized to pH=7 using concentrated hydrochloric acid. After that, the AX solution was destarched using α-amylase from *Bacillus lichenformis* (Sigma-Aldrich Incorporated; Saint Louis, MO; Termamyl 120; 1186 Units mg^{-1} protein; 19.8 mg protein mL^{-1}; A-3403-1MU). Next, 0.25 mL of α-amylase was added to one liter of AX solution, and was heated for three hours at 90 °C. After destarching, the AX was further acidified to pH=6 using concentrated hydrochloric acid. Next, the solution was deproteined using protease from *Bacillus amyloliquefaciens* (Sigma-Aldrich Incorporated; Saint Louis, MO; P1236-50 mL; Lot No. SLBG5002V, PC Code:1001778189, ≥0.8 units g^{-1}). This solution was stirred for three hours at 50 °C. To inactivate all enzymes, the solution was heated to 100 °C for 10 minutes. The AX was then fractionated using 950 g kg^{-1} ethanol in a 2:1 (v v^{-1}) ratio of ethanol to AX solution for one hour at 23 °C. After stirring for one hour, the solutions were filtered using a Büchner funnel and Whatman No. 4 filter paper under vacuum. The solids were washed with 950 g kg^{-1} ethanol after filtering and allowed to dry at 23 °C overnight in the fume hood.

Following ethanol fractionation, dialysis was performed to remove species with molecular weights below 12 kDa. To do this, each type of AX was individually homogenized along with at least two liters of distilled water using a Polytron Homogenizer with a Polytron 93, PTA 205 tip and a PCU 13 power control unit. The dialysis membranes used were Spectra Por 2 Dialysis Membranes from Spectrum Laboratories Incorporated (12 to 14 kDa; Lot No. 9200228). Dialysis was performed at 23 °C in 10 gallon buckets of distilled water for 72 hours. The water was changed at least twice daily to maintain a favorable state of osmotic pressure. Sodium azide was used to keep the growth of microorganisms to a minimum (0.5 g per 10 gallons of distilled water). After 72 hours of dialysis, all samples were freeze-dried in a VirTis Freeze Dryer (Model No. 10-MRSM; Serial No. SM-1028).

3.3.3. Chemical Characterizations of Starting Materials and Arabinoxylan

These methods are the same as those used in chapter two beginning on page 50, but described briefly here for reference. The proximate compositions of all starting materials and AX fractions and particle sizes of all starting materials were determined using official methods as seen in Table 3.1. The analyses performed included: particle size range (AACC International 2011), moisture content (AACC International 1999a), total ash (AACC International 1999c), total N (AACC International 1999d), total starch (AACC International 1999b) and total lipid (milled materials only) (AOCS 2009).

Table 3.1. Standard methods for analysis of particle size range and proximate composition.

Parameter	Method	Method Number
Particle Size	Determination of Particle Size Distribution	AACCI[a] Method 55-60.01
Moisture	Moisture-Air Oven Method	AACCI[a] Method 44-15.02
Total Ash	Basic Ash Method	AACCI[a] Method 08-01.01
Total Protein	Crude Protein-Combustion (Leco)	AACCI[a] Method 46-30.01
Total Starch	Total Starch Assay Procedure (Megazyme)	AACCI[a] Method 76-13.01
Total Lipid	Oil Content	AOCS[b] Method Ba 3-38

[a] American Association of Cereal Chemists International, [b] American Oil Chemists' Society

Alditol acetates analysis was used to determine the sugar composition, total AX content, and arabinose to xylose ratio (A:X) in the samples (Blankeney et al. 1983). After sample preparation, the analysis of the sugar composition was done using gas chromatography with a flame ionization detector (GC-FID). The gas chromatograph used was a Hewlett Packard 5890 Series II GC system with a flame ionization detector (Agilent Technologies, Incorporated Santa Clara, CA). The column used was a SupelcoSP-2380 fused silica capillary column (30 m x 0.25 mm x 2 µm) (Supelco Bellefonte, PA). The parameters under which testing was completed were as follows: 827372 Pa flow pressure, 100 °C oven temperature, 0.8 mL min^{-1} flow rate, detector temperature of 250 °C, and injector temperature of 230 °C. Helium was used as the carrier gas.

High performance size exclusion chromatography with a multi-angle light scattering detector and a refractive index detector (HPSEC-MALS-RI) were used to determine the weight average molecular weight (Mw) and polydispersity index (PI) of all AX species, film solutions at each step of the process, and films. The method developed by Mendis and Simsek (2015) was used for these analyses. Samples were prepared by dissolving 4 mg in deionized water by heating at 40 °C with continuous stirring for 60 minutes. All samples were then filtered through 5 µm filter paper and analyzed using HPSEC-MALS-RI. The high performance liquid chromatograph (HPLC) used was an Agilent 1200 with a Wyatt Dawn Helios-II multi- angle light scattering detector (MALS) and a refractive index detector (RI). There were two columns used including a Shodex OHpak guard column and a SB 806-HQ column. Pullulan with a Mw of 300 kDa was used a standard for normalizing the MALS detector. Throughout this process, the mobile phase used was water with a flow rate of 0.5 mL min^{-1}. All calculations were performed using Astra 6.0.5 software and a 3rd order Debye plot with second-order polynomial fit (Wyatt Technology 2016). The proportional change in the RI with changes in polymer concentration for AX were assumed to be 0.146 as previously published in research by Dervilly et al. (2000).

To determine the types of linkages present in each type of AX extract, nuclear magnetic resonance spectroscopy (NMR) was used. This was done following the method of Mendis and Simsek (2015). The samples were dissolved in 600 µL of deuterium oxide while heating at 40 °C under continuous stirring by a Reacti-therm III (Thermo Scientific, USA). The samples were then freeze-dried on a Labconco Freeze Zone 4.5 Freeze Dryer (Labconco Corporation Kansas City, MO; Cat No. 7751070; Serial No. 070975444 Rev. S.). This cycle of dissolving and freeze drying was repeated twice more before one final dissolution in 650 µL deuterium oxide before and analysis at 80 °C on a 400 MHz spectrometer (Bruker AV3 HD 400 MHZ NMR that had a 5 mm PABBO BB/19F-!H/D Z-GRD Z probe). All data obtained was analyzed with TopSpin 3.2 software (Bruker BioSpin Corporation 2015).

3.3.4. Film Casting and Drying

Film solutions were made by first creating a 26.7 g kg^{-1} solution of AX in deionized water. The AX solutions were stirred for 24 hours on a Lab Line Multi Magnestir (Model No. 1278). After stirring for 24 hours, the solutions were divided into the appropriate number of test tubes for the number of films being made and heated for 15 minutes at 90 °C in a Thermo Scientific Reacti-Therm III (Model No. TS-18823). After 15 minutes, either sorbitol or glycerol was added to each test tube at 100, 250, or 500 g kg^{-1} of the AX solution. The sorbitol was purchased from Sigma-Aldrich Incorporated (Saint Louis, MO) and was BioUltra Grade (≥ 995 g kg^{-1}). The glycerol was also purchased from Sigma-Aldrich Incorporated (Saint Louis, MO) and was ACS Reagent Grade (≥ 995 g kg^{-1}). The test tubes were then vortexed on a Vortex Mixer (Serial No. 061220025). Next, the test tubes were heated for 10 minutes at 90 °C. The film solutions were then cast onto polystyrene petri dishes (VWR, 100 X 15mm, Cat No. 25384-094). After casting, the films were dried at 60 °C for eight hours. The films were then allowed to finish drying overnight at 23 °C on the bench top. Once dried, all films were stored at 49 % relative humidity (RH) in a Dry Keeper (Sanplatec Corporation, Catalog No. H42056-0001). The RH was maintained using Boveda 49 % RH packs (Item No. B49-60-48).

3.3.5. Mechanical Characterizations of Arabinoxylan Films

Before determination of the mechanical strengths of all films, they were conditioned for at least 48 hours at 23 °C and 50 % RH. These are the conditions set forth by the American Society for Testing and Materials (ASTM) in all the standard methods used in this research for analyses of films. Proper conditioning ensured that all films and their properties could be properly analyzed for significance.

3.3.5.1. Moisture Content

The moisture content of each film was determined in triplicate following the method of Garcia et al. (2004).To begin, the initial masses of three films from each treatment were determined and recorded. Next, all films were placed into a 110 °C oven for 24 hours. After 24 hours, the final mass of each film was determined. The moisture content of each film was determined using Equation 3.1.

$$Film\ Moisture\ Content = \left(\frac{Inital\ Mass - Final\ Mass}{Initial\ Mass}\right) * 100\ g\ kg^{-1} \qquad (3.1)$$

3.3.5.2. Water Solubility

The percentage of water soluble material in each film was determined in duplicate following a modified method of Garcia et al. (2004).To begin, each film was carefully cut into a 2 cm x 3 cm piece and the initial mass was recorded. Next, each film was placed into 80 mL of distilled water in a capped glass jar. The jars were then placed onto a Lab-Linee Orbit Shaker (Model No. 3520) and shaken at 75 rpm for one hour. After one hour, each sample was filtered through an Endecotts Limited (London, England) Steel Mesh No. 325 (Aperature: 45 microns). The solid material remaining was dried at 100 °C for 10 hours and the final mass of the material was determined. The percentage of water soluble material was determined using Equation 3.2.

$$Percent\ Water\ Soluble\ Material = \left(\frac{Inital\ Mass - Final\ Mass}{Initial\ Mass}\right) * 100g\ kg^{-1} \tag{3.2}$$

3.3.5.3. Puncture Resistance

The puncture resistance of each film was determined using a modified version of ASTM D7192-10 (ASTM International 2010). The test conditions were 23 ± 2 °C and 50 ± 10 % RH. Testing was done in quadruplicate using a Texture Technologies Group TA-XT2i Texture Analyzer (Scarsdale, NY) and a 2 mm diameter stainless probe with a flat head. To hold the samples, a Texture Analyzer tortilla extensibility platform was used in conjunction with two polycarbonate disks with an outer diameter of 120 mm and an inner diameter of 15 mm. The speed of testing was 33 mm sec^{-1}. The peak load, deflection, energy required to reach the peak load, and total energy absorbed were determined using Texture Exponent 32 software (Texture Technologies Corporation 2016). The thickness of all samples was determined with a Mitutoyo 2416F thickness gage in five places on each film and recorded to the nearest 0.001 inch.

3.3.5.4. Tensile Strength

The peak tensile strength, breaking factor, percent elongation, and modulus of elasticity were determined for all treatments five times. The method followed was ASTM D882-12 (ASTM International 2002). An Instron 2710-004 tensile test machine with a load cell of 100 N and Instron Bluehill 2.1 software were used for these analyses (Instron 2004). The testing conditions were 23 °C, a strain rate of 51 mm min^{-1}, and an initial grip separation of 25.4 mm. The thickness of all films was determined using a Mitutoyo 2416F gage in five places and recorded to the nearest 0.001 inch.

3.3.5.5. Tear Resistance

The tear resistance of all treatments was determined according to ASTM D1004-13 (ASTM International 2013b). This testing was done five times for each type of film using an Instron 5545 Tensile Tester with a 100 N Load Cell. The thicknesses of all films were determined in five places on each film using a Mitutoyo 2416F thickness gage and recorded to the nearest 0.001 inch. The testing conditions for all tear resistance tests were 23 °C, an initial grip separation of 25.4 mm, and a 51 mm min^{-1} rate of grip separation. The average tear resistance and maximum extension for all samples was determined using Instron Bluehill 2.1 software (Instron 2004).

3.3.5.6. Contact Angle

The contact angle of all treatments was determined by following ASTM D7334-08(2013) in at least duplicate for both distilled water and mineral oil for both sides of the films (ASTM International 2013a). The mineral oil used was from Sigma-Aldrich Incorporated (Saint Louis, MO; 330779-1L; CAS No. 042-47-5; EC No. 232-455-8) and has 10-20 ppm Vitamin E added as a stabilizer. A Dynamic Contact Angle Analyzer by First Ten Angstroms 125 (Serial No. 980806) with a CCD Camera was used. To determine the contact angles and wetting tensions of all treatments, First Ten Angstroms 32 Video 2.0 software was used (First Ten Angstroms 2000).

3.3.5.7. Water Vapor Permeability

The water vapor transmission rate (WVTR) and permeance of each treatment was determined according to ASTM E96/E96M-15 in triplicate (ASTM International 2015). The water method was used, and the RH and temperature were recorded every 30 minutes. The thickness of all films were determined using a Mitutoyo 2416F thickness gage in five places and recorded to the nearest 0.001 inch. During testing, the mass of each sample was determined using a Mettler Toledo New Classic MF analytical balance (Model No. ML203E/03). The test assembly was as follows: a 100 mm polystyrene petri dish with 20 mL distilled water (water was 13 mm from specimen), two steel washers with inner diameters of 2 1/8 inch and outer diameters of 4 ½ inch with plain finishes (Grainger Item No. 22UE14; Model No. U38402.200.0001) used to hold the specimen flat, and parafilm wax was used to secure the specimen and seal the entire apparatus. Equations 3.3 and 3.4 were used to determine the WVTR and water vapor permeance respectively. In Equation 3.3, the variables are as follows: G = mass change (in grams), t = time in hours, $G\ t^{-1}$ = slope of straight line in grams per hour, A = test area in square meters. In Equation 3.4, the variables are as follows: WVT = water vapor transmission rate, Δp = vapor pressure difference in mm Hg, S = saturation vapor pressure at test temperature in mm Hg, R_1 = RH at the source, R_2 = RH at the vapor sink.

$$Water\ Vapor\ Transmission\ Rate = \frac{G}{tA} \tag{3.3}$$

$$Permeance = \frac{WVT}{\Delta p} = \frac{WVT}{S(R_1 - R_2)} \tag{3.4}$$

3.3.6. Statistical Analysis

This experiment utilized completely random design with a factorial arrangement. The AX source was the first factor, the type of plasticizer was the second factor, and the plasticizer level was the third factor in the arrangement. The analysis of all data was completed using Statistical Analysis Software version 9.3 (SAS Institute Inc. 2011). This software was used to produce the analysis of variance for each data set. The data was then further analyzed using Fischer’s protected least significant difference and correlations between the chemical characteristics of the films and their mechanical properties.

3.4. Results and Discussion

3.4.1. Starting Materials and Extracted Arabinoxylan

3.4.1.1. Particle Size Range and Composition

The particle size range for all starting materials (milled WB, MB, and DDG) is provided in Table 3.2. The particle size range of all three starting materials was 177 to 250 microns, as seen by the materials remaining on top of screen 60 and screen 80. These particle sizes are overall smaller than the commercial average for both WB and MB. The average particle size for WB is 278 to 609 microns (Zhang and Moore 1999), and the majority of commercially produced MB is larger than 250 microns (Agger and Meyer 2012).

Table 3.2. Particle size range for wheat bran, maize bran, and dried distillers grain.

Material	Screen 60	Screen 80
	(g kg^{-1})	
WB[a]	459	469
MB[b]	971	16
DDG[c]	396	443

[a] Wheat bran
[b] Maize bran
[c] Dreid distillers grain

The proximate compositions of the starting materials are shown in Table 3.3. The analyses completed included moisture content, ash content, lipid content, total starch, sugar profile, and A:X. The compositions of the starting materials were very close to compositions previously established. Firstly, WB usually is about 20 g kg^{-1} ash (Carvajal-Millan et al. 2007), 150 to 220 g kg^{-1} protein (Maes and Delcour 2002), 100 to 200 g kg^{-1} starch (Zhang et al. 2011), 300 g kg^{-1} AX with an A:X of 0.57-1.07 (Zhang et al. 2014; Zhang et al. 2011). The WB utilized in this research had higher ash and AX, but other that all components fell within the typical ranges. Secondly, MB is about 20 g kg^{-1} ash, 100 to 130 g kg^{-1} protein, and 90 to 230 g kg^{-1} starch (Carvajal-Millan et al. 2007). The AX content of MB is around 300 to 400 g kg^{-1} (Saeed et al. 2011; Zhang and Whistler 2004), and the A:X is 0.40 to 0.66 (Agger et al. 2010). The MB used in this research was very similar aside from being comparatively low in protein (estimated by N content), but high in AX. Thirdly, DDG is usually 300 g kg^{-1} protein, 50 g kg^{-1} starch, 100 g kg^{-1} lipid, and 90 g kg^{-1} AX (Xiang et al. 2014; Zarrinbakhsh et al. 2013). The DDG used in this research similar aside from being was relatively low in starch and lipid, but high in AX.

After extraction and purification of the AX, the materials obtained were higher in AX content than AX alkaline extracted by other research groups. This was due to a novel combination of extraction and purification methods utilized in this research as described in chapter two page of this manuscript. The AX extracted from the WB had a purity of 730 g kg^{-1}, which is much higher than the purity of the WB AX extracted by Maes and Delcour (2002). In addition, the purity of the MB AX was 850 g kg^{-1} and the DDG AX had a purity of 580 g kg^{-1}. Also, the ash contents of the extracted AX were lower than previously published work on extracting AX including the work of Xiang et al. (2014), which cites DDG AX with an ash content of 54 g kg^{-1}. The N contents of the AX alkaline extracted in this work were also lower than previously published works including WB AX with a N content of 190 g kg^{-1} (Maes and Delcour 2002), MB AX with a N content of 70 g kg^{-1} (Kale et al. 2010), and DDG AX with a N content of 170 g kg^{-1} (Reis et al. 2015). These differences in purity were due to the effectiveness of the purification techniques used.

Table 3.3. Proximate composition (dry weight basis) of wheat bran, maize bran, dried distillers grain, wheat bran arabinoxylan, maize bran arabinoxylan, and dried distillers grain arabinoxylan.

	Proximate composition (g kg^{-1})								
Material	Moisture	Ash	N	Lipid	Starch	Arabinoxylan	Arabinose	Xylose	A:X[e]
Milled materials									
WB[a]	84.5	67.9	179.4	40.7	126.2	409.2	190.8	274.2	0.70
MB[b]	96.1	7.6	49.1	24.3	128.5	683.7	263.2	513.7	0.51
DDG[c]	93.3	68.4	286.8	85.6	39.8	179.2	84.7	118.9	0.71
LSD (*P*≤0.05)[d]	-	0.1	7.4	9.8	3.3	14.1	6.5	9.5	0.01
LSD (*P*≤0.01)	-	0.3	13.5	18.0	4.8	25.8	11.8	17.5	0.02
Extracted arabinoxylan									
WB AX[a]	126.2	85.5	136.2	-	15.0	729.4	280.0	548.9	0.51
MB AX[b]	111.1	12.2	38.9	-	3.3	847.1	325.1	637.5	0.51
DDG AX[c]	63.1	18.9	149.5	-	3.1	580.5	223.5	436.2	0.51
LSD (*P*≤0.05)[d]	13.9	0.6	13.1	-	1.1	29.7	10.2	23.7	-
LSD (*P*≤0.01)	25.6	1.1	24.0	-	1.5	54.5	18.8	43.6	-

[a] Wheat bran
[b] Maize bran
[c] Dried distillers grain
[d] Least significant difference
[e] Arabinose to xylose ratio

3.4.1.2. Chemical Characterization

Both the Mw and PI were determined using HPSEC-MALS-RI for the film solutions and films every step of the process (heating, addition of plasticizer, and after drying). Table 3.4 provides the Mw and PI for the solutions and films for all materials (WB, MB, and DDG). When the information is further broken down by treatment (heating, solution with plasticizer, film type, etc.) as seen in Table 3.5, there is a clear trend. The decrease in Mw as the solutions are heated indicates that a hydrolysis reaction took place during this portion of the film solution processing. When the film solutions were dried, there was a significant (*P*≤0.01) increase in the Mw of the film materials. This trend is indicative of an increase in the number of intermolecular interactions between AX polymers. One such interaction could be formation of cross-linkages (Berlanga-Reyes et al. 2011). Hydrogen bonding between the plasticizer and AX polymer could also be taking place. The PI decreased when the film solutions were dried. This is indicative of a decrease in the variation of the Mw for AX present in the films after curing.

Table 3.4. Molecular weight and polydispersity index for film solutions throughout the film solution development process and films made from one type of plasticizer in varying amounts (glycerol or sorbitol; 100, 250, or 500 g kg^{-1}) and one type of arabinoxylan (wheat bran arabinoxylan, maize bran arabinoxylan, or dried distillers grain arabinoxylan).

Material	Material type	Treatment	Molecular weight (Da)	Polydispersity index
WB[a]	Solutions	24 hours stirring	7116667	1.59
WB	Solutions	90 °C	6053333	1.55
WB	Solutions	100 g kg^{-1} Glycerol	6966667	1.66
WB	Solutions	250 g kg^{-1} Glycerol	7150000	1.74
WB	Solutions	500 g kg^{-1} Glycerol	7960000	2.02
WB	Solutions	100 g kg^{-1} Sorbitol	6410000	1.44
WB	Solutions	250 g kg^{-1} Sorbitol	6906667	1.33
WB	Solutions	500 g kg^{-1} Sorbitol	8270000	1.51
WB	Films	100 g kg^{-1} Glycerol	14533333	1.71
WB	Films	250 g kg^{-1} Glycerol	16300000	1.86
WB	Films	500 g kg^{-1} Glycerol	19833333	2.63
WB	Films	100 g kg^{-1} Sorbitol	12833333	1.33
WB	Films	250 g kg^{-1} Sorbitol	14500000	1.29
WB	Films	500 g kg^{-1} Sorbitol	19166667	1.50
MB[b]	Solutions	24 hours stirring	7703333	1.27
MB	Solutions	90 °C	4440000	1.20
MB	Solutions	100 g kg^{-1} Glycerol	1713333	1.55
MB	Solutions	250 g kg^{-1} Glycerol	1690000	1.63
MB	Solutions	500 g kg^{-1} Glycerol	1600000	1.36
MB	Solutions	100 g kg^{-1} Sorbitol	3066667	1.11
MB	Solutions	250 g kg^{-1} Sorbitol	2553333	1.14
MB	Solutions	500 g kg^{-1} Sorbitol	1660000	1.51
MB	Films	100 g kg^{-1} Glycerol	9620000	1.39
MB	Films	250 g kg^{-1} Glycerol	11433333	1.65
MB	Films	500 g kg^{-1} Glycerol	12966667	1.93
MB	Films	100 g kg^{-1} Sorbitol	9366667	1.48
MB	Films	250 g kg^{-1} Sorbitol	10600000	1.46
MB	Films	500 g kg^{-1} Sorbitol	14200000	1.55
DDG[c]	Solutions	24 hours stirring	5900000	1.04
DDG	Solutions	90 °C	4463333	1.80
DDG	Solutions	100 g kg^{-1} Glycerol	5176667	1.57
DDG	Solutions	250 g kg^{-1} Glycerol	5593333	1.93

Table 3.4. Molecular weight and polydispersity index for film solutions throughout the film solution development process and films made from one type of plasticizer in varying amounts (glycerol or sorbitol; 100, 250, or 500 g kg^{-1}) and one type of arabinoxylan (wheat bran arabinoxylan, maize bran arabinoxylan, or dried distillers grain arabinoxylan) (continued).

Material	Material type	Treatment	Molecular weight (Da)	Polydispersity index
DDG	Solutions	500 g kg^{-1} Glycerol	7070000	1.96
DDG	Solutions	100 g kg^{-1} Sorbitol	5040000	2.11
DDG	Solutions	250 g kg^{-1} Sorbitol	5950000	1.88
DDG	Solutions	500 g kg^{-1} Sorbitol	7170000	1.75
DDG	Films	100 g kg^{-1} Glycerol	9433333	1.36
DDG	Films	250 g kg^{-1} Glycerol	10466667	1.65
DDG	Films	500 g kg^{-1} Glycerol	12400000	2.10
DDG	Films	100 g kg^{-1} Sorbitol	7540000	1.43
DDG	Films	250 g kg^{-1} Sorbitol	8850000	1.40
DDG	Films	500 g kg^{-1} Sorbitol	13300000	1.44
LSD ($P \leq 0.05$)[d]			59535	0.23
LSD ($P \leq 0.01$)			78906	0.31

[a] Wheat bran
[b] Maize bran
[c] Dried distillers grain
[d] Least significant difference

Table 3.5. Molecular weight and polydispersity index for film solutions throughout the film solution development process and films made from one type of plasticizer in varying amounts (glycerol or sorbitol; 100, 250, or 500 g kg^{-1}) and one type of arabinoxylan (wheat bran arabinoxylan, maize bran arabinoxylan, or dried distillers grain arabinoxylan). These values have been averaged by treatment type.

Material type	Treatment	Molecular weight (Da)	Polydispersity index
Solutions	24 hr stirring	6906667	1.30
Solutions	90° C	4985556	1.52
Solutions	100 g kg^{-1} Glycerol	4618889	1.59
Solutions	250 g kg^{-1} Glycerol	4811111	1.77
Solutions	500 g kg^{-1} Glycerol	5543333	1.78
Solutions	100 g kg^{-1} Sorbitol	4838889	1.55
Solutions	250 g kg^{-1} Sorbitol	5136667	1.45
Solutions	500 g kg^{-1} Sorbitol	5700000	1.59
Films	100 g kg^{-1} Glycerol	11195556	1.48
Films	250 g kg^{-1} Glycerol	12733333	1.72
Films	500 g kg^{-1} Glycerol	15066667	2.22
Films	100 g kg^{-1} Sorbitol	9913333	1.41
Films	250 g kg^{-1} Sorbitol	11316667	1.38
Films	500 g kg^{-1} Sorbitol	15555556	1.49
LSD ($P \leq 0.05$)[a]		34373	0.13
LSD ($P \leq 0.01$)		45557	0.18

[a] Least significant difference

Table 3.6 provides the resonance peak locations as well as the relative integral for each peak in each type of AX extract. All abundances were determined relative to the amount of unsubstituted xylose present in the sample (seen by the unsubstituted xylose having a relative integral of 1.00). In WB AX, the three most abundant anomeric protons included the anomeric proton of the unsubstituted xylose, the anomeric proton of arabinose that is C-(O)-3 linked to disubstituted xylose, the anomeric proton of arabinose C-(O)-2 linked to disubstituted xylose that has an adjoining disubstituted xylose. Whereas, in MB AX, the three most abundant types of anomeric protons included the anomeric proton of monosubstituted xylose, unsubstituted xylose, and the anomeric proton of arabinose that is C-(O)-3 linked to disubstituted xylose. Lastly, the three types of anomeric protons that are the most abundant included the anomeric proton of disubstituted xylose, the anomeric proton of the monosubstituted xylose, and the anomeric proton of arabinose that is C-(O)-2 linked to disubstituted xylose that has an adjoining disubstituted xylose. These differences in linkages influenced the mechanical properties of the films made from these three types of AX.

Table 3.6. ^{1}H nuclear magnetic resonance peak relative abundances (relative to the total abundance of all anomeric protons) for wheat bran, maize bran, and dried distillers grain arabinoxylan.

	Arabinoxylan type		
	WB[a]	MB[b]	DDG[c]
Anomeric proton	Anomeric proton Abundance (g kg^{-1})		
Anomeric Proton of Arabinose C-(O)-3 Linked to Anomeric Xylose	63.4	80.7	69.9
Anomeric Proton of Arabinose C-(O)-3 to Disubstituted Xylose	167.3	162.8	141.7
Anomeric Proton of Arabinose C-(O)-2 to Disubstituted Xylose with Adjoining Disubstituted Xylose	147.1	141.1	166.4
Anomeric Proton of Arabinose C-(O)-2 to Disubstituted Xylose	32.5	21.2	30.9
Anomeric Proton of Disubstituted Xylose	96.4	81.1	268.0
Anomeric Proton of Monosubstituted Xylose	122.3	269.0	202.4
Anomeric Proton of Unsubstituted Xylose	371.0	244.1	129.5

[a] Wheat bran
[b] Maize bran
[c] Dried distillers grain

3.4.2. Mechanical Properties of Arabinoxylan Films

3.4.2.1. Moisture Content

The moisture content of a film can impact the mechanical properties of said film, so the moisture contents of all films were determined and analyzed for significance ($P \leq 0.01$) or ($P \leq 0.05$). The moisture contents of all films are given in Table 3.7. In previously published work, one trend noted is that films made with glycerol have higher moisture contents than films made with sorbitol (Antoniou et al. 2014). This is because glycerol is more hydrophilic than sorbitol. This trend was also seen in the AX films in this research and seen in Table 3.8, where it is shown that the moisture contents of the films made with glycerol were significantly ($P \leq 0.01$) higher than those made with sorbitol. In addition, the moisture content of the films increased significantly ($P \leq 0.01$) as the level of plasticizer increased from 100 to 500 g kg^{-1} When averaged across AX type used in the films, the moisture contents of the MB AX films were significantly ($P \leq 0.01$) lower than that of both the WB AX and DDG AX films. However, the differences between the WB AX films and the MB AX films were not significantly ($P \leq 0.01$) different. The moisture (or water) content of the films demonstrated the presence of water, which was a secondary plasticizer in the films. However, for simplicity in this thesis, the effects of water as a plasticizer have been disregarded. The plasticizing effects of only sorbitol and glycerol will be discussed.

Table 3.7. Moisture contents of arabinoxylan films.

Film composition	Moisture content (g kg^{-1})
WB AX[a] + 100 g kg^{-1} Sorbitol	109.5 ± 18.6
WB AX+ 250 g kg^{-1} Sorbitol	111.3 ± 2.8
WB AX+ 500 g kg^{-1} Sorbitol	115.6 ± 1.8
WB AX+ 100 g kg^{-1} Glycerol	120.8 ± 7.9
WB AX+ 250 g kg^{-1} Glycerol	160.0 ± 5.1
WB AX+ 500 g kg^{-1} Glycerol	289.7 ± 7.0
MB AX[b] + 100 g kg^{-1} Sorbitol	110.4 ± 3.2
MB AX + 250 g kg^{-1} Sorbitol	97.4 ± 0.8
MB AX+ 500 g kg^{-1} Sorbitol	101.6 ± 2.7
MB AX+ 100 g kg^{-1} Glycerol	103.0 ± 6.3
MB AX+ 250 g kg^{-1} Glycerol	193.2 ± 8.8
MB AX+ 500 g kg^{-1} Glycerol	213.3 ± 14.4
DDG AX[c] + 100 g kg^{-1} Sorbitol	104.8 ± 4.9
DDG AX+ 250 g kg^{-1} Sorbitol	101.9 ± 12.8
DDG AX+ 500 g kg^{-1} Sorbitol	106.2 ± 4.5
DDG AX+ 100 g kg^{-1} Glycerol	134.2 ± 7.7
DDG AX+ 250 g kg^{-1} Glycerol	119.2 ± 1.3
DDG AX+ 500 g kg^{-1} Glycerol	371.1 ± 7.4

[a] Wheat bran arabinoxylan
[b] Maize bran arabinoxylan
[c] Dried distillers grain arabinoxylan

Table 3.8. Mean separation by main factor for the moisture content of arabinoxylan films.

Main factor	Moisture content ($g\ kg^{-1}$)
WB[a]	151.2
MB[b]	136.5
DDG[c]	156.2
LSD ($P \leq 0.05$)[d]	5.4
LSD ($P \leq 0.01$)	7.3
Glycerol	189.4
Sorbitol	106.5
LSD ($P \leq 0.05$)	4.4
LSD ($P \leq 0.01$)	5.9
100 $g\ kg^{-1}$ Plasticizer	113.9
250 $g\ kg^{-1}$ Plasticizer	130.5
500 $g\ kg^{-1}$ Plasticizer	199.6
LSD ($P \leq 0.05$)	5.4
LSD ($P \leq 0.01$)	7.2

[a] Wheat bran arabinoxylan
[b] Maize bran arabinoxylan
[c] Dried distillers grain arabinoxylan
[d] Least significant difference

There are four significant ($P \leq 0.01$) interactions between main factors that affect the moisture content of the AX films: AX type by plasticizer type, AX type by plasticizer level, plasticizer type by plasticizer level, and the three-way interaction between all three main factors. Table 3.9 contains the mean separation of all two-way interactions, and Table 3.10 contains the mean separation for the three-way interaction. The most notable interaction is the three way interaction, which is also depicted in Figures 3.1 and 3.2. This three-way interaction is indicative of variation in one or more of the two-way interactions over the levels of the third variable. The interaction of AX type and plasticizer level for the films made with glycerol was a crossover response. Whereas the interaction of AX type and plasticizer level for the films made with sorbitol was a crossover response for the DDG and MB films and a diverging response for the MB and WB films.

Table 3.9. Mean separation for each two-way interaction affecting the moisture content of the arabinoxylan films.

Arabinoxylan type	Plasticizer type	Plasticizer level (g kg^{-1})	Moisture content (g kg^{-1})
WB[a]	Glycerol		190.2
WB	Sorbitol		112.2
MB[b]	Glycerol		169.8
MB	Sorbitol		103.1
DDG[c]	Glycerol		208.2
DDG	Sorbitol		104.3
LSD ($P \leq 0.05$)[d]			7.7
LSD ($P \leq 0.01$)			10.3
WB		100	115.2
WB		250	135.7
WB		500	202.7
MB		100	106.7
MB		250	145.3
MB		500	157.5
DDG		100	119.5
DDG		250	110.6
DDG		500	238.7
LSD ($P \leq 0.05$)			9.4
LSD ($P \leq 0.01$)			12.6
	Glycerol	100	119.3
	Glycerol	250	157.5
	Glycerol	500	291.4
	Sorbitol	100	108.2
	Sorbitol	250	103.5
	Sorbitol	500	107.8
	LSD ($P \leq 0.05$)		7.7
	LSD ($P \leq 0.01$)		10.3

[a] Wheat bran
[b] Maize bran
[c] Dried distillers grain
[d] Least significant difference

Table 3.10. Three-way interaction between arabinoxylan type and plasticizer type and plasticizer level for film moisture content.

Arabinoxylan type	Plasticizer type	Plasticizer level (g kg^{-1})	Moisture content (g kg^{-1})
WB[a]	Glycerol	100	120.8
WB	Glycerol	250	160.0
WB	Glycerol	500	289.7
MB[b]	Glycerol	100	103.0
MB	Glycerol	250	193.2
MB	Glycerol	500	213.3
DDG[c]	Glycerol	100	134.2
DDG	Glycerol	250	119.2
DDG	Glycerol	500	371.1
WB	Sorbitol	100	109.5
WB	Sorbitol	250	111.3
WB	Sorbitol	500	115.6
MB	Sorbitol	100	110.4
MB	Sorbitol	250	97.4
MB	Sorbitol	500	101.6
DDG	Sorbitol	100	104.8
DDG	Sorbitol	250	101.9
DDG	Sorbitol	500	106.2
LSD ($P \leq 0.05$)[d]			13.3
LSD ($P \leq 0.01$)			17.8

[a] Wheat bran
[b] Maize bran
[c] Dried distillers grain
[d] Least significant difference

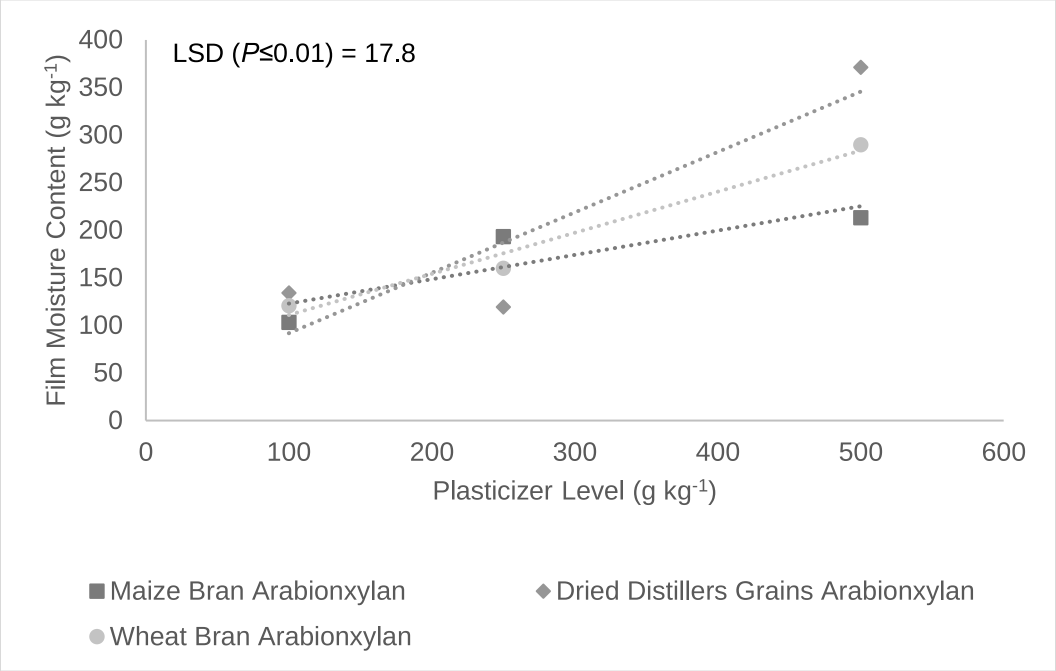

Figure 3.1. Interaction of arabinoxylan type and plasticizer level for all films made with glycerol for the moisture content of arabinoxylan films.

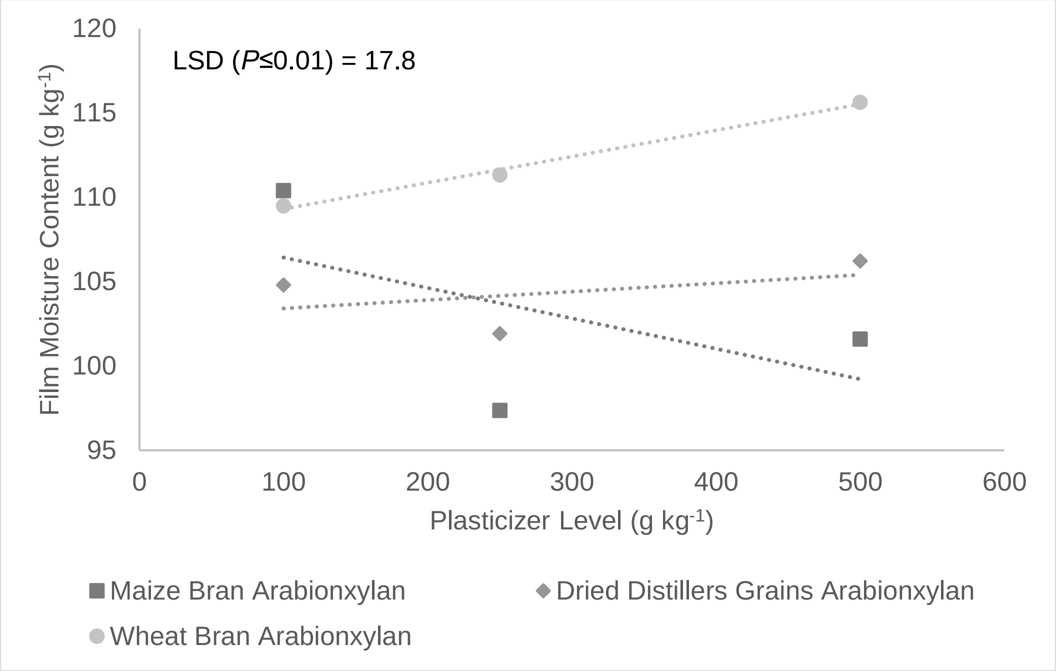

Figure 3.2. Interaction of arabinoxylan type and plasticizer level for all films made with sorbitol for the moisture content of arabinoxylan films.

There were also significant (*P*≤0.05) correlations between the moisture contents of the AX films and some other factors, as seen in Table 3.11. Firstly, there was a significant (*P*≤0.01) correlation between the level of glycerol present and the moisture content. The correlation was positive, which indicated that as the level of glycerol increased, so did the moisture content of the film. This relationship has also been documented by the work of Thomazine et al. (2005). Secondly, there was a significant (*P*≤0.05) correlation between the Mw of the film solutions when heated and the moisture contents of films made with sorbitol. This correlation showed that as the Mw of the heated solutions increased, so did the moisture content of the films the solutions were used to make. Thirdly, there was a significant (*P*≤0.05) positive correlation between the PI of the films and the moisture content of the films made with glycerol. This showed that as the PI of films made with glycerol increased, so did the moisture content.

Table 3.11. Correlations between plasticizer level, molecular weight, and polydispersity indexes and the moisture content of arabinoxylan films for each type of plasticizer.

	Moisture content (g kg^{-1})
	Films made with sorbitol
Plasticizer level	0.025
Polydispersity index for 24 hours of stirring	0.631
Molecular weight for 24 hours of stirring	0.056
Polydispersity index for heated solutions	0.153
Molecular weight for heated solutions	0.737*
Polydispersity index for solutions with plasticizer	-0.132
Molecular weight for solutions with plasticizer	0.658
Polydispersity index for films	-0.233
Molecular weight for films	0.568
	Films made with glycerol
Plasticizer level	0.858**
Polydispersity index for 24 hours of stirring	-0.071
Molecular weight for 24 hours of stirring	-0.179
Polydispersity index for heated solutions	0.184
Molecular weight for heated solutions	0.008
Polydispersity index for solutions with plasticizer	0.485
Molecular weight for solutions with plasticizer	0.324
Polydispersity index for films	0.770*
Molecular weight for films	0.420

* Significant at *P*≤0.05
** Significant at *P*≤0.01

3.4.5.2. Water Solubility

Films made from AX for the potential use in food packaging must not be too water soluble, or they will dissolve when near the foods they are storing. Previous research showed that water solubility is dependent upon multiple characteristics of the AX films including the A:X. As the A:X decreases, the AX structure increases in crystallinity, which results in a lower content of water soluble material in the AX film (Heikkinen et al. 2013). The increase in crystallinity results in less surface area of the AX polymer that water can interact with. The percentage of all water-soluble material that was in each type of AX film analyzed during this research is shown in Table 3.12. The water solubility of these films is dependent upon the water entering the polymer chains and disruption of all intermolecular interactions such as hydrogen bonding and Van der Walls forces (Nazan Turhan and Şahbaz 2004).

The majority of plastics used to manufacture the plastic bags used at supermarkets (and similar locations) are made from low-density polyethylene and polyvinyl chloride (Michelot et al. 2017). These plastics are used because they can prevent the transmission of gases such as oxygen. However, these materials can interact with the materials they store if they are compatible. These materials have very low water solubility. Ulutan and Balkose (1996) determined that the water solubility of polyvinyl chloride is very low, which makes it good for use in bags that will be used many times. Bags are slowly being developed out of biopolymers such as chitosan (Kittur et al. 1998). These materials have a variety of materials properties depending upon composition and processing.

Table 3.12. Water soluble material in films made from alkaline extracted arabinoxylan and a plasticizer (either glycerol or sorbitol) at one of the following levels: 100, 250, or 500 g kg^{-1}.

Film composition	Water soluble material (g kg^{-1})
WB AX[a] + 100 g kg^{-1} Sorbitol	547 ± 4
WB AX+ 250 g kg^{-1} Sorbitol	360 ± 23
WB AX+ 500 g kg^{-1} Sorbitol	440 ± 33
WB AX+ 100 g kg^{-1} Glycerol	380 ± 47
WB AX+ 250 g kg^{-1} Glycerol	305 ± 1
WB AX+ 500 g kg^{-1} Glycerol	342 ± 18
MB AX[b] + 100 g kg^{-1} Sorbitol	879 ± 3
MB AX + 250 g kg^{-1} Sorbitol	863 ± 66
MB AX+ 500 g kg^{-1} Sorbitol	705 ± 67
MB AX+ 100 g kg^{-1} Glycerol	956 ± 30
MB AX+ 250 g kg^{-1} Glycerol	717 ± 14
MB AX+ 500 g kg^{-1} Glycerol	625 ± 55
DDG AX[c] + 100 g kg^{-1} Sorbitol	937 ± 21
DDG AX+ 250 g kg^{-1} Sorbitol	873 ± 11
DDG AX+ 500 g kg^{-1} Sorbitol	893 ± 32
DDG AX+ 100 g kg^{-1} Glycerol	826 ± 8
DDG AX+ 250 g kg^{-1} Glycerol	845 ± 59
DDG AX+ 500 g kg^{-1} Glycerol	732 ± 66

[a] Wheat bran arabinoxylan
[b] Maize bran arabinoxylan
[c] Dried distillers grain arabinoxylan

Table 3.13 provides the percentage of water soluble material in the AX films averaged across the type of AX used in the films. The DDG AX films had the highest amount of water soluble material (851 g kg^{-1}), followed by the MB AX films (791 g kg^{-1}) and lastly the WB AX films (395 g kg^{-1}); these are significantly ($P \leq 0.01$) different from one another. When the water solubility of the films was averaged across the type of plasticizer used, films made with sorbitol were significantly ($P \leq 0.01$) more water soluble than those made with glycerol. This result is the same as that found in films made with varying levels of sorbitol or glycerol by Müller et al. (2008). These differences were most likely due to the increased plasticizing ability of glycerol as compared to sorbitol and the smaller molecular size of glycerol. However, these differences could have also been the result of the initial moisture contents of the films or the Mw of the films after drying. Sorbitol disrupts the AX chains more than glycerol, which increases water solubility.

In addition, there was a decrease in water soluble material present in the films as the plasticizer level increased. This trend was also identified by Nazan Turhan and Şahbaz in the films they created (2004). The decerase in the percentage of water soluble material as plasticizer level increased could be due to the increase in intermolecular interactions which limits the interaction of the AX polymers with water.

Table 3.13. Mean separation for each main factor influencing the water solubility of arabinoxylan films.

Arabinoxylan type	Plasticizer type	Plasticizer level (g kg^{-1})	Water soluble material (g kg^{-1})
WB[a]			395.5
MB[b]			790.6
DDG[c]			851.0
LSD ($P≤0.05$)[d]			33.0
LSD ($P≤0.01$)			45.2
	Glycerol		636.4
	Sorbitol		721.7
	LSD ($P≤0.05$)		26.9
	LSD ($P≤0.01$)		36.9
		100	754.0
		250	660.5
		500	622.7
		LSD ($P≤0.05$)	33.0
		LSD ($P≤0.01$)	45.2

[a] Wheat bran
[b] Maize bran
[c] Dried distillers grain
[d] Least significant difference

There are two significant ($P≤0.05$) interactions that impact the water solubility of the AX films including the two-way material by plasticizer level interaction, and the three-way interaction between AX type, plasticizer type, and plasticizer level. Table 3.14 shows the percentage of water soluble material averaged across the two-way interaction. The significance of this interaction was that the effect of the AX type on the water solubility of the films was dependent upon the level of plasticizer used to make the film. In addition, this two-way interaction demonstrates the presence of increased intermolecular interactions that took place as the level of plasticizer was increased. This two-way interaction is also illustrated in Figure 3.3. The three-way interaction between all factors (AX type, plasticizer type, and plasticizer level) is shown in Table 3.15 and Figures 3.4 and 3.5. The interaction of AX type and plasticizer level for films made with glycerol showed a converging response for MB AX and DDG AX films over the three levels of plasticizers. For this same interaction, a converging response was also seen for MB AX films and WB AX films, but a very similar response (minimal to no interaction) for DDG AX and WB AX films. The interaction of AX type and plasticizer level over all films made with sorbitol showed a diverging response for DDG AX and WB AX films, a diverging response for MB AX films and DDG AX films, and no interaction for DDG AX and WB AX films. These three-way interactions demonstrated that the percentage of water soluble material present in the AX films depends upon all three factors.

Table 3.14. Interaction between arabinoxylan type and plasticizer level that affects the percentage of water soluble material in arabinoxylan films.

Arabinoxylan type	Plasticizer level (g kg^{-1})	Water soluble material (g kg^{-1})
WB[a]	100	463.1
WB	250	332.6
WB	500	390.9
MB[b]	100	917.2
MB	250	790.0
MB	500	664.7
DDG[c]	100	881.6
DDG	250	858.9
DDG	500	812.5
LSD ($P \leq 0.05$)[d]		57.1
LSD ($P \leq 0.01$)		78.3

[a] Wheat bran
[b] Maize bran
[c] Dried distillers grain
[d] Least significant difference

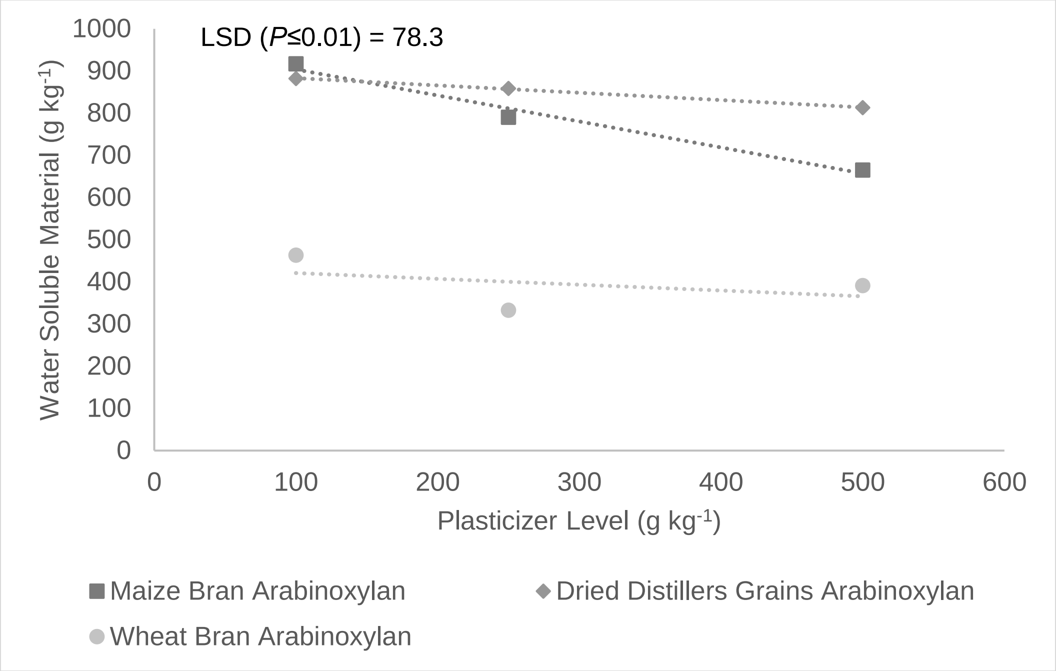

Figure 3.3. Two-way interaction between arabinoxylan type and plasticizer level for percentage of water soluble material in arabinoxylan films.

Table 3.15. Three-way interaction between arabinoxylan type, plasticizer type, and plasticizer level of arabinoxylan films that affects the percentage of water soluble material in the films.

Arabinoxylan type	Plasticizer type	Plasticizer level (g kg^{-1})	Water soluble material (g kg^{-1})
WB[a]	Glycerol	100	379.5
WB	Glycerol	250	305.3
WB	Glycerol	500	341.9
MB[b]	Glycerol	100	955.5
MB	Glycerol	250	717.2
MB	Glycerol	500	625.0
DDG[c]	Glycerol	100	826.1
DDG	Glycerol	250	845.2
DDG	Glycerol	500	732.2
WB	Sorbitol	100	546.7
WB	Sorbitol	250	360.0
WB	Sorbitol	500	439.9
MB	Sorbitol	100	878.9
MB	Sorbitol	250	862.9
MB	Sorbitol	500	704.5
DDG	Sorbitol	100	937.2
DDG	Sorbitol	250	872.6
DDG	Sorbitol	500	892.9
LSD (P≤0.05)[d]			80.8
LSD (P≤0.01)			110.7

[a] Wheat bran

[b] Maize bran

[c] Dried distillers grain

[d] Least significant difference

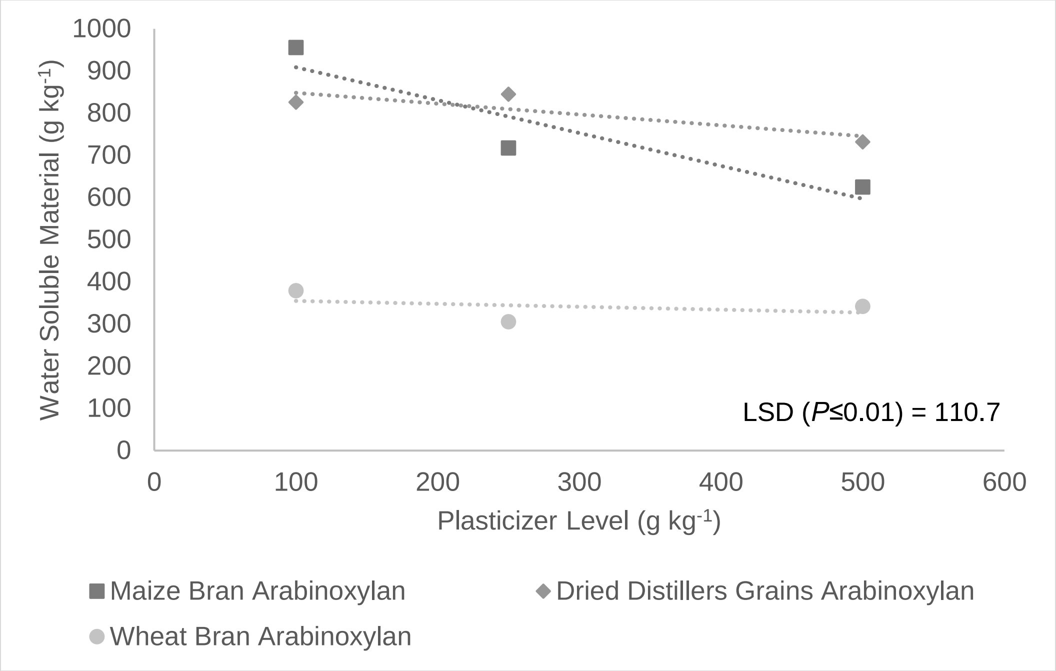

Figure 3.4. Interaction of arabinoxylan type and plasticizer level over the plasticizer glycerol for the percentage of water soluble material in arabinoxylan films.

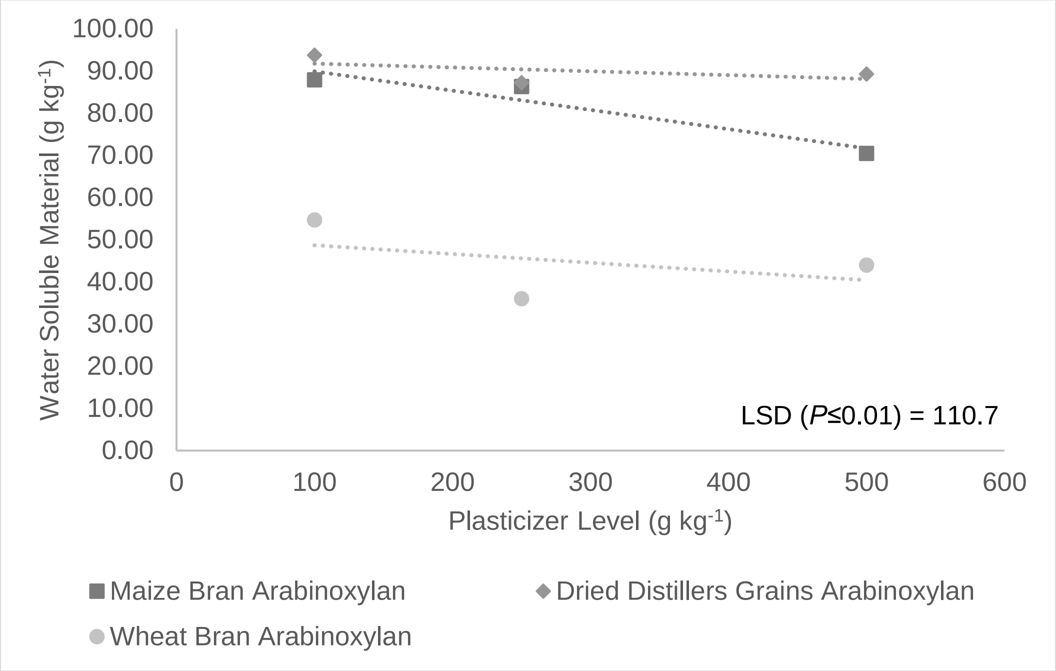

Figure 3.5. Interaction of arabinoxylan type and plasticizer level over the plasticizer sorbitol for the percentage of water soluble material in arabinoxylan films.

There were also significant (*P*≤0.05, *P*≤0.01, and *P*≤0.001) correlations between the Mw and PI of film solutions throughout the film making process and the percentage of water soluble material present in the films as seen in Table 3.16. There were significant (*P*≤0.01 for glycerol films and *P*≤0.001 for sorbitol films) correlations between the PI of the film solution after stirring in water for 24 hours and the percentage of water soluble material in the film. This correlation was negative, which means that as the PI decreased for these solutions, the percentage of water soluble material in the film after drying increased. In addition, there were also significant (*P*≤0.001) correlations between the Mw of the film solution after heating and the percentage of water soluble material in the film. This correlation was also negative, indicating that as the Mw of the film solution decreased, the water solubility of the film increased. The increase in water solubility due to a lower Mw was also noted by Nazan Turhan and Şahbaz (2004). There was one final significant (*P*≤0.05 for films made with sorbitol and *P*≤0.001 for films made with glycerol) negative correlation between Mw and film water solubility. This correlation was between the Mw of the films and their water solubility. This shows that as the Mw of the films increased, their water solubility decreased. In addition, there was a significant (*P*≤0.05) correlation between an increase in the presence of disubstituted xylose and an increase in the amount of water soluble material present in the film. This was due to an increase in the spaces between the polymers that allowed water to enter and dissolve the material. When disubstituted xylose is present, the AX polymers cannot align as closely as if there was no disubstituted xylose.

Table 3.16. Correlations between plasticizer level, molecular weight, and polydispersity indexes and the water soluble material in arabinoxylan films divided by type of plasticizer.

	Water soluble material
	Films made with sorbitol
Polydispersity index for 24 hours of stirring	-0.919***
Molecular weight for 24 hours of stirring	-0.350
Polydispersity index for heated solutions	0.087
Molecular weight for heated solutions	-0.931***
Polydispersity index for solutions with plasticizer	0.323
Molecular weight for solutions with plasticizer	-0.477
Polydispersity index for films	0.394
Molecular weight for films	-0.784*
R1	0.483
R2	0.627
R3	0.758*
R4	0.623
R5	0.760*
R6	0.649
R7	-0.601
	Films made with glycerol
Polydispersity index for 24 hours of stirring	-0.863**
Molecular weight for 24 hours of stirring	-0.244
Polydispersity index for heated solutions	-0.015
Molecular weight for heated solutions	-0.919***
Polydispersity index for solutions with plasticizer	-0.239
Molecular weight for solutions with plasticizer	-0.597
Polydispersity index for films	-0.628
Molecular weight for films	-0.911***
R1	0.380
R2	0.530
R3	0.672*
R4	0.526
R5	0.674*
R6	0.553
R7	-0.503

* Significant at *P*≤0.05
** Significant at *P*≤0.01
*** Significant at *P*≤0.001
R1 = Arabinose C-(O)-3 Linked to Anomeric Xylose, R2 = Anomeric Proton of Arabinose C-(O)-3 to Disubstituted Xylose, R3 = Anomeric Proton of Arabinose C-(O)-2 to Disubstituted Xylose with Adjoining Disubstituted Xylose, R4 = Anomeric Proton of Arabinose C-(O)-2 to Disubstituted Xylose, R5 = Anomeric Proton of Disubstituted Xylose, R6 = Anomeric Proton of Monosubstituted Xylose, R7 = Anomeric Proton of Xylopyranosyl Unit

3.4.5.3. Puncture Resistance

Puncture resistance is critical for AX films that will be utilized as the basis of food packaging material. The resistance, extensibility, total energy required to puncture, and energy to peak energy required are given in Table 3.17. When these values were averaged by the type of AX used, as seen in Table 3.18, there were clear trends in puncture resistance characteristics. Firstly, WB AX films had the highest puncture resistance (8.34 N), followed by MB AX films (6.02 N), and finally DDG AX films (3.46 N); all puncture resistances were significantly ($P \leq 0.01$) different from each other. Secondly, the extensibility of all films, when averaged by AX type, were insignificantly different. Thirdly, the total energy required to puncture the films as well as the energy to the peak energy required to puncture the films followed the same trend as puncture resistance.

Table 3.17. Puncture resistance, extensibility, total energy, and energy to peak puncture resistance of arabinoxylan films.

Film composition	Resistance (N)	Extensibility (mm)	Total energy (J)	Energy to peak (J)
WB AX[a] + 100 g kg^{-1} Sorbitol	5.25 ± 0.51	39.57 ± 0.15	0.0058 ± 0.001	0.0033 ± 0.001
WB AX + 250 g kg^{-1} Sorbitol	8.79 ± 0.40	38.81 ± 0.21	0.0120 ± 0.001	0.0075 ± 0.001
WB AX + 500 g kg^{-1} Sorbitol	10.09 ± 1.36	37.35 ± 0.68	0.0220 ± 0.008	0.0163 ± 0.006
WB AX + 100 g kg^{-1} Glycerol	9.70 ± 0.43	37.51 ± 0.61	0.0213 ± 0.003	0.0158 ± 0.004
WB AX + 250 g kg^{-1} Glycerol	8.37 ± 0.21	34.97 ± 2.92	0.0350 ± 0.024	0.0253 ± 0.016
WB AX + 500 g kg^{-1} Glycerol	7.85 ± 1.26	35.93 ± 1.65	0.0210 ± 0.008	0.0182 ± 0.009
MB AX[b] + 100 g kg^{-1} Sorbitol	6.20 ± 1.07	39.40 ± 0.74	0.0063 ± 0.004	0.0040 ± 0.003
MB AX + 250 g kg^{-1} Sorbitol	6.78 ± 1.13	36.94 ± 0.58	0.0160 ± 0.003	0.0128 ± 0.002
MB AX + 500 g kg^{-1} Sorbitol	6.72 ± 1.48	37.62 ± 0.23	0.0118 ± 0.003	0.0072 ± 0.003
MB AX + 100 g kg^{-1} Glycerol	4.63 ± 1.76	39.28 ± 0.48	0.0055 ± 0.003	0.0033 ± 0.002
MB AX + 250 g kg^{-1} Glycerol	7.59 ± 0.39	33.43 ± 2.80	0.0330 ± 0.014	0.0310 ± 0.015
MB AX + 500 g kg^{-1} Glycerol	4.19 ± 0.15	35.18 ± 1.05	0.0098 ± 0.001	0.0085 ± 0.001
DDG AX[c] + 100 g kg^{-1} Sorbitol	0.73 ± 0.33	40.43 ± 0.61	0.0005 ± 0.001	0.0003 ± 0.001
DDG AX + 250 g kg^{-1} Sorbitol	1.36 ± 0.37	40.40 ± 0.09	0.0008 ± 0.001	0.0005 ± 0.001
DDG AX + 500 g kg^{-1} Sorbitol	7.28 ± 0.69	35.01 ± 0.75	0.0243 ± 0.005	0.0215 ± 0.005
DDG AX + 100 g kg^{-1} Glycerol	4.19 ± 1.04	37.98 ± 1.29	0.0058 ± 0.003	0.0058 ± 0.003
DDG AX + 250 g kg^{-1} Glycerol	4.20 ± 0.84	36.78 ± 1.04	0.0133 ± 0.007	0.0103 ± 0.006
DDG AX + 500 g kg^{-1} Glycerol	3.01 ± 0.44	34.05 ± 1.82	0.0088 ± 0.003	0.0075 ± 0.003

[a] Wheat bran arabinoxylan

[b] Maize bran arabinoxylan

[c] Dried distillers grain arabinoxylan

Table 3.18. Mean separation for the puncture resistance, extensibility, total energy, and energy to peak puncture resistance of arabinoxylan films.

Arabinoxylan type	Plasticizer type	Plasticizer level (g kg^{-1})	Resistance (N)	Extensibility (mm)	Total energy (J)	Energy to peak (J)
WB[a]			8.34	37.22	0.0201	0.015
MB[b]			6.02	36.97	0.0137	0.0114
DDG[c]			3.46	37.44	0.0089	0.0078
LSD ($P \leq 0.05$)[d]			0.52	0.74	0.0040	0.0037
LSD ($P \leq 0.01$)			0.70	0.98	0.0060	0.0049
	Glycerol		5.97	36.03	0.0170	0.0144
	Sorbitol		5.91	38.39	0.0110	0.0083
	LSD ($P \leq 0.05$)		0.43	0.60	0.0036	0.0030
	LSD ($P \leq 0.01$)		0.57	0.80	0.0048	0.0040
		100	5.12	39.03	0.0075	0.0054
		250	6.18	36.89	0.0183	0.0147
		500	6.52	35.71	0.0169	0.0140
		LSD ($P \leq 0.05$)	0.52	0.74	0.0040	0.0037
		LSD ($P \leq 0.01$)	0.70	0.98	0.0060	0.0049

[a] Wheat bran
[b] Maize bran
[c] Dried distillers grain
[d] Least significant difference

When the puncture resistances of all films were averaged across plasticizer type, it was demonstrated that films made with sorbitol had insignificantly higher puncture resistances than the films made with glycerol, but they were significantly ($P \leq 0.01$) more extensible. Films made with sorbitol also required significantly ($P \leq 0.01$) more energy to be punctured. These trends for films made with either glycerol or sorbitol were also noted by Thomazine et al. (2005). When averaged by plasticizer level, the trend demonstrated in resistance to puncture was that the resistance increased as the level of plasticizer increased. The increase in puncture resistance was significant ($P \leq 0.01$) when going from 100 g kg^{-1} plasticizer to either 250 g kg^{-1} or 500 g kg^{-1} plasticizer. However, the increase in puncture resistance was not significant ($P \leq 0.01$) between films made with 250 g kg^{-1} or 500 g kg^{-1} plasticizer. This trend in puncture resistance was the same trend observed in films made with varying levels of glycerol by Nur Hanani et al. (2013). A second trend demonstrated is that extensibility decreased significantly ($P \leq 0.01$) as the level of plasticizer increased. This trend was the opposite of the trend seen in previously published work. Both Nur Hanani et al. (2013) and Thomazine et al. (2005) noted an increase in extensibility as the level of plasticizer was increased. This discrepency de due to the difference in the geometries of the probes used. The probe used in this research was flat, which could have resulted a different force distribution than a domed probe. Total energy and energy to peak followed the same trend: films made with 250 g kg^{-1} plasticizer were the highest in energy values, followed by films made with 500 g kg^{-1} plasticizer, and finally films made with 100 g kg^{-1} plasticizer.

There were four significant ($P \leq 0.05$) interactions for the puncture resistances of the AX films as seen in Tables 3.19 and 3.20. These interactions included AX type by plasticizer type, AX type by plasticizer level, plasticizer type by plasticizer level, and the three-way interaction between all three factors. The three-way interaction between AX type and plasticizer level for films made with glycerol demonstrated very little to no interaction as seen in Figure 3.6. Conversely, the three-way interaction of AX type and plasticizer type for films made with sorbitol showed multiple effects as seen in Figure 3.7. Firstly, there were converging responses between MB AX and WB AX films over varying levels of plasticizer for films made with sorbitol. Secondly, there were also converging responses for the MB AX films and DDG AX films over varying levels of plasticizer for sorbitol films. Thirdly, it did not appear that there was an interaction between WB AX films and DDG AX films over the levels of plasticizer for films made with sorbitol.

The puncture resistance of materials used in food packaging vary widely depending upon composition and processing. Puncture resistance also depends upon the geometry of the material. For example, the puncture resistance of polyvinyl chloride is around 20 J mm^{-1} when a thickness of 5 mm is utilized (Whittle et al. 2013). Chitosan films are often studied as a possible replacement for polyvinyl chloride bags (Czaja-Jagielska et al. 2011). When these films are prepared with acetic acid, they have a puncture resistance of 311 N mm^{-1} (Zivanovic et al. 2005). This puncture resistance is much higher than the puncture resistances of the AX films developed in this research.

Table 3.19. Mean separation for the significant two-way interactions affecting the puncture resistance of arabinoxylan films.

Arabinoxylan type	Plasticizer type	Plasticizer level (g kg^{-1})	Puncture resistance (N)
WB[a]	Glycerol		5.47
WB	Sorbitol		6.57
MB[b]	Glycerol		3.80
MB	Sorbitol		3.12
DDG[c]	Glycerol		8.64
DDG	Sorbitol		8.04
LSD ($P \leq 0.05$)[d]			0.74
LSD ($P \leq 0.01$)			0.98
WB		100	39.34
WB		250	35.18
WB		500	36.40
MB		100	39.20
MB		250	38.59
MB		500	34.53
DDG		100	38.54
DDG		250	36.89
DDG		500	36.21
LSD ($P \leq 0.05$)			1.28
LSD ($P \leq 0.01$)			1.70
	Glycerol	100	6.17
	Glycerol	250	6.72
	Glycerol	500	5.01
	Sorbitol	100	4.06
	Sorbitol	250	5.64
	Sorbitol	500	8.03
	LSD ($P \leq 0.05$)		0.74
	LSD ($P \leq 0.01$)		0.98

[a] Wheat bran
[b] Maize bran
[c] Dried distillers grain
[d] Least significant difference

Table 3.20. Three-way interactions between arabinoxylan type, plasticizer type, and plasticizer level affecting the puncture resistance of arabinoxylan films.

Arabinoxylan type	Plasticizer type	Plasticizer level (g kg^{-1})	Puncture resistance (N)
WB[a]	Glycerol	100	9.70
WB	Glycerol	250	8.37
WB	Glycerol	500	7.85
MB[b]	Glycerol	100	4.63
MB	Glycerol	250	7.59
MB	Glycerol	500	4.19
DDG[c]	Glycerol	100	4.19
DDG	Glycerol	250	4.20
DDG	Glycerol	500	3.01
WB	Sorbitol	100	5.25
WB	Sorbitol	250	8.79
WB	Sorbitol	500	10.09
MB	Sorbitol	100	6.20
MB	Sorbitol	250	6.78
MB	Sorbitol	500	6.72
DDG	Sorbitol	100	0.73
DDG	Sorbitol	250	1.36
DDG	Sorbitol	500	7.28
LSD ($P \leq 0.05$)[a]			1.28
LSD ($P \leq 0.01$)			1.71

[a] Wheat bran
[b] Maize bran
[c] Dried distillers grain
[d] Least significant difference

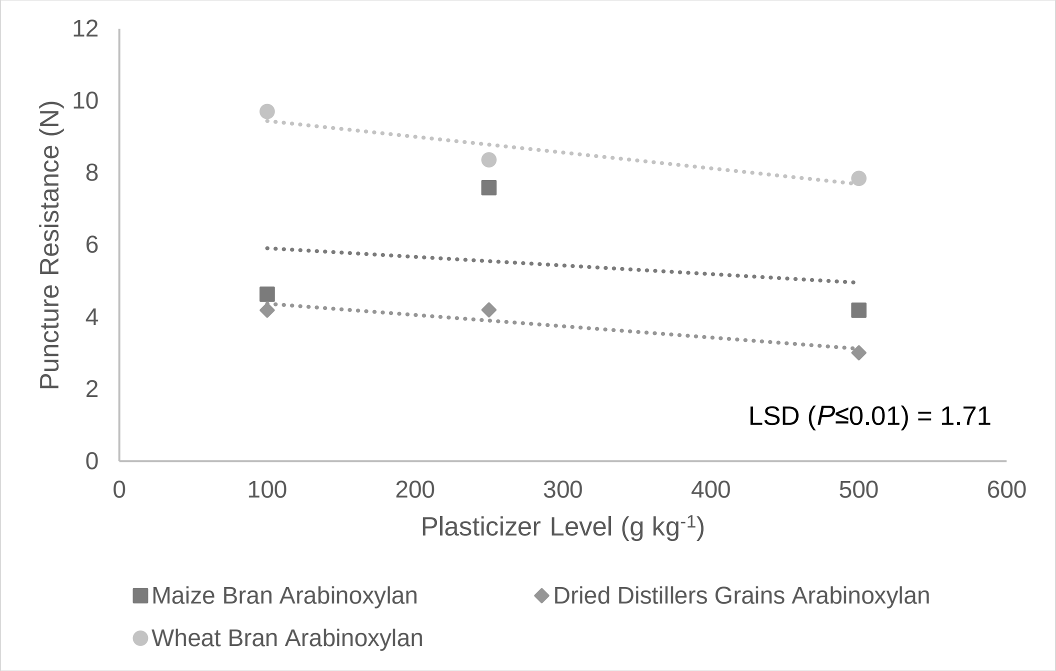

Figure 3.6. Interaction of arabinoxylan type and plasticizer level for films made with glycerol for the puncture resistance of arabinoxylan films.

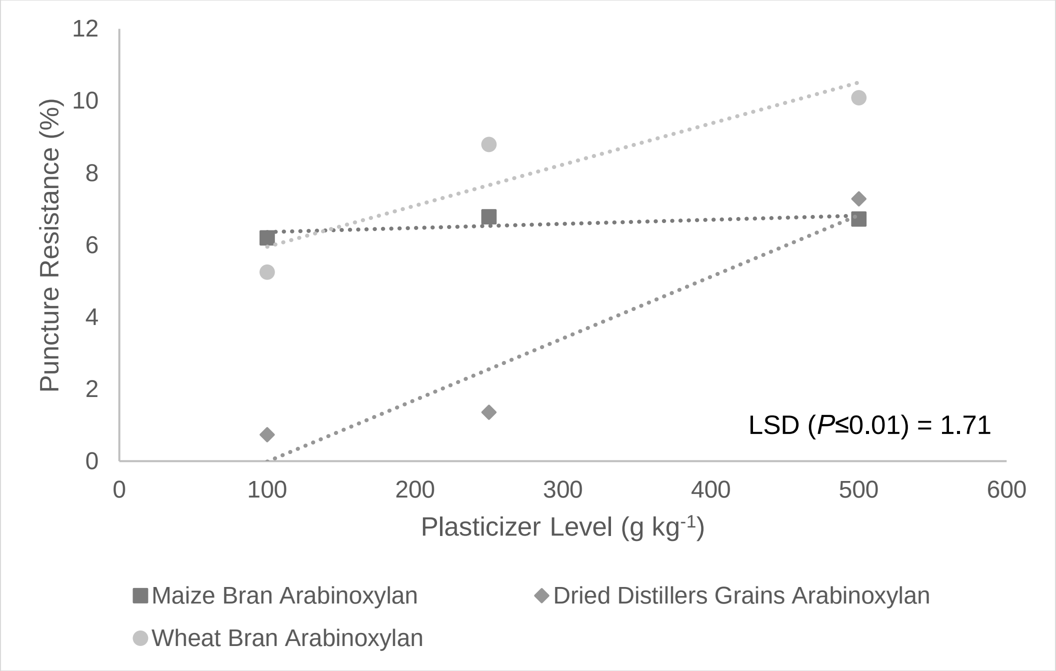

Figure 3.7. Interaction of arabinoxylan type and plasticizer level for films made with sorbitol for the puncture resistance of arabinoxylan films.

There was only one significant (*P*≤0.05) interaction for the extensibility of the AX films, and it was the interaction between AX type and plasticizer level. The significance of this interaction was that the effect of the type of AX on the extensibility of the film was influenced by the level of plasticizer in the film as seen in Table 3.21. Figure 3.8 shows that there were multiple true interactions between AX type and plasticizer level. Firstly, there was a converging response between the extensibility of MB AX films and DDG AX films over increasing plasticizer levels. Secondly, there was a converging response between the extensibility of WB AX films and DDG AX films over increasing plasticizer levels. Thirdly, there did not appear to be an interaction between the extensibility of WB AX films and MB AX films over increasing plasticizer levels.

Table 3.21. Interaction between arabinoxylan type and plasticizer level affecting the extensibility of arabinoxylan films.

Arabinoxylan type	Plasticizer level (g kg^{-1})	Extensibility (mm)
WB[a]	100	38.54
WB	250	36.89
WB	500	36.21
MB[b]	100	39.34
MB	250	35.18
MB	500	36.40
DDG[c]	100	39.20
DDG	250	38.59
DDG	500	34.53
LSD ($P \leq 0.05$)[d]		1.28
LSD ($P \leq 0.01$)		1.70

[a] Wheat bran
[b] Maize bran
[c] Dried distillers grain
[d] Least significant difference

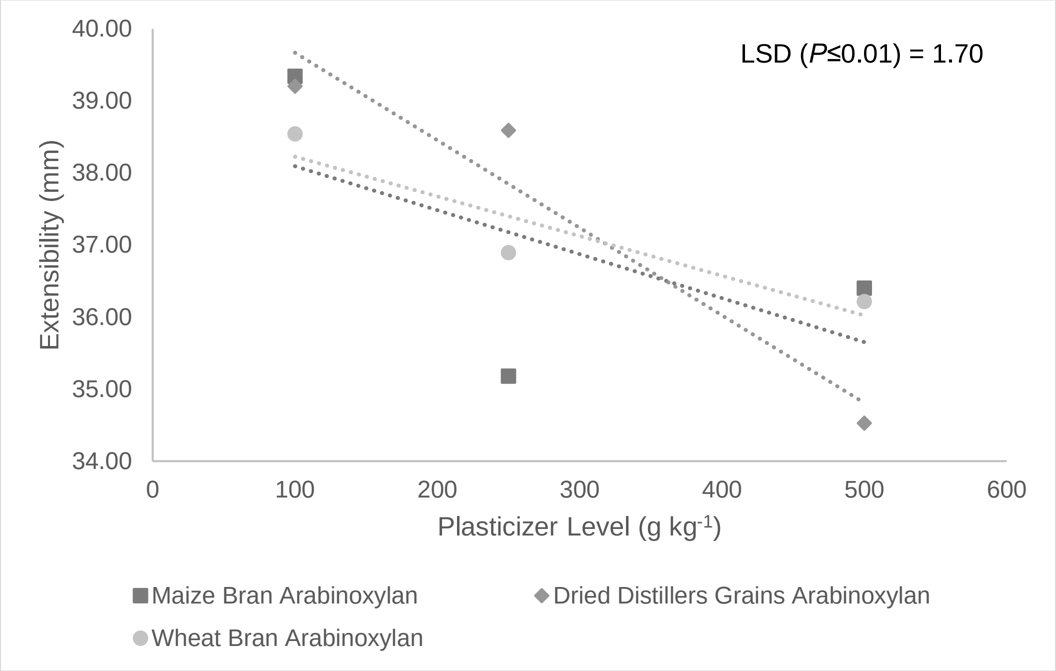

Figure 3.8. Interaction of arabinoxylan type and plasticizer level for the extensibility of arabinoxylan films.

There were multiple significant (*P*≤0.01 or *P*≤0.05) correlations between the plasticizer level, Mw, and PI of the AX films and their puncture resistance characteristics as seen in Table 3.22. Firstly, for both films made with sorbitol and those made with glycerol, there was a significant (*P*≤0.05 for sorbitol and *P*≤0.01 for glycerol) correlation between the PI of the film solutions after they were mixed with water for 24 hours and the puncture resistance of the films. These two things were positively correlated, which means that as the PI index of the solutions increased, so did the puncture resistance of the films. This is most likely due to an increase in the level of interaction between polymers of different sizes that created a network that was puncture resistant. Secondly, there was a significant (*P*≤0.01) correlation between the Mw for the film solutions after heating and the puncture resistance of the glycerol films. The correlation seen is that as the Mw increased, the puncture resistance also increased. The larger AX polymers were more resistant to puncture. Thirdly, there was a significant (*P*≤0.01) positive correlation between the Mw of the sorbitol films and their puncture resistance. Fourthly, there was a significant (*P*≤0.05) negative correlation between the plasticizer level and the extensibility of the AX films. This means that as the plasticizer level increased, the extensibility of the films decreased. However, this correlation was not in accordance with previously published research (Nur Hanani et al. 2013; Thomazine et al. 2005).

Table 3.22. Correlations between plasticizer level, molecular weight, and polydispersity indexes and the puncture resistance characteristics of arabinoxylan films made with either sorbitol or glycerol.

	Resistance (N)	Extensibility (mm)	Total energy (J)	Energy to peak (J)
		Films made with sorbitol		
Plasticizer level	0.557	-0.766 *	0.776 *	0.782 *
Polydispersity index for 24 hours of stirring	0.668 *	0.007	0.236	0.091
Molecular weight for 24 hours of stirring	0.573	-0.132	0.179	0.077
Polydispersity index for heated solutions	-0.434	0.156	-0.126	-0.058
Molecular weight for heated solutions	0.509	0.079	0.192	0.070
Polydispersity index for solutions with plasticizer	-0.648	0.231	-0.288	-0.200
Molecular weight for solutions with plasticizer	0.197	-0.064	0.275	0.254
Polydispersity index for films	0.114	-0.365	0.283	0.316
Molecular weight for films	0.849 **	-0.533	0.733 *	0.641
		Films made with glycerol		
Plasticizer level	-0.241	-0.712 *	0.044	0.104
Polydispersity index for 24 hours of stirring	0.886 **	-0.089	0.676 *	0.576
Molecular weight for 24 hours of stirring	0.462	-0.080	0.377	0.372
Polydispersity index for heated solutions	-0.230	0.062	-0.203	-0.233
Molecular weight for heated solutions	0.832 **	-0.066	0.622	0.503
Polydispersity index for solutions with plasticizer	0.076	-0.299	0.229	0.229
Molecular weight for solutions with plasticizer	0.311	-0.113	0.242	0.156
Polydispersity index for films	0.212	-0.596	0.323	0.341
Molecular weight for films	0.628	-0.417	0.599	0.546

* Significant at $P \leq 0.05$
** Significant at $P \leq 0.01$

There were also significant ($P \leq 0.05$) correlations between the relative abundances of linkages in the AX used to make the films and the puncture resistance of the films as seen in Table 3.23. The main trend in significant ($P \leq 0.05$) correlations was that as the abundance of substituted xylose present in the AX structure increased, the puncture resistance of the AX film decreased. In accordance, there was also a significant ($P \leq 0.05$) correlation between increased puncture resistances when there was an increase in unsubstituted xylose. This is due to increased strength associated with more closely aligned AX polymers, which was hindered when substituted xylose is present.

Table 3.23. Correlation between puncture resistances of arabinoxylan films made with either sorbitol or glycerol and the relative abundances of linkages between arabinose and xylose.

Resonance peak of anomeric proton	Puncture resistance
	Films made with sorbitol
R1	-0.630
R2	-0.677*
R3	-0.702*
R4	-0.676*
R5	-0.703*
R6	-0.683*
R7	0.670*
	Films made with glycerol
R1	-0.575
R2	-0.691*
R3	-0.790*
R4	-0.688*
R5	-0.792*
R6	-0.708*
R7	0.671*

* Significant at $P \leq 0.05$

R1 = Arabinose C-(O)-3 Linked to Anomeric Xylose, R2 = Anomeric Proton of Arabinose C-(O)-3 to Disubstituted Xylose, R3 = Anomeric Proton of Arabinose C-(O)-2 to Disubstituted Xylose with Adjoining Disubstituted Xylose, R4 = Anomeric Proton of Arabinose C-(O)-2 to Disubstituted Xylose, R5 = Anomeric Proton of Disubstituted Xylose, R6 = Anomeric Proton of Monosubstituted Xylose, R7 = Anomeric Proton of Xylopyranosyl Unit

3.4.5.4. Tensile Strength

The tensile properties of a polymeric material, such as AX films depend upon many factors including the order of the polymers in the material. As the organization of the polymers in the polymer material becomes more ordered, the modulus and tensile strength of the polymeric material increase (Nagendra et al. 2000). These trends were clearly reflected in the tensile properties of the polymer films created in this experiment. Figure 3.9 provides an example of a tensile strain vs tensile stress curve for the AX films analyzed. From the overall shape of this curve, it was evident that this AX film exhibited ductile characteristics. However, some of the films exhibited brittle characteristics. Table 3.24 provides numerical values for all tensile properties of all AX films, and all films had similar curves to the one shown below, however they did vary in overall properties depending upon film composition.

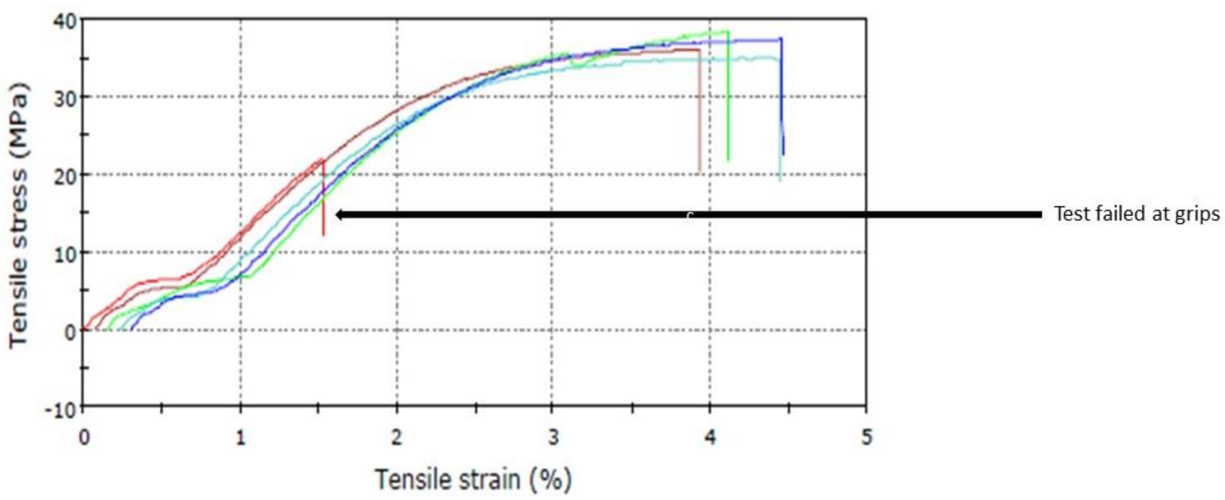

Figure 3.9. Tensile stress-strain curves for wheat bran arabinoxylan films plasticized with 100 g kg^{-1} glycerol.

Table 3.24. Tensile strength and related parameters of arabinoxylan films.

Film composition	Maximum tensile strength (MPa)	Modulus (MPa)	Breaking factor (N m^{-1})	Elongation (%)
WB AX[a] + 100 g kg^{-1} Sorbitol	23.22 ± 4.63	1455.19 ± 350.17	1116.35 ± 253.89	1.87 ± 0.47
WB AX + 250 g kg^{-1} Sorbitol	20.86 ± 3.29	663.69 ± 299.40	1180.68 ± 185.00	6.45 ± 0.86
WB AX + 500 g kg^{-1} Sorbitol	12.85 ± 0.39	1687.01 ± 116.58	842.39 ± 47.47	26.52 ± 2.00
WB AX + 100 g kg^{-1} Glycerol	26.62 ± 0.45	1381.70 ± 219.83	1563.28 ± 106.70	4.05 ± 0.15
WB AX + 250 g kg^{-1} Glycerol	17.73 ± 1.30	1280.22 ± 523.47	1204.83 ± 126.35	2.44 ± 0.53
WB AX + 500 g kg^{-1} Glycerol	1.22 ± 0.18	308.53 ± 73.60	30.23 ± 27.50	30.22 ± 2.46
MB AX[b] + 100 g kg^{-1} Sorbitol	18.61 ± 3.34	825.30 ± 310.34	556.59 ± 113.18	1.42 ± 0.16
MB AX + 250 g kg^{-1} Sorbitol	13.31 ± 1.39	669.56 ± 170.90	552.88 ± 59.91	2.28 ± 0.71
MB AX + 500 g kg^{-1} Sorbitol	13.58 ± 2.98	728.57 ± 176.92	537.18 ± 121.65	34.32 ± 13.54
MB AX + 100 g kg^{-1} Glycerol	29.31 ± 2.89	936.78 ± 415.68	856.88 ± 69.12	2.43 ± 0.37
MB AX + 250 g kg^{-1} Glycerol	14.48 ± 0.60	1345.14 ± 200.59	409.09 ± 46.71	9.12 ± 4.00
MB AX + 500 g kg^{-1} Glycerol	0.25 ± 0.03	910.17 ± 38.66	79.48 ± 4.02	225.28 ± 9.39
DDG AX[c] + 100 g kg^{-1} Sorbitol	6.29 ± 0.81	807.24 ± 131.48	290.98 ± 22.66	0.61 ± 0.22
DDG AX + 250 g kg^{-1} Sorbitol	6.31 ± 0.89	1037.83 ± 172.86	290.98 ± 50.82	2.29 ± 3.22
DDG AX + 500 g kg^{-1} Sorbitol	7.02 ± 0.74	5.85 ± 0.44	319.91 ± 37.41	18.29 ± 6.46
DDG AX + 100 g kg^{-1} Glycerol	9.31 ± 2.54	1277.42 ± 56.61	403.25 ± 111.23	1.20 ± 0.45
DDG AX + 250 g kg^{-1} Glycerol	6.28 ± 0.83	1106.02 ± 67.08	263.45 ± 37.44	0.83 ± 0.21
DDG AX + 500 g kg^{-1} Glycerol	1.32 ± 0.07	21.45 ± 3.02	6.16 ± 6.07	54.28 ± 6.04

[a] Wheat bran arabinoxylan
[b] Maize bran arabinoxylan
[c] Dried distillers grain arabinoxylan

Table 3.25 provides the tensile properties of all films averaged by the type of AX used. The trend seen was that WB AX films had the highest tensile strength, indicated by the maximum tensile strength, and breaking factor. Following the WB AX films, the MB AX films were the next strongest, and the DDG AX films were the least strong. The strength of a polymer film such as these AX films is usually dependent on the crystallinity of the material (Nagendra et al. 2000). The differences in the strengths of all AX films when averaged across AX type were significantly (*P*≤0.01) different. A similar trend was seen for the moduli of these films. The WB AX films had moduli that were significantly (*P*≤0.01) larger than that of both the MB AX films and the DDG AX films. The moduli of these films were determined by the slope of the first portion of the stress-strain curve due to elongation of the sample obtained from tensile strength analysis. The portion of the stress-strain curve due to slack in the sample was disregarded for the modulus calculation. The natural logarithm of the moduli was taken for separation of the means as the variances of the moduli were uneven. This provided information about the response of the AX films to these forces. The trend seen in the elongations of the films is that the MB AX films are the most elastic, followed by DDG AX films, and then WB AX films. The MB AX films had significantly (*P*≤0.01) larger elongations than the WB AX and DDG AX films.

Table 3.25. Mean separation of tensile properties of arabinoxylan films.

Arabinoxylan type	Plasticizer type	Plasticizer level (g kg^{-1})	Maximum tensile strength (MPa)	Modulus (MPa)[e]	Ln Modulus (MPa)	Breaking factor (N m^{-1})	Elongation (%)
WB[a]			17.08	1164.4	7.06	989.63	11.92
MB[b]			14.92	902.6	6.81	498.68	45.81
DDG[c]			6.09	709.3	6.56	244.70	12.92
LSD (*P*≤0.05)[d]			1.05	-	0.12	52.26	2.39
LSD (*P*≤0.01)			1.39	-	0.16	69.36	3.17
	Glycerol		11.84	975.2	6.88	535.18	36.65
	Sorbitol		13.56	875.6	6.77	620.16	10.45
	LSD (*P*≤0.05)		0.85	-	0.10	42.67	1.95
	LSD (*P*≤0.01)		1.13	-	0.13	56.63	2.59
		100	18.89	1113.9	7.02	780.10	1.93
		250	13.16	1052.0	6.96	650.32	3.9
		500	6.04	610.3	6.41	302.56	64.82
		LSD (*P*≤0.05)	1.05	-	0.12	52.26	2.39
		LSD (*P*≤0.01)	1.39	-	0.16	69.36	3.17

[a] Wheat bran
[b] Maize bran
[c] Dried distillers grain
[d] Least significant difference
[e] Mean separation was performed on the natural logarithm of the moduli values due to unequal variances of the raw data

The tensile properties of the AX films were also averaged across the type of plasticizer used. Films plasticized with glycerol had significantly ($P \leq 0.01$) lower strength than those plasticized with sorbitol. This is the same trend was observed by other researchers including Thomazine et al. (2005). The reason for this trend is that glycerol plasticizes the films better than sorbitol, which results in a higher level of polymer chain disruption and lower mechanical strength. Congruently, the elongation of films made with glycerol was significantly ($P \leq 0.01$) higher than films made with sorbitol.

The effect of the level of plasticization on the tensile properties of the films showed clear trends that are in accordance with the trends previously demonstrated for the tensile properties of the AX films. Essentially, increasing the level of plasticizer used in the films increased the disorder of the polymer chains, and in doing so decreased the strength of the films (Nagendra et al. 2000). In the films analyzed, the result of this trend was demonstrated by the significant ($P \leq 0.01$) decrease in mechanical strength of the AX films. In addition, as the level of plasticizer was increased, the elongation significantly ($P \leq 0.01$) increased.

There were numerous significant ($P \leq 0.05$) two- and three-way interactions for the three main factors (AX type, plasticizer type, and plasticizer level) and their effects on the tensile properties of the AX films. Table 3.26 provides the mean separations for all significant ($P \leq 0.05$) three-way interactions. Table 3.27 provides the mean separations for all significant ($P \leq 0.05$) two-way interactions that affected the tensile properties of the AX films. The two most relevant parameters to food packaging are the maximum tensile strength and elongation. This is because these two factors will determine the breaking point and characteristics at break. Figures 3.10 and 3.11 provide a graphic representation for the three-way interactions for the tensile strength of the films. It is notable that there a crossover response regardless of AX type for all films made with glycerol. However, there was a converging response for the effect of AX type and plasticizer level for films made with glycerol when the AX type is DDG AX and WB AX or DDG AX and MB AX. For films made with sorbitol, there was a converging response for the effects of the interactions of AX type and plasticizer level for films made with sorbitol on the tensile strength. Overall, these three-way interactions demonstrate that for both sorbitol and glycerol and all levels of AX type, as the plasticizer level increased, the tensile strength decreased. The elongation of the AX films was also affected by these three factors. Firstly, the effect of the MB AX and plasticizer level for both types of plasticizers showed a crossover response with the two other types of AX. Secondly, there were converging responses for the DDG AX and MB AX films made with sorbitol and those made with glycerol as the level of plasticizer was increased. The overall trend seen was that the effect of the interaction between AX type and level of plasticizer for a given type of plasticizer was an increase in elongation as the level of plasticizer increases. These interactions are shown in Figures 3.12 and 3.13.

Table 3.26. Mean separation for the three-way interaction between arabinoxylan type, plasticizer type, and plasticizer level affecting the tensile properties of arabinoxylan films.

Arabinoxylan type	Plasticizer type	Plasticizer level ($g\ kg^{-1}$)	Maximum tensile strength (MPa)	Modulus (MPa)[f]	Ln Modulus (MPa)	Breaking factor ($N\ m^{-1}$)	Elongation (%)
WB[a]	Glycerol	100	26.62	1381.7	7.23	1563.28	4.05
WB	Glycerol	250	17.73	1490.0	7.31	1204.83	2.44
WB	Glycerol	500	1.22	308.5	5.73	30.23	30.22
MB[b]	Glycerol	100	29.31	936.8	6.84	856.88	2.43
MB	Glycerol	250	14.48	1345.1	7.20	409.09	9.12
MB	Glycerol	500	0.25	910.2	6.81	79.48	225.28
DDG[c]	Glycerol	100	9.31	1277.4	7.15	403.25	1.20
DDG	Glycerol	250	6.28	1106.0	7.01	263.45	0.83
DDG	Glycerol	500	1.32	21.5	3.07	6.16	54.28
WB	Sorbitol	100	23.22	1455.2	7.28	1116.35	1.87
WB	Sorbitol	250	20.86	663.7	6.50	1180.68	6.45
WB	Sorbitol	500	12.85	1687.0	7.43	842.39	26.52
MB	Sorbitol	100	18.61	825.3	6.72	556.59	1.42
MB	Sorbitol	250	13.31	669.6	6.51	552.88	2.28
MB	Sorbitol	500	13.58	728.6	6.59	537.18	34.32
DDG	Sorbitol	100	6.29	807.2	6.69	184.48	0.61
DDG	Sorbitol	250	6.31	1037.8	6.94	290.98	2.29
DDG	Sorbitol	500	7.02	5.9	1.77	319.91	18.29
LSD ($P \leq 0.05$)[d]			2.56	-	0.29	128.00	5.86
LSD ($P \leq 0.01$)			3.40	-	0.39	169.89	7.77

[a] Wheat bran

[b] Maize bran

[c] Dried distillers grain

[d] Least significant difference

[e] Not significant ($P \leq 0.05$)

[f] Mean separation was performed on the natural logarithm of the moduli values due to unequal variances of the raw data

Table 3.27. Mean separation of the significant ($P \leq 0.05$) two-way interactions affecting the tensile properties of arabinoxylan films.

Arabinoxylan type	Plasticizer type	Plasticizer level (g kg^{-1})	Maximum tensile strength (MPa)	Modulus (MPa)[f]	Ln Modulus (MPa)	Breaking factor (N m^{-1})	Elongation (%)
WB[a]	Glycerol		15.19	1060.1	6.97	NS[e]	12.23
WB	Sorbitol		18.97	1268.6	7.15	NS	11.61
MB[b]	Glycerol		14.68	1064.0	6.97	NS	78.94
MB	Sorbitol		15.17	741.1	6.61	NS	12.67
DDG[c]	Glycerol		5.64	801.6	6.69	NS	18.77
DDG	Sorbitol		6.54	617.0	6.42	NS	7.07
LSD ($P \leq 0.05$)[d]			1.48	-	0.17	NS	3.38
LSD ($P \leq 0.01$)			1.96	-	0.23	NS	4.49
WB		100	24.92	1418.5	7.26	1339.82	2.96
WB		250	19.29	1076.9	6.98	1192.75	4.44
WB		500	7.03	997.8	6.91	436.31	28.37
MB		100	23.96	881.0	6.78	706.73	1.93
MB		250	13.89	1007.4	6.92	480.98	5.70
MB		500	6.92	819.4	6.71	308.33	129.80
DDG		100	7.80	1042.3	6.95	293.87	0.91
DDG		250	6.30	1071.9	6.98	277.21	1.56
DDG		500	4.17	13.7	2.61	163.03	36.29
LSD ($P \leq 0.05$)			1.81	-	0.21	90.51	4.14
LSD ($P \leq 0.01$)			2.40	-	0.28	120.13	5.50
	Glycerol	100	21.75	1198.6	7.09	941.14	2.56
	Glycerol	250	12.83	1313.7	7.18	625.79	4.13
	Glycerol	500	0.93	413.4	6.02	38.62	103.26
	Sorbitol	100	16.04	1029.2	6.94	619.14	1.30
	Sorbitol	250	13.49	790.4	6.67	674.85	3.67
	Sorbitol	500	11.15	807.1	6.69	566.49	26.38
	LSD ($P \leq 0.05$)		1.48	-	0.17	73.90	3.38
	LSD ($P \leq 0.01$)		1.96	-	0.23	98.08	4.49

[a] Wheat bran

[b] Maize bran

[c] Dried distillers grain

[d] Least significant difference

[e] Not significant ($P \leq 0.05$)

[f] Mean separation was performed on the natural logarithm of the moduli values due to unequal variances of the raw data

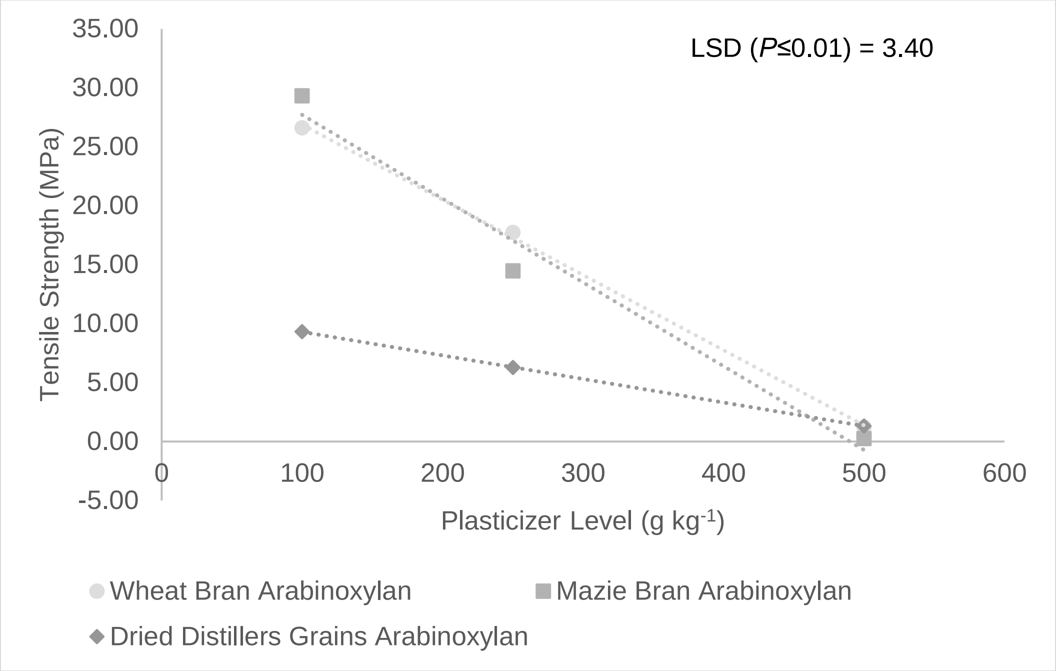

Figure 3.10. Effects of the interaction between arabinoxylan type and plasticizer level on the tensile strength for all films made with glycerol.

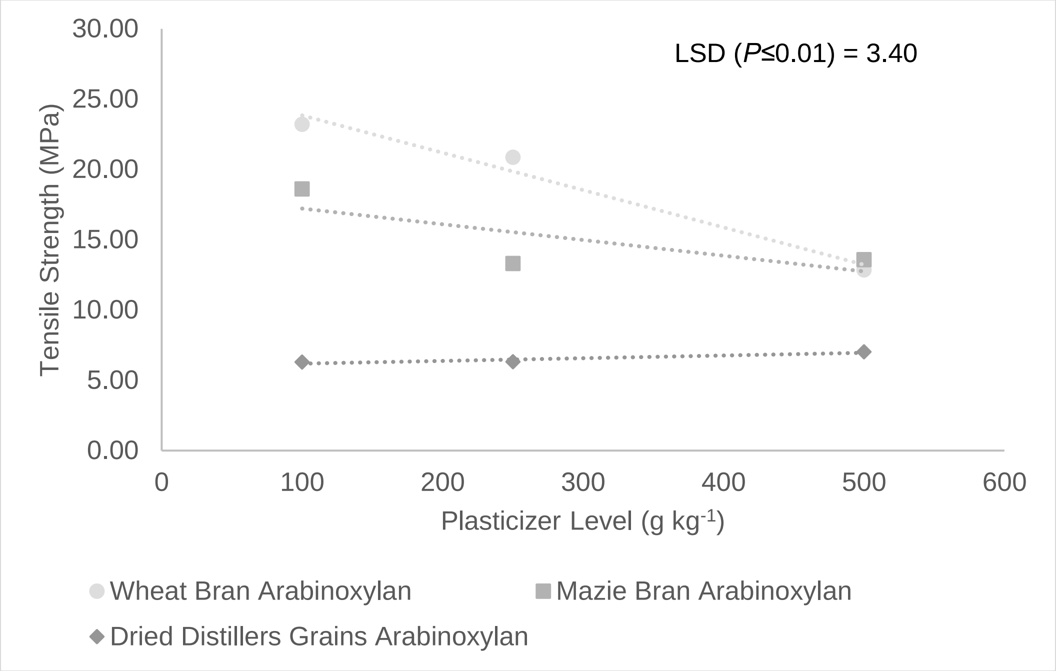

Figure 3.11. Effects of the interaction between arabinoxylan type and plasticizer level on the tensile strength for all films made with sorbitol.

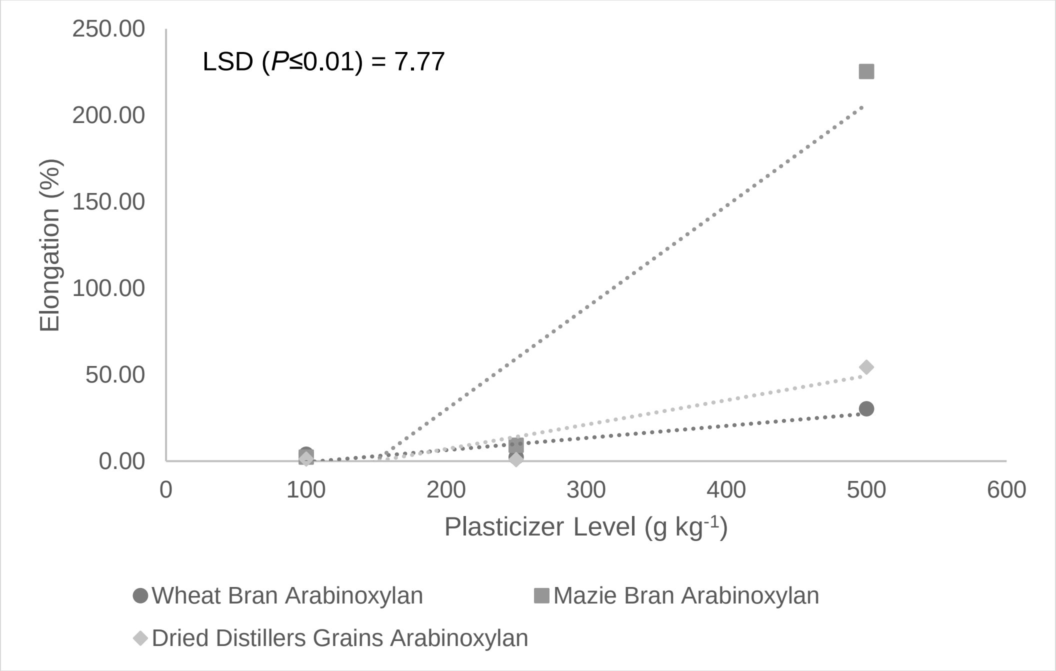

Figure 3.12. Effects of the interaction between arabinoxylan type and plasticizer level on the elongation for all films made with glycerol.

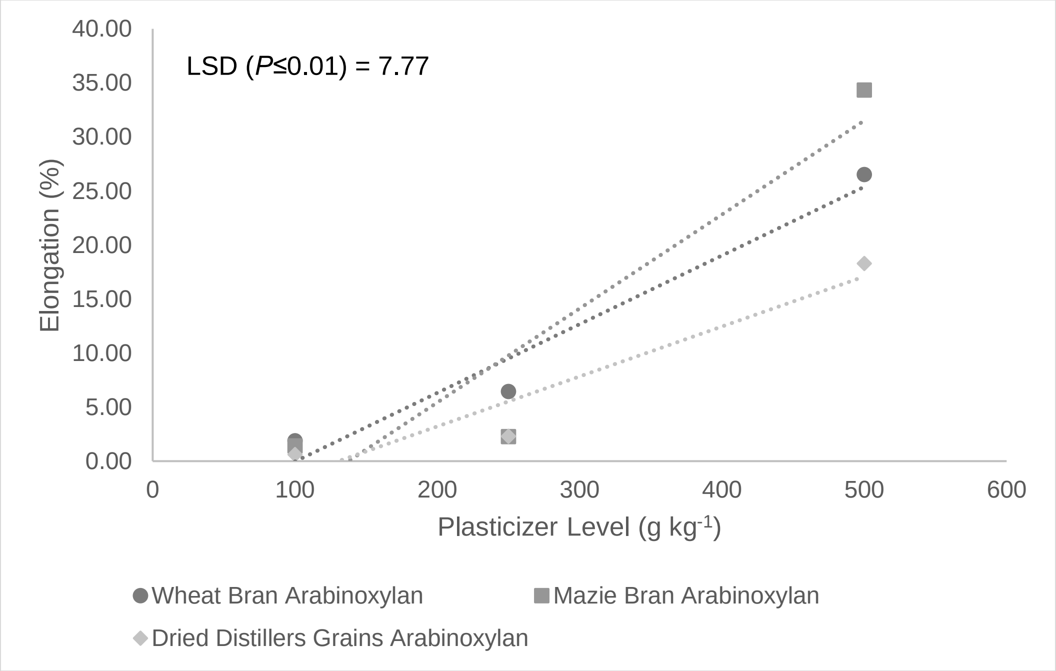

Figure 3.13. Effects of the interaction between arabinoxylan type and plasticizer level on the elongation for all films made with sorbitol.

Tensile properties of the materials currently being used in food packaging such as polyvinyl chloride have varying tensile strengths. For example, polyvinyl chloride has a tensile strength of about 52 MPa when it is about 5 mm thick (Whittle et al. 2001). Chitosan films have been known to have even higher tensile strengths at around 106 MPa (Zivanovic et al. 2005). The tensile strengths of both of these materials are much higher than that of the AX films developed in this research.

There were significant ($P \leq 0.01$ or $P \leq 0.05$) correlations between the tensile properties of the AX films and the plasticizer level of the film for films made with glycerol as seen in Table 3.28. Firstly, for films made with glycerol, there was a significant ($P \leq 0.01$) negative correlation between the plasticizer level and the maximum tensile strength of the film. This implied that as the plasticizer level increased, the maximum tensile strength decreased. This was also true for the tensile strength and the breaking factor of the films. This correlation was also negative, so as the plasticizer level increased, the breaking factor decreased. Significant ($P \leq 0.05$ or $P \leq 0.01$ or $P \leq 0.001$) correlations were present between the tensile properties of films made with sorbitol and other factors such as plasticizer level. There were no significant ($P \leq 0.001$) correlations between plasticizer level and both the modulus and the elongation of the films. The correlation between the elongation of the AX film and the plasticizer level (for films made with sorbitol) were positively correlated. This correlation was significant ($P \leq 0.001$) for films made with sorbitol but not those made with glycerol, which indicates a difference in the plasticizing characteristics of these two materials.

Table 3.28. Correlations between tensile properties of arabinoxylan films and other factors including plasticizer level, molecular weight, and polydispersity index.

	Maximum tensile strength (MPa)	Breaking factor (N m^{-1})	Elongation (%)
Plasticizer level	-0.82**	-0.72*	0.64
PI[a] for 24 hours of stirring	0.36	0.56	-0.08
Mw[b] for 24 hours of stirring	0.40	0.28	0.30
PI for heated solutions	-0.34	-0.13	-0.38
Mw for heated solutions	0.22	0.54	-0.26
PI for solutions with plasticizer	-0.33	-0.25	-0.46
Mw for solutions with plasticizer	-0.18	0.14	-0.38
PI for films	-0.59	-0.41	0.28
Mw for films	-0.21	0.06	0.08
Plasticizer level	-0.33	-0.08	0.90***
PI for 24 hours of stirring	0.83**	0.96***	0.14
Mw for 24 hours of stirring	0.71*	0.52	0.20
PI for heated solutions	-0.53	-0.27	-0.18
Mw for heated solutions	0.63	0.89**	0.07
PI for solutions with plasticizer	-0.75*	-0.57	0.03
Mw for solutions with plasticizer	-0.05	0.32	0.00
PI for films	-0.40	-0.53	0.61
Mw for films	0.30	0.59	0.73*

[a] Polydispersity index
[b] Molecular weight
* Significant at $P\leq0.05$
** Significant at $P\leq0.01$
*** Significant at $P\leq0.001$

There were also significant ($P\leq0.05$ or higher) correlations between the PI of the film solutions after stirring with water for 24 hours and the mechanical strengths (maximum tensile strength and breaking factor) of the AX films made with sorbitol. The negative correlation between the PI of the film solutions after stirring with sorbitol and tensile strength were also significant ($P\leq0.05$). This is because as the variation in the Mw of AX polymers increased, there was a decrease in the uniformity of the material, which resulted in decreased mechanical strength. Thirdly, there were also correlations between the Mw of the films and film solutions and the tensile properties of the AX films. The first significant ($P\leq0.05$) correlation was between the Mw of the films solution after stirring for 24 hours and the maximum tensile strength. This correlation was positive because as the Mw increased, so did the tensile strength of the films. In addition, there was also a significant ($P\leq0.01$) positive correlation between the Mw of the heated film solutions and the breaking factor of the films made with sorbitol. Lastly, there was a significant

($P \leq 0.05$) correlation between the Mw of the AX films and the elongation of the films. This correlation was positive, which indicates that the elongation of the films increased with an increase in film Mw.

3.4.5.5. Tear Resistance

Tear resistance is a measure of the resistance of a material to propagation of a tear, which is of the upmost importance when creating materials that could be used for packaging foods. This is because tearing of the packaging would result in possibly requiring a new package to house the food or throwing the food away due to spoilage. Table 3.29 provides the average load required for tear propagation to occur as well as the extension of the material before tear propagation for all films analyzed in this research. The load required before propagation of a tear is also known as a material's resistance to tearing. Thus, the higher the load, the higher the tear resistance. Also, the higher the tear resistance, the lower the extensibility.

Table 3.29. Load and extension at tear initiation for arabinoxylan films.

Film composition	Load (N)	Extension (mm)
WB AX[a] + 100 g kg^{-1} Sorbitol	0.82 ± 0.37	0.45 ± 0.12
WB AX + 250 g kg^{-1} Sorbitol	1.42 ± 0.60	0.20 ± 0.04
WB AX + 500 g kg^{-1} Sorbitol	1.94 ± 0.13	0.85 ± 0.18
WB AX + 100 g kg^{-1} Glycerol	1.38 ± 0.26	0.26 ± 0.06
WB AX + 250 g kg^{-1} Glycerol	1.35 ± 0.40	0.91 ± 0.58
WB AX + 500 g kg^{-1} Glycerol	0.85 ± 0.11	4.05 ± 0.62
WB AX + 100 g kg^{-1} Sorbitol	1.27 ± 0.04	0.23 ± 0.04
MB AX[b] + 250 g kg^{-1} Sorbitol	1.53 ± 0.25	0.44 ± 0.11
MB AX + 500 g kg^{-1} Sorbitol	1.22 ± 0.14	1.30 ± 0.40
MB AX + 100 g kg^{-1} Glycerol	1.73 ± 0.57	0.77 ± 0.26
MB AX + 250 g kg^{-1} Glycerol	1.48 ± 0.51	2.25 ± 0.34
MB AX + 500 g kg^{-1} Glycerol	0.32 ± 0.07	48.43 ± 8.65
DDG AX[c] + 100 g kg^{-1} Sorbitol	0.33 ± 0.07	0.27 ± 0.08
DDG AX + 250 g kg^{-1} Sorbitol	0.65 ± 0.11	0.42 ± 0.13
DDG AX + 500 g kg^{-1} Sorbitol	1.58 ± 0.16	1.84 ± 0.26
DDG AX + 100 g kg^{-1} Glycerol	0.95 ± 0.29	0.29 ± 0.07
DDG AX + 250 g kg^{-1} Glycerol	1.46 ± 0.26	2.14 ± 0.90
DDG AX + 500 g kg^{-1} Glycerol	0.44 ± 0.07	27.50 ± 5.35

[a] Wheat bran arabinoxylan
[b] Maize bran arabinoxylan
[c] Dried distillers grain arabinoxylan

In Table 3.30, the tear resistances of all films are averaged across the type of AX used (WB, MB, or DDG), the type pf plasticizer used (glycerol or sorbitol), and the level of plasticizer used (100, 250, or 500 g kg^{-1}). The general trend seen was that WB AX films had the highest tear resistance, followed by MB

AX films, and lastly the DDG AX films. However, the difference in the average tear resistances of the WB AX films and the MB AX films was not significant ($P \leq 0.01$). In addition, the trend observed in average extensibility was that the MB AX films were the most extensible, followed by the DDG AX films, and finally the WB AX films. The differences in the extensibilities of these films were significant ($P \leq 0.01$). When the tear resistances and extensions of AX films were averaged across the type of plasticizer, there was a decrease in tear resistance but an increase in extension when glycerol was used instead of sorbitol. This difference in tear resistance was not significantly ($P \leq 0.01$) different. However, the difference in extension was significantly ($P \leq 0.01$) different. These differences were most likely due to the small size of glycerol that allowed it to insert more fully into the AX chains. This chain disruption results in a decrease in strength, but an increase in flexibility. Similarly, as the level of plasticizer used in the AX films increased, so did the extension observed. An increase in plasticizer level resulted in increased disruption of the polymer chain but increased flexibility. This was also seen in the significant ($P \leq 0.01$) decrease in tear resistance when the plasticizer level was increased from 250 g kg^{-1} to 500 g kg^{-1}. These results are in accordance with those published by Hong-rui et al. (2014).

Table 3.30. Mean separation of tear resistance properties of arabinoxylan films.

Arabinoxylan type	Plasticizer type	Plasticizer level (g kg^{-1})	Load (N)	Extension (mm)
WB[a]			1.29	1.12
MB[b]			1.26	8.91
DDG[c]			0.90	5.41
LSD ($P \leq 0.05$)[d]			0.15	1.25
LSD ($P \leq 0.01$)			0.21	1.65
	Glycerol		1.11	9.62
	Sorbitol		1.20	0.67
	LSD ($P \leq 0.05$)		0.13	1.02
	LSD ($P \leq 0.01$)		0.17	1.35
		100	1.08	0.38
		250	1.32	1.06
		500	1.06	13.99
		LSD ($P \leq 0.05$)	0.15	1.25
		LSD ($P \leq 0.01$)	0.21	1.65

[a] Wheat bran arabinoxylan
[b] Maize bran arabinoxylan
[c] Dried distillers grain arabinoxylan
[d] Least significant difference

There were multiple significant ($P \leq 0.05$) interactions for the tear resistance and extensibility of the AX films. The significant ($P \leq 0.05$) interactions that affected the tear resistance of the films included AX type by plasticizer level, plasticizer type by plasticizer level, and the three-way interaction between AX type, plasticizer type, and plasticizer level. Firstly, the significance ($P \leq 0.05$) of the two-way interactions is that they demonstrate that the effects of these factors combine to impact the tear resistance and extensibility of the materials. The mean separation for these two-way interactions is shown in Table 3.31.

Table 3.31. Mean separation for the significant ($P \leq 0.05$) two-way interactions affecting the tear resistance of arabinoxylan films.

Arabinoxylan type	Plasticizer type	Plasticizer level ($g\ kg^{-1}$)	Load (N)	Extension (mm)
WB[a]		100	1.10	NS
WB		250	1.39	NS
WB		500	1.39	NS
MB[b]		100	1.50	NS
MB		250	1.50	NS
MB		500	0.77	NS
DDG[c]		100	0.64	NS
DDG		250	1.06	NS
DDG		500	1.01	NS
LSD ($P \leq 0.05$)[d]			0.27	NS
LSD ($P \leq 0.01$)			0.36	NS
	Glycerol	100	1.36	NS
	Glycerol	250	1.43	NS
	Glycerol	500	0.54	NS
	Sorbitol	100	0.81	NS
	Sorbitol	250	1.20	NS
	Sorbitol	500	1.58	NS
	LSD ($P \leq 0.05$)		0.22	NS
	LSD ($P \leq 0.01$)		0.29	NS
WB	Glycerol		NS[e]	1.74
WB	Sorbitol		NS	0.50
MB	Glycerol		NS	17.15
MB	Sorbitol		NS	0.66
DDG	Glycerol		NS	9.98
DDG	Sorbitol		NS	0.83
LSD ($P \leq 0.05$)				1.76
LSD ($P \leq 0.01$)				2.34

[a] Wheat bran
[b] Maize bran
[c] Dried distillers grain
[d] Least significant difference
[e] Not significant ($P \leq 0.05$)

The three-way interaction between AX type, plasticizer type, and plasticizer level was also significant ($P \leq 0.05$) for both the tear resistance and elongation of the films as seen in Table 3.32. The interaction of AX type and plasticizer level over all films made with glycerol is shown in Figure 3.14. It is clear from this figure that there was an interaction between the level of plasticizer and type of AX used for the MB AX films and WB AX films. Films made with MB AX or the DDG AX also show the same crossover response. However, there was no interaction between plasticizer level and AX type for the WB AX films and DDG AX films for films made with glycerol. This indicates that the effects of AX type and plasticizer level on the tear resistance of the films were influenced by the use of glycerol. The combined interaction of these factors resulted in an overall decrease in tear resistance as the plasticizer level increased. In Figure 3.15., the interaction of AX type and plasticizer level for films made with sorbitol are shown. Similar to the films made with glycerol, there were crossover responses for AX films made with MB AX and DDG AX, as well as for films made with MB AX and WB AX. In addition, there was no true interaction for films made with WB AX and DDG AX over all levels of plasticizer for films made with sorbitol. These trends show that the tear resistance of these AX films was affected by the levels of plasticizer and type of AX when sorbitol was used as the plasticizer. Overall, there appears to have been an increase in tear resistance for films made with sorbitol as the plasticizer level increased. The three-way interaction for the extension of AX films is graphically represented in Figure 3.16 for AX type and increasing levels of plasticizer for films made with glycerol. Figure 3.17 shows the same three-way interaction but for films made with sorbitol instead of glycerol. Both figures show the same crossover response for the three types of AX over increasing levels of plasticizer. As the level of plasticizer increased, so did the extension of the AX films. However, the exact extension of the films was affected by the interaction of all three main factors (AX type, plasticizer type, and plasticizer level).

In work published by Zivanovic et al. (2005), it was stated that chitosan films do not have a tendency to tear. However, tear resistances as low as 1.9 N have been noted by others for chitosan films (Kittur et al. 1998). This tear resistance is comparable to those of the AX films developed in this research. Polyvinyl chloride has a much higher tear resistance (around 10 kN m^{-1}), but this is highly dependent upon extrusion temperature (Rabinovitch 2003).

Table 3.32. Mean separation for the three-way interaction between arabinoxylan type, plasticizer type, and plasticizer level for tear resistance properties of arabinoxylan films.

Arabinoxylan type	Plasticizer type	Plasticizer level (g kg $^{-1}$)	Load (N)	Extension (mm)
WB[a]	Glycerol	100	1.38	0.26
WB	Glycerol	250	1.35	0.91
WB	Glycerol	500	0.85	4.05
MB[b]	Glycerol	100	1.73	0.77
MB	Glycerol	250	1.48	2.25
MB	Glycerol	500	0.32	48.43
DDG[c]	Glycerol	100	0.95	0.29
DDG	Glycerol	250	1.46	2.14
DDG	Glycerol	500	0.44	27.50
WB	Sorbitol	100	0.82	0.45
WB	Sorbitol	250	1.42	0.20
WB	Sorbitol	500	1.94	0.85
MB	Sorbitol	100	1.27	0.23
MB	Sorbitol	250	1.53	0.44
MB	Sorbitol	500	1.22	1.30
DDG	Sorbitol	100	0.33	0.27
DDG	Sorbitol	250	0.65	0.42
DDG	Sorbitol	500	1.58	1.79
LSD ($P \leq 0.05$)[a]			0.38	3.05
LSD ($P \leq 0.01$)			0.50	4.05

[a] Wheat bran
[b] Maize bran
[c] Dried distillers grain
[d] Least significant difference

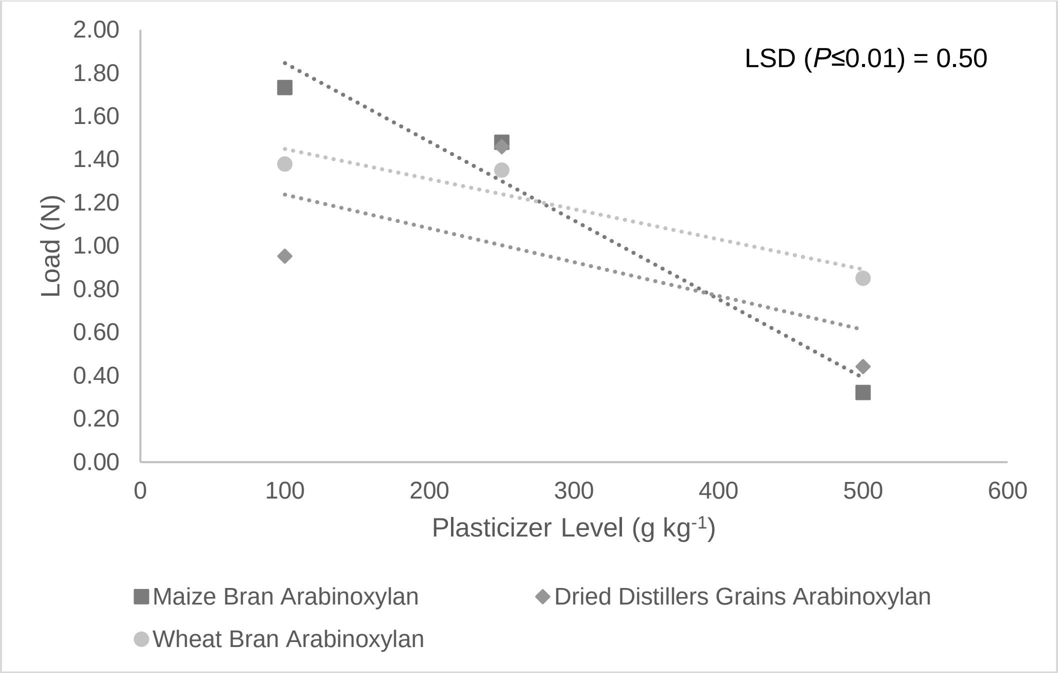

Figure 3.14. Three-way interaction for the load of arabinoxylan type and plasticizer levels for arabinoxylan films made with glycerol.

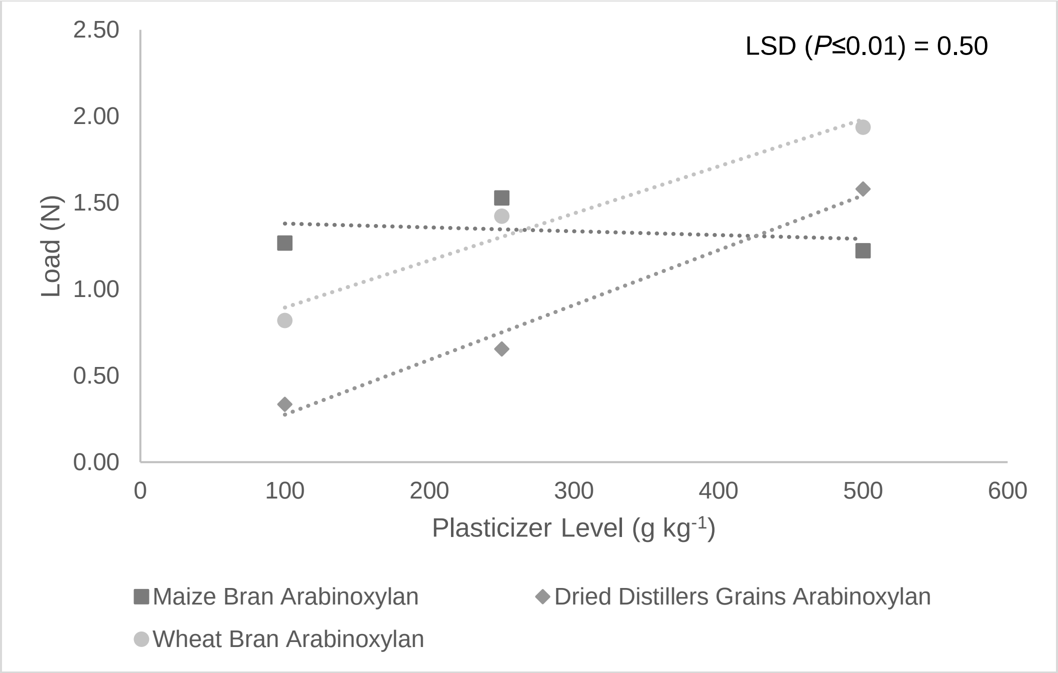

Figure 3.15. Three-way interaction for the load of arabinoxylan type and plasticizer levels for arabinoxylan films made with sorbitol.

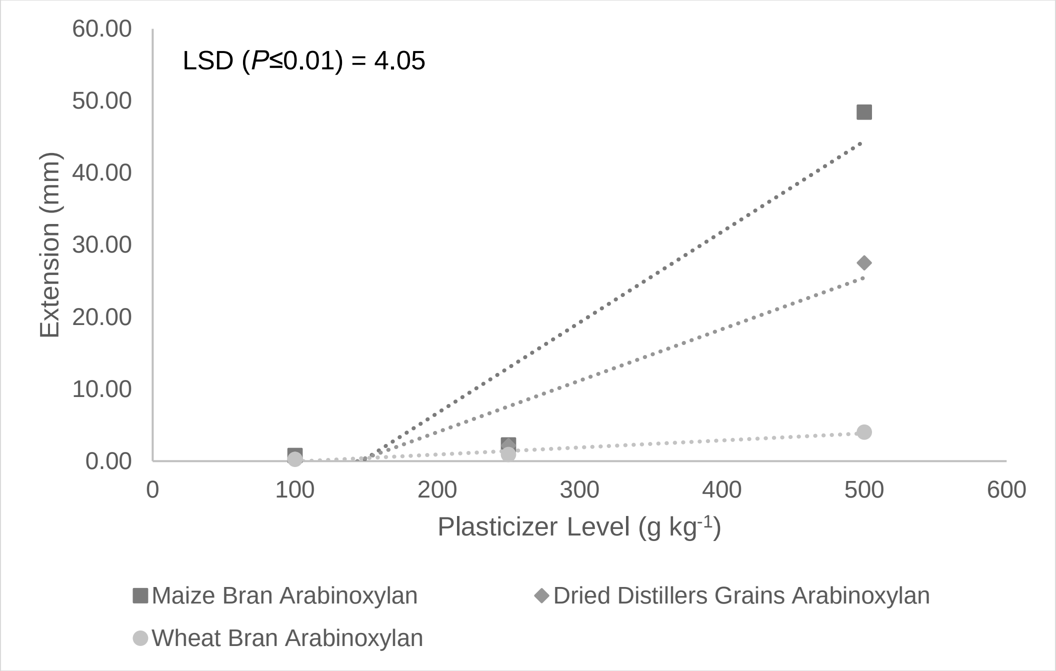

Figure 3.16. Three-way interaction for the extension of arabinoxylan type and plasticizer levels for arabinoxylan films made with glycerol.

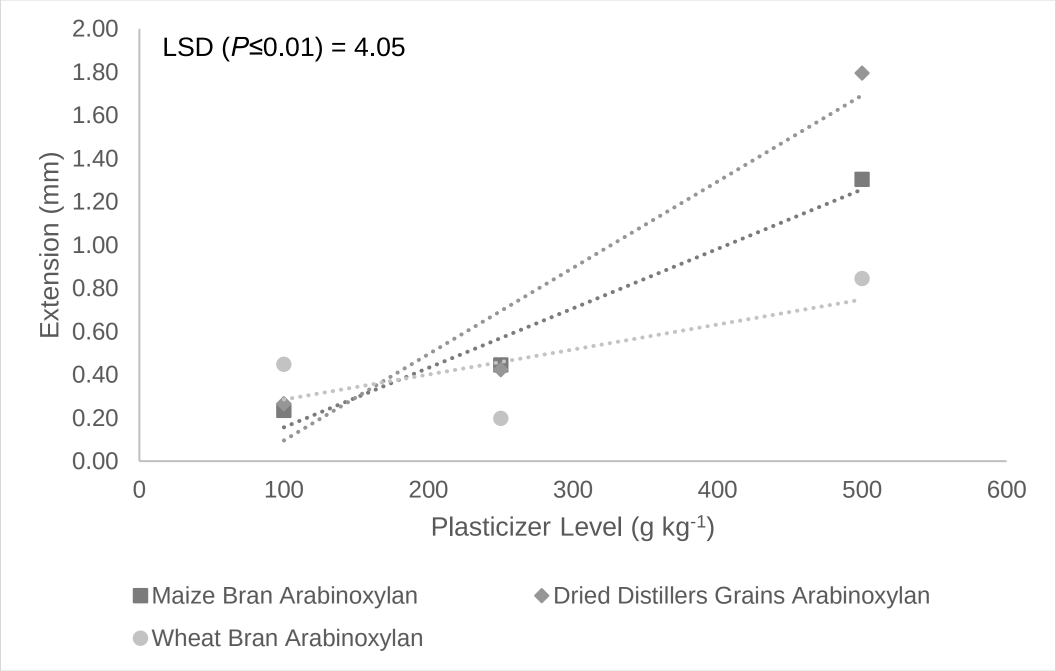

Figure 3.17. Three-way interaction for the extension of arabinoxylan type and plasticizer levels for arabinoxylan films made with sorbitol.

There were two significant (*P*≤0.05) correlations present between the tear resistance characteristics of the AX films and their chemical properties as seen in Table 3.33. Firstly, there was a significant (*P*≤0.01 for films made with sorbitol and *P*≤0.05 for films made with glycerol) correlation between the plasticizer level used in the films and the extension of the films. This correlation shows that as the plasticizer level increased, so did the extension of the film. As previously discussed, this is in accordance with the work of Hong-rui et al. (2014). Similarly, the load withstood before tear propagation was significantly (*P*≤0.05) correlated to the plasticizer level of glycerol films, and as the plasticizer level increased, the load before propagation of the tear decreased. This was due to the disruption of the polymer chains by the addition of plasticizer. The final significant (*P*≤0.05) correlation was between the Mw of the AX films made with sorbitol and their tear resistance loads. This correlation shows that as the Mw increased, so did the puncture resistance of the films. When the films were composed of larger AX polymers, there were fewer fracture points due to gaps between the AX polymers, which resulted in an increased resistance to propagation of a tear.

Table 3.33. Correlations between tear resistance characteristics (load and extension) and the plasticizer level, polydispersity index, and molecular weights of arabinoxylan films and arabinoxylan film solutions.

	Load (N)	Extension (mm)
	Films made with sorbitol	
Plasticizer level	0.655	0.836**
PI[a] for 24 hours of stirring	0.438	-0.258
Mw[b] for 24 hours of stirring	0.464	-0.173
PI for heated solutions	-0.387	0.111
Mw for heated solutions	0.287	-0.222
PI for solutions with plasticizer	-0.585	0.205
Mw for solutions with plasticizer	0.169	0.083
PI for films	0.281	0.466
Mw for films	0.760*	0.430
	Films made with glycerol	
Plasticizer level	-0.782*	0.713*
PI[a] for 24 hours of stirring	0.202	-0.241
Mw[b] for 24 hours of stirring	0.222	0.111
PI for heated solutions	-0.188	-0.213
Mw for heated solutions	0.128	-0.351
PI for solutions with plasticizer	-0.009	-0.303
Mw for solutions with plasticizer	-0.147	-0.284
PI for films	-0.538	0.306
Mw for films	-0.235	0.001

* Significant at *P*≤0.05

** Significant at *P*≤0.01

3.4.5.6. Contact Angle

The contact angle and wetting tension of a material provide information about their hydrophilicity. The first liquid used in contact angle determination was water. Due to the polarity of this liquid, as the contact angle decreased, the hydrophilicity of the films increased (Phan The et al. 2009). The surface wettability is a measure of the ability of the film to be hydrated by the liquid used. Some of the AX films had a high hydrophilicity, and as such it was difficult to measure the contact angle and surface wetting tension using water (Peroval et al. 2002). Thus, mineral oil was also used to determine surface hydrophobicity of the films. The contact angles and wetting tensions of all films were determined for both sides of the films. The smooth side was the side that was facing the petri dish during film drying, and the rough side was exposed to the air during drying. An example of the image obtained during the measurement of contact angle with water is shown in Figure 3.18. Table 3.34 provides the contact angles and wetting tensions for all AX films.

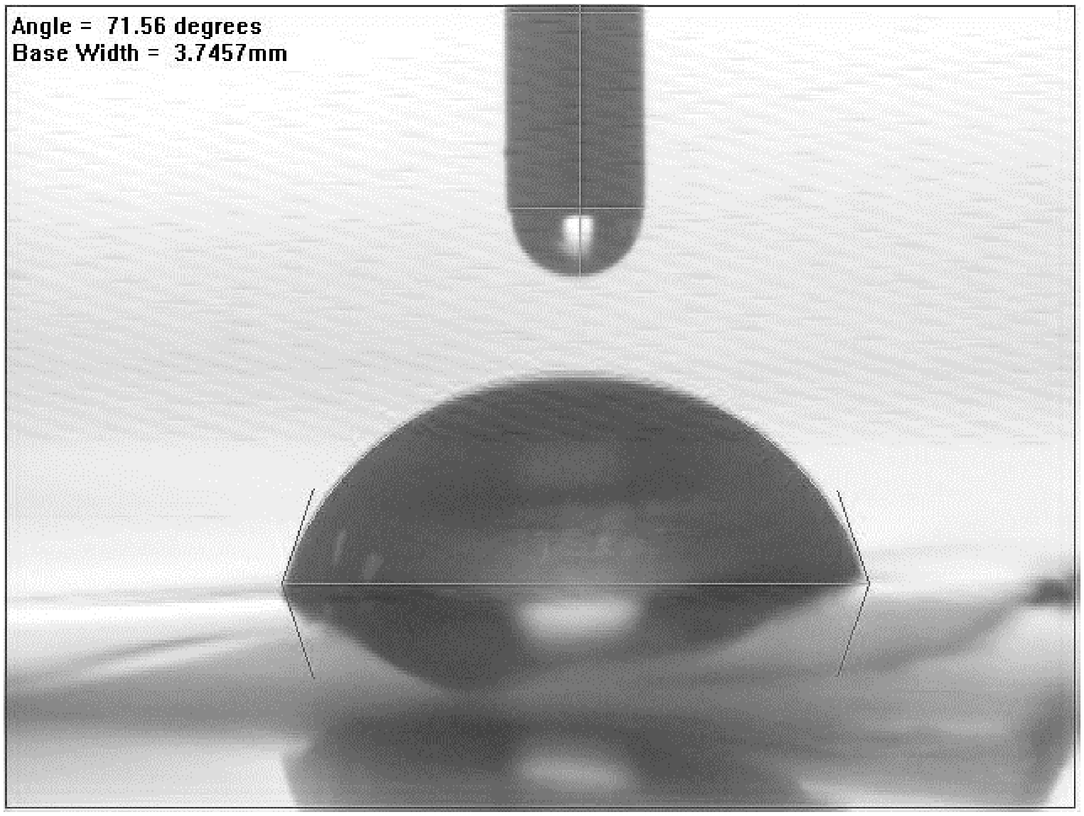

Figure 3.18. Contact angle (with water) of a maize bran arabinoxylan film plasticized with 100 g kg^{-1} glycerol.

Table 3.34. Contact angles and wetting tensions of arabinoxylan films with water.

Film composition	Contact angle (deg)	Wetting tension (mN m^{-1})
	Smooth side of films	
WB AX[a] + 100 g kg^{-1} Sorbitol	62.81 ± 0.66	33.27 ± 0.75
WB AX + 250 g kg^{-1} Sorbitol	63.97 ± 1.68	31.94 ± 1.91
WB AX + 500 g kg^{-1} Sorbitol	66.59 ± 0.34	30.02 ± 1.15
WB AX + 100 g kg^{-1} Glycerol	76.71 ± 0.68	17.02 ± 1.24
WB AX + 250 g kg^{-1} Glycerol	101.30 ± 0.47	-14.26 ± 0.58
WB AX + 500 g kg^{-1} Glycerol	102.01 ± 0.39	-15.15 ± 0.49
MB AX[b] + 100 g kg^{-1} Sorbitol	71.03 ± 0.78	23.66 ± 0.93
MB AX + 250 g kg^{-1} Sorbitol	71.52 ± 0.69	23.08 ± 0.84
MB AX + 500 g kg^{-1} Sorbitol	74.25 ± 1.04	19.77 ± 1.27
MB AX + 100 g kg^{-1} Glycerol	71.10 ± 0.66	23.58 ± 0.79
MB AX + 250 g kg^{-1} Glycerol	82.25 ± 3.00	9.81 ± 3.77
MB AX + 500 g kg^{-1} Glycerol	64.45 ± 0.51	31.40 ± 0.58
DDG AX[c] + 100 g kg^{-1} Sorbitol	54.23 ± 2.67	42.54 ± 2.75
DDG AX + 250 g kg^{-1} Sorbitol	52.49 ± 0.01	44.33 ± 0.01
DDG AX + 500 g kg^{-1} Sorbitol	62.29 ± 0.75	33.85 ± 0.85
DDG AX + 100 g kg^{-1} Glycerol	61.27 ± 3.56	34.96 ± 3.97
DDG AX + 250 g kg^{-1} Glycerol	46.67 ± 1.21	49.96 ± 1.12
DDG AX + 500 g kg^{-1} Glycerol	53.88 ± 0.61	42.91 ± 0.62
	Rough side of films	
WB AX + 100 g kg^{-1} Sorbitol	97.12 ± 2.35	-9.02 ± 2.96
WB AX + 250 g kg^{-1} Sorbitol	128.64 ± 5.15	-45.37 ± 5.11
WB AX + 500 g kg^{-1} Sorbitol	97.15 ± 0.40	-9.06 ± 0.51
WB AX + 100 g kg^{-1} Glycerol	68.56 ± 4.39	26.58 ± 5.19
WB AX + 250 g kg^{-1} Glycerol	105.31 ± 1.79	-19.21 ± 2.19
WB AX + 500 g kg^{-1} Glycerol	107.44 ± 1.92	-21.81 ± 2.33
MB AX + 100 g kg^{-1} Sorbitol	80.61 ± 0.34	11.88 ± 0.43
MB AX + 250 g kg^{-1} Sorbitol	72.24 ± 1.58	22.21 ± 1.91
MB AX + 500 g kg^{-1} Sorbitol	76.64 ± 0.06	16.83 ± 0.08
MB AX + 100 g kg^{-1} Glycerol	95.31 ± 4.09	-6.73 ± 5.16
MB AX + 250 g kg^{-1} Glycerol	100.66 ± 2.01	-13.46 ± 2.50
MB AX + 500 g kg^{-1} Glycerol	75.39 ± 3.44	18.35 ± 4.22
DDG AX + 100 g kg^{-1} Sorbitol	70.57 ± 0.04	24.22 ± 0.04
DDG AX + 250 g kg^{-1} Sorbitol	71.57 ± 0.71	23.02 ± 0.85
DDG AX + 500 g kg^{-1} Sorbitol	71.72 ± 1.48	22.83 ± 1.78
DDG AX + 100 g kg^{-1} Glycerol	67.84 ± 0.15	27.47 ± 0.18
DDG AX + 250 g kg^{-1} Glycerol	75.50 ± 1.34	18.23 ± 1.64
DDG AX + 500 g kg^{-1} Glycerol	55.53 ± 0.86	41.21 ± 0.89

[a] Wheat bran arabinoxylan

[b] Maize bran arabinoxylan

[c] Dried distillers grain arabinoxylan

To determine the significant (*P*≤0.05 or *P*≤0.01) differences between the contact angles and wetting tensions of AX films with water, mean separation was performed and is shown in Table 3.35. To begin, the differences in these materials properties was determined by the type of AX used in the films. For both the smooth and rough sides of the AX films, the contact angles were significantly (*P*≤0.01) different between the three types of AX used. The contact angle decreased moving from WB AX to MB AX to DDG AX. This indicates that the hydrophilicity increased in the corresponding order: WB AX, MB AX, and DDG AX. This hydrophilicity was also shown in the wetting tensions of these films. For both the smooth and rough sides of the films, the wetting tension increased significantly (*P*≤0.01) in the following order: WB AX, MB AX, DDG AX. When the contact angles and wetting tensions of the smooth side of the film were compared to those of the rough side, the smooth side had a smaller contact angle and larger wetting tension. This indicates that the smooth side of the films were more hydrophilic than the rough side.

The hydrophilicity of the films was also averaged across the type of plasticizer used to make the film. For both the smooth and rough sides of the AX films, the contact angle was higher for films made with glycerol than those made with sorbitol. This difference was significant (*P*≤0.01) for the smooth side of the films, and *P*≤0.05 for the rough side of the films) for all films. In addition, the wetting tensions of the smooth sides of the films made with glycerol were significantly (*P*≤0.01) lower than those of the films made with sorbitol. There were no significant (*P*≤0.01) differences between the wetting tensions of the rough sides of the films when averaged across the type of plasticizer used. Overall, the smooth sides of the AX films were more hydrophilic than the rough sides, indicated by the increase in contact angles when moving from the smooth side to the rough side of the films.

Table 3.35. Mean separation of contact angles and wetting tensions of arabinoxylan films with water.

Arabinoxylan type	Plasticizer type	Plasticizer level ($g\ kg^{-1}$)	Contact angle (deg)	Wetting tension ($mN\ m^{-1}$)
			Smooth side of films	
WB[a]			78.90	13.81
MB[b]			72.43	21.88
DDG[c]			55.14	41.42
LSD (*P*≤0.05)[d]			1.25	1.45
LSD (*P*≤0.01)			1.71	1.99
	Glycerol		73.29	20.03
	Sorbitol		64.35	31.38
	LSD (*P*≤0.05)		1.02	1.19
	LSD (*P*≤0.01)		1.40	1.63
		100	66.19	29.17
		250	69.70	24.14
		500	70.58	23.80
		LSD (*P*≤0.05)	1.25	1.45
		LSD (*P*≤0.01)	1.71	1.99
			Rough side of films	
WB			100.70	-12.98
MB			83.47	8.18
DDG			68.79	26.16
LSD (*P*≤0.05)			2.01	2.34
LSD (*P*≤0.01)			2.75	3.21
	Glycerol		83.50	7.85
	Sorbitol		85.14	6.39
	LSD (*P*≤0.05)		1.64	1.91
	LSD (*P*≤0.01)		2.25	2.62
		100	80.00	12.40
		250	92.32	-2.43
		500	80.64	11.39
		LSD (*P*≤0.05)	2.01	2.34
		LSD (*P*≤0.01)	2.75	3.21

[a] Wheat bran
[b] Maize bran
[c] Dried distillers grain
[d] Least significant difference

When the hydrophilicity of the AX films was averaged across the level of plasticizer used, the smooth sides of the films and the rough sides had different results. For the smooth side of the films, a decrease in hydrophilicity was observed as plasticizer content increased. There was an increase in contact angle and a decrease in wetting tension as the level of plasticizer used increased. These results show the same trend as those of previously published work with variation in plasticizer levels and surface hydrophilicity (Casariego et al. 2008). However, when the hydrophilicity of the rough side of the films was analyzed, a different trend was seen. The contact angle for the rough side of the AX films was significantly ($P \leq 0.01$) higher for films made with 250 g kg^{-1} plasticizer than those made with 100 g kg^{-1} and 500 g kg^{-1} plasticizer. The difference in contact angle for the rough side of the films made with 100 g kg^{-1} and those made with 500 g kg^{-1} were not significantly ($P \leq 0.01$) different. In accordance with this trend, the wetting tension was not significantly ($P \leq 0.01$) different for the rough sides of films made with 100 g kg^{-1} plasticizer and those made with 500 g kg^{-1} plasticizer. However, the wetting tension was significantly ($P \leq 0.01$) lower for the films made with 250 g kg^{-1} plasticizer than those made with either 100 or 500 g kg^{-1}. This variation in trend could be due to the variation in surface characteristics in the rough sides of the films because they were exposed to an environment that was less controlled than the smooth sides of the films.

All two- and three-way interactions between the main factors of the film composition including AX type, plasticizer type, and plasticizer level were significant ($P \leq 0.05$). Mean separation for all two-way interactions is shown in Table 3.36. The significance of these two-way interactions is that when two of the factors interacted, the contact angle and wetting tension of the films were affected by these interactions rather than solely the influence of each factor individual.

Table 3.36. Mean separation for the significant ($P\le0.05$) two-way interactions affecting the hydrophilicity of the arabinoxylan films measured with water.

Arabinoxylan type	Plasticizer type	Plasticizer level (g kg^{-1})	Contact angle (deg)	Wetting tension (mN m^{-1})
			Smooth side of films	
WB[a]	Glycerol		93.34	-4.13
WB	Sorbitol		64.45	31.74
MB[b]	Glycerol		72.60	21.60
MB	Sorbitol		72.27	22.17
DDG[c]	Glycerol		53.94	42.61
DDG	Sorbitol		56.34	40.24
LSD ($P\le0.05$)[d]			1.77	2.06
LSD ($P\le0.01$)			2.42	2.82
WB		100	69.76	25.14
WB		250	82.63	8.84
WB		500	84.30	7.44
MB		100	71.06	23.62
MB		250	76.89	16.44
MB		500	69.35	25.58
DDG		100	57.75	38.75
DDG		250	49.58	47.14
DDG		500	58.09	38.38
LSD ($P\le0.05$)			2.17	2.52
LSD ($P\le0.01$)			2.97	3.45
	Glycerol	100	69.69	25.19
	Glycerol	250	76.74	15.17
	Glycerol	500	73.45	19.72
	Sorbitol	100	62.69	33.16
	Sorbitol	250	62.66	33.12
	Sorbitol	500	67.71	27.88
	LSD ($P\le0.05$)		1.77	2.06
	LSD ($P\le0.01$)		2.42	2.82
			Rough side of films	
WB	Glycerol		93.77	-4.81
WB	Sorbitol		107.64	-21.15
MB	Glycerol		90.45	-0.61
MB	Sorbitol		76.49	16.97
DDG	Glycerol		66.29	28.97
DDG	Sorbitol		71.29	23.36
LSD ($P\le0.05$)			2.84	3.31
LSD ($P\le0.01$)			3.89	4.54
WB		100	82.84	8.78
WB		250	116.97	-32.29
WB		500	102.29	-15.43
MB		100	87.96	2.57
MB		250	86.45	4.38
MB		500	76.01	17.59
DDG		100	69.20	25.84
DDG		250	73.53	20.63
DDG		500	63.62	32.02
LSD ($P\le0.05$)			3.48	4.06
LSD ($P\le0.01$)			4.77	5.56
	Glycerol	100	77.23	15.77
	Glycerol	250	93.82	-4.81
	Glycerol	500	79.45	12.58
	Sorbitol	100	82.77	9.03
	Sorbitol	250	90.82	-0.05
	Sorbitol	500	81.83	10.20
	LSD ($P\le0.05$)		2.84	3.31
	LSD ($P\le0.01$)		3.89	4.54

[a] Wheat bran

[b] Maize bran

[c] Dried distillers grain

[d] Least significant difference

In addition to the significant ($P \leq 0.05$) two-way interactions, the three-way interaction of all main factors is also significant ($P \leq 0.05$) as seen in Table 3.37. These correlations for the contact angles are also graphically represented by Figures 3.19 through 3.22. The three-way interactions for wetting tension are shown in Figures 3.23 through 3.26. From these figures, the responses of each factor in the three-way interactions are shown. Firstly, in the films made with glycerol, the effect of the interaction between AX type and plasticizer level had a diverging response for the MB AX and WB AX as plasticizer level increased for contact angle. For the rough side of the films, there was a crossover response for all types of AX. Secondly, when sorbitol was used instead of glycerol, there was a converging response for the interaction of AX type and plasticizer level for films made with glycerol for DDG AX films and WB AX films for the contact angle of the smooth side of the films. The three-way interaction influencing the contact angles of the rough side of the films had no true interaction. However, the responses for wetting tension are the inverse of those for contact angle. For example, for the smooth side of films made with glycerol, the response of MB AX films over increasing levels of plasticizer showed an increase in contact angle, but a decrease in wetting tension. This relationship was present because as contact angle increased, showing a decrease in hydrophilicity, the wetting tension decreased.

Table 3.37. Mean separation for the significant ($P \le 0.05$) three-way interaction between arabinoxylan type, plasticizer type, and plasticizer level affecting the hydrophilicity of the arabinoxylan films measured with water.

Arabinoxylan type	Plasticizer type	Plasticizer level (g kg^{-1})	Contact angle (deg)	Wetting tension (mN m^{-1})
			Smooth side of films	
WB[a]	Glycerol	100	76.71	17.02
WB	Glycerol	250	101.30	-14.26
WB	Glycerol	500	102.01	-15.15
MB[b]	Glycerol	100	71.10	23.58
MB	Glycerol	250	82.25	9.81
MB	Glycerol	500	64.45	31.40
DDG[c]	Glycerol	100	61.27	34.96
DDG	Glycerol	250	46.67	49.96
DDG	Glycerol	500	53.88	42.91
WB	Sorbitol	100	62.81	33.27
WB	Sorbitol	250	63.97	31.94
WB	Sorbitol	500	66.59	30.02
MB	Sorbitol	100	71.03	23.66
MB	Sorbitol	250	71.52	23.08
MB	Sorbitol	500	74.25	19.77
DDG	Sorbitol	100	54.23	42.54
DDG	Sorbitol	250	52.49	44.33
DDG	Sorbitol	500	62.29	33.85
LSD ($P \le 0.05$)[d]			3.06	3.56
LSD ($P \le 0.01$)			4.20	4.88
			Rough side of films	
WB	Glycerol	100	68.56	26.58
WB	Glycerol	250	105.31	-19.21
WB	Glycerol	500	107.44	-21.81
MB	Glycerol	100	95.31	-6.73
MB	Glycerol	250	100.66	-13.46
MB	Glycerol	500	75.39	18.35
DDG	Glycerol	100	67.84	27.47
DDG	Glycerol	250	75.50	18.23
DDG	Glycerol	500	55.53	41.21
WB	Sorbitol	100	97.12	-9.02
WB	Sorbitol	250	128.64	-45.37
WB	Sorbitol	500	97.15	-9.06
MB	Sorbitol	100	80.61	11.88
MB	Sorbitol	250	72.24	22.21
MB	Sorbitol	500	76.64	16.83
DDG	Sorbitol	100	70.57	24.22
DDG	Sorbitol	250	71.57	23.02
DDG	Sorbitol	500	71.72	22.83
LSD ($P \le 0.05$)			4.92	5.74
LSD ($P \le 0.01$)			6.74	7.86

[a] Wheat bran

[b] Maize bran

[c] Dried distillers grain

[d] Least significant difference

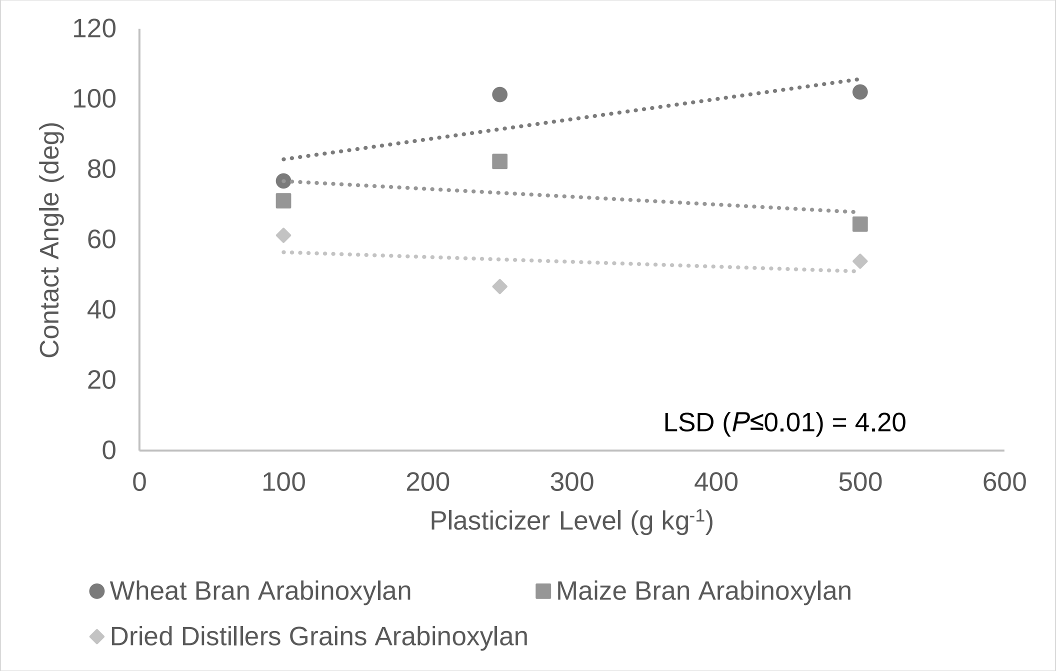

Figure 3.19. Three-way interaction for contact angle with water of the smooth side of films made with glycerol.

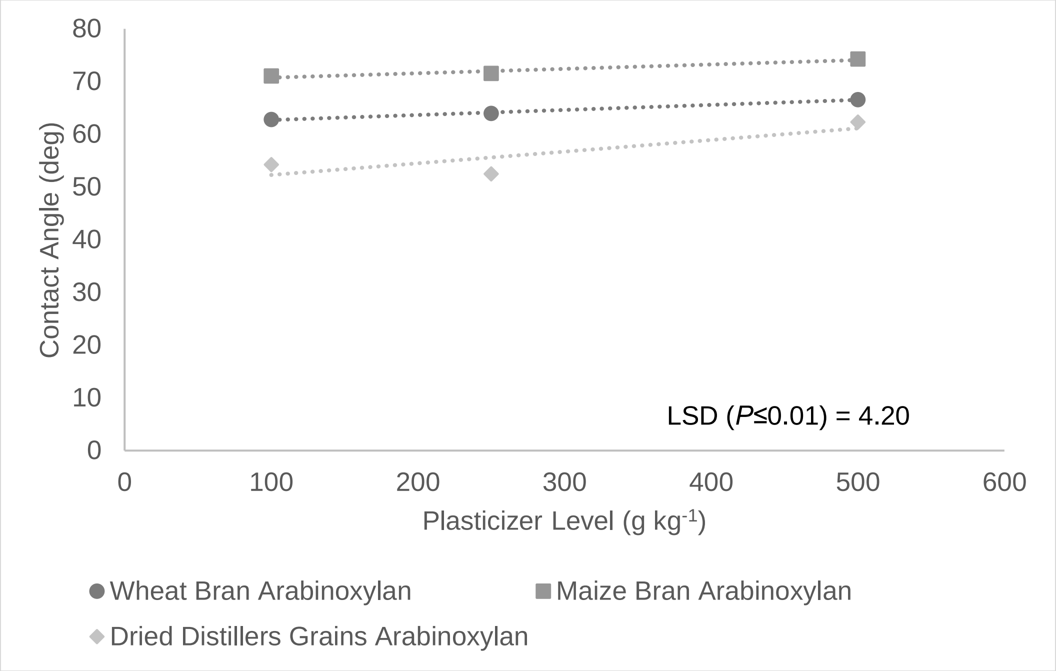

Figure 3.20. Three-way interaction for contact angle with water of the smooth side of films made with sorbitol.

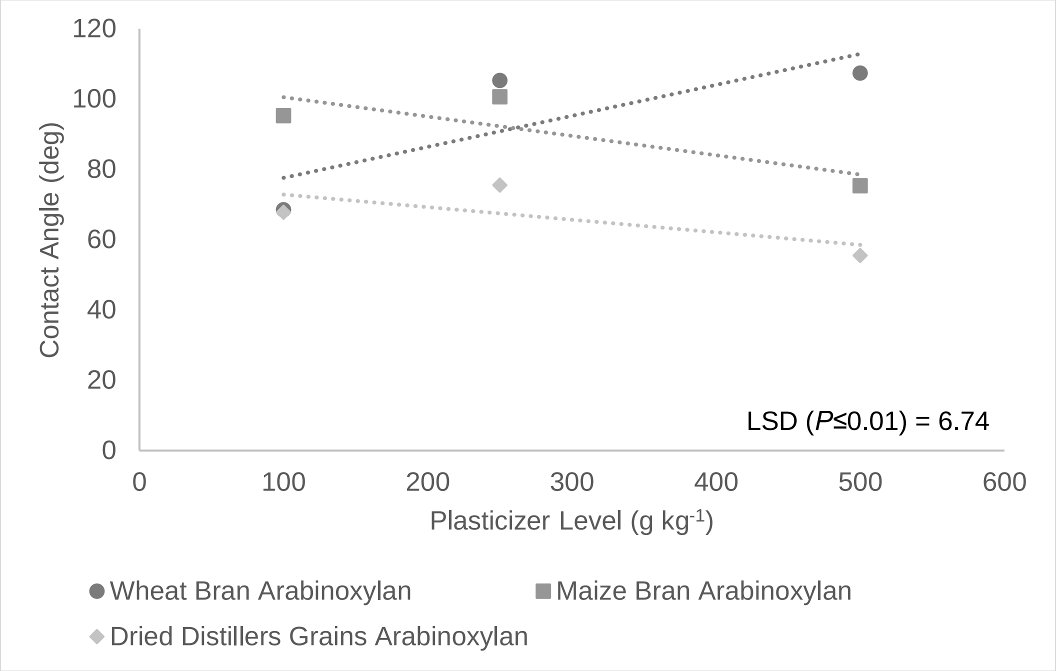

Figure 3.21. Three-way interaction for contact angle with water of the rough side of films made with glycerol.

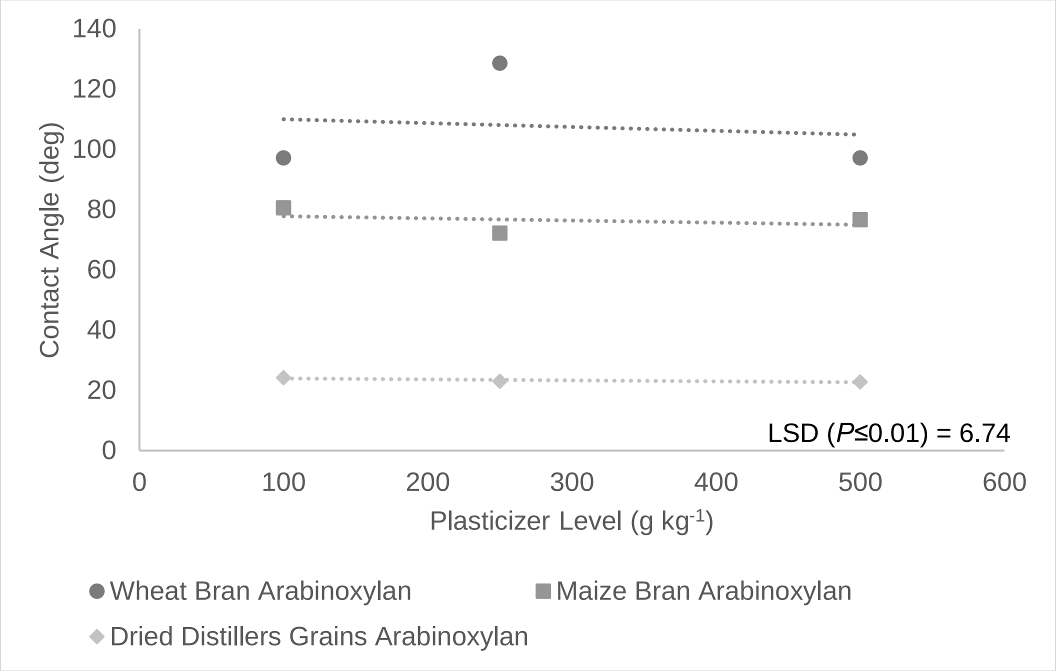

Figure 3.22. Three-way interaction for contact angle with water of the rough side of films made with sorbitol.

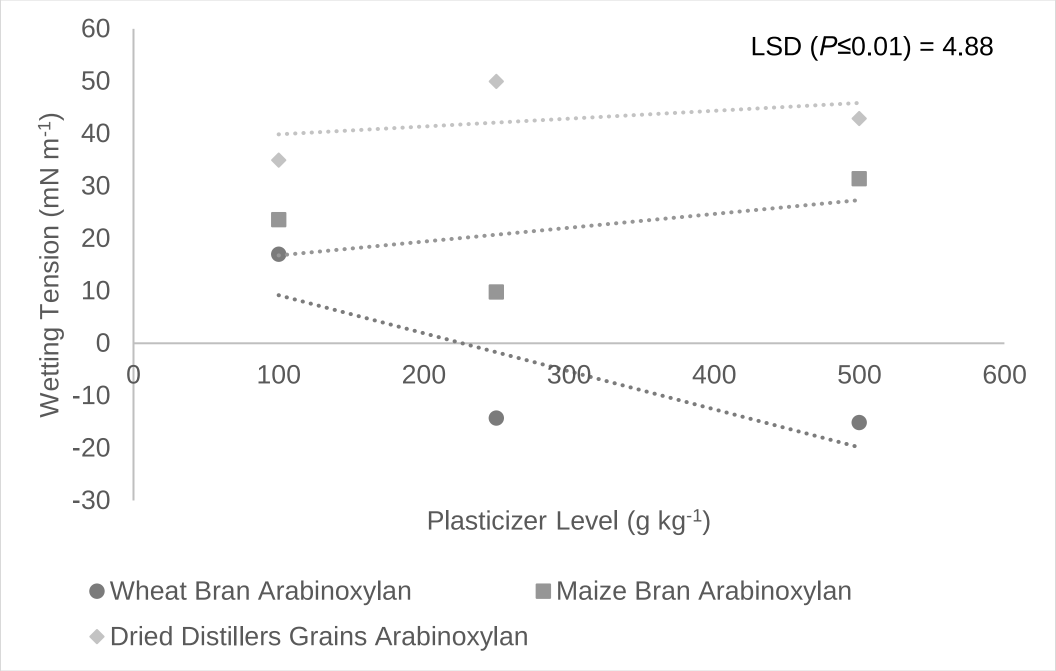

Figure 3.23. Three-way interaction for wetting tension with water of the smooth side of films made with glycerol.

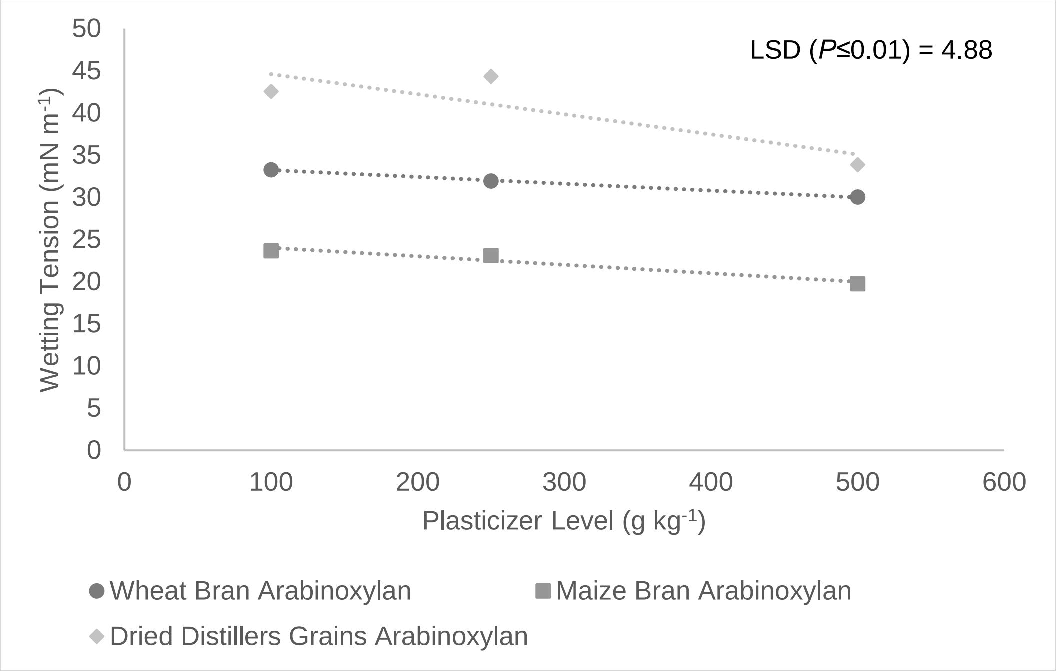

Figure 3.24. Three-way interaction for wetting tension with water of the smooth side of films made with sorbitol.

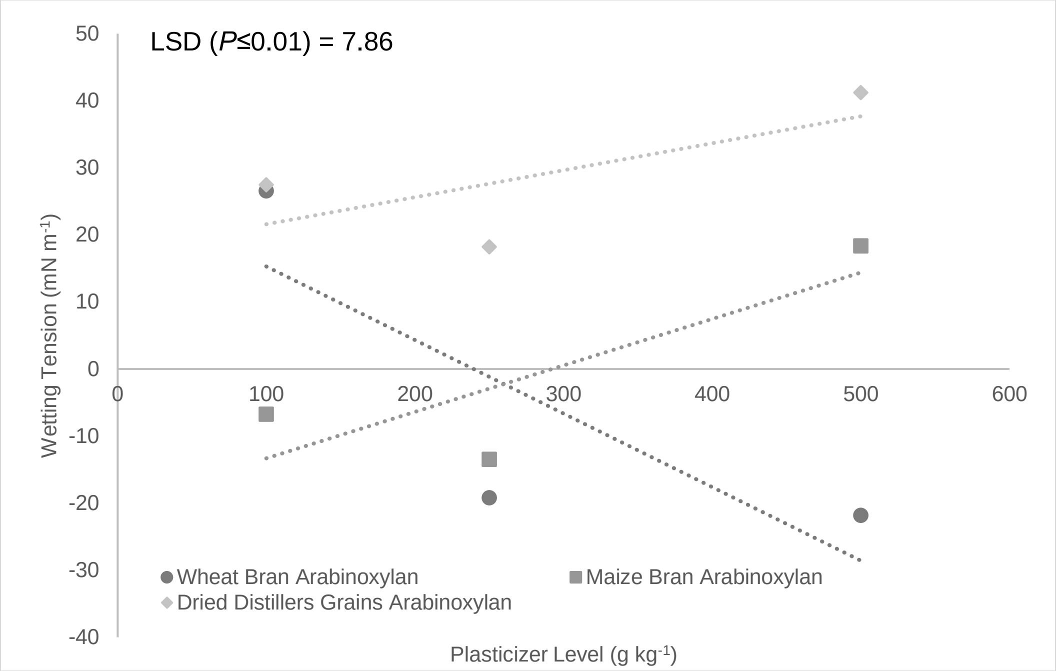

Figure 3.25. Three-way interaction for wetting tension with water of the rough side of films made with glycerol.

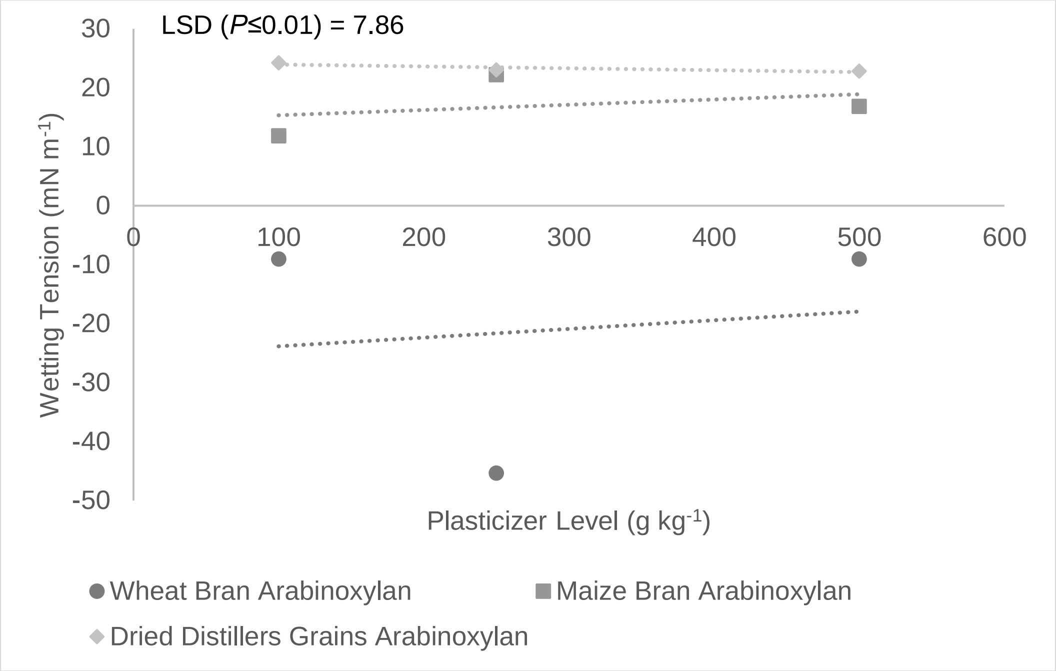

Figure 3.26. Three-way interaction for wetting tension with water of the rough side of films made with sorbitol.

There were many correlations present between the hydrophilic characteristics of the AX films and their chemical properties. These correlations are shown in Table 3.38 for the smooth sides of the films. To begin, there was a significant ($P \leq 0.001$) correlation between the Mw of the film solutions after stirring for 24 hours and the contact angle and wetting tension of the films made with sorbitol. As the Mw of these solutions increased, the hydrophobicity of the film increased. There was also a significant ($P \leq 0.001$) correlation between the PI of the film solutions after heating and an increase in their hydrophilicity. Similarly, the PI for the solutions after the addition of plasticizer was significantly ($P \leq 0.01$) correlated to an increase in hydrophilicity of the films made with sorbitol. For the films made with glycerol, there were also three notable correlations between the hydrophilicity of the films and either the PI or Mw. Firstly, the PI after the film solutions were stirred for 24 hours was significantly ($P \leq 0.01$) correlated to a decrease in the hydrophilicity of the films. Secondly, the Mw of the solutions after heating was significantly ($P \leq 0.05$) correlated to a decrease in the hydrophilicity of the films made with glycerol. Thirdly, there was a significant ($P \leq 0.05$) correlation between the Mw of the films and a decrease in their hydrophilicity as the Mw of the films increase. Correlations between the linkage types and the hydrophilicity were also analyzed. There were two types of notable correlations present. Firstly, there was a significant ($P \leq 0.05$ or $P \leq 0.01$) correlation between an increase in the substitution of the AX and an increase in hydrophilicity. Secondly, there was a significant ($P \leq 0.05$ or $P \leq 0.01$) correlation between a decrease in the substitution of the AX and an increase in hydrophobicity.

There were also correlations between the hydrophilicity of the rough side of the AX films and their chemical properties, as seen in Table 3.39. To begin, there was a significant ($P \leq 0.01$) correlation between the PI of the film solutions after stirring for 24 hours and a decrease in hydrophilicity in films made with sorbitol. This correlation shows that as the PI increased, the hydrophilicity decreased. In addition, there was a significant ($P \leq 0.01$) correlation between an increase in the Mw of the film solutions after heating and a decrease in hydrophilicity. For the rough side of films made with glycerol, there were no notable correlations between PI or Mw and the hydrophilicity of the films. The same correlations between linkage and hydrophilicity were present for the rough sides of the films as for the smooth sides of the films.

Table 3.38. Correlations between hydrophilicity (measured with water) of the smooth side of arabinoxylan films and other chemical characteristics.

	Contact angle (deg)	Wetting tension (mN m^{-1})
	Films made with sorbitol	
Polydispersity index for 24 hours of stirring	0.393	-0.358
Molecular weight for 24 hours of stirring	0.905***	-0.904***
Polydispersity index for heated solutions	-0.917***	0.928***
Molecular weight for heated solutions	-0.001	0.044
Polydispersity index for solutions with plasticizer	-0.807**	0.802**
Molecular weights for solutions with plasticizer	-0.546	0.590
Polydispersity index for films	0.492	-0.495
Molecular weight for films	0.408	-0.363
R1	-0.867**	0.860**
R2	-0.794*	0.779*
R3	-0.682*	0.660
R4	-0.796*	0.782*
R5	-0.680*	0.658
R6	-0.779*	0.763*
R7	0.810**	-0.797*
	Films made with glycerol	
Polydispersity index for 24 hours of stirring	0.878**	-0.873**
Molecular weight for 24 hours of stirring	0.561	-0.539
Polydispersity index for heated solutions	-0.348	0.323
Molecular weight for heated solutions	0.769*	-0.775*
Polydispersity index for solutions with plasticizer	0.101	-0.135
Molecular weights for solutions with plasticizer	0.249	-0.272
Polydispersity index for films	0.446	-0.463
Molecular weight for films	0.772*	-0.784*
R1	-0.660	0.639
R2	-0.757*	0.739*
R3	-0.832**	0.818**
R4	-0.754*	0.736*
R5	-0.833**	0.819**
R6	-0.770*	0.753*
R7	0.740*	-0.722*

* Significant at $P \leq 0.05$

** Significant at $P \leq 0.01$

R1 = Arabinose C-(O)-3 Linked to Anomeric Xylose, R2 = Anomeric Proton of Arabinose C-(O)-3 to Disubstituted Xylose, R3 = Anomeric Proton of Arabinose C-(O)-2 to Disubstituted Xylose with Adjoining Disubstituted Xylose, R4 = Anomeric Proton of Arabinose C-(O)-2 to Disubstituted Xylose, R5 = Anomeric Proton of Disubstituted Xylose, R6 = Anomeric Proton of Monosubstituted Xylose, R7 = Anomeric Proton of Xylopyranosyl Unit

Table 3.39. Correlations between hydrophilicity (measured with water) of the rough side of arabinoxylan films and other chemical characteristics.

	Contact angle (deg)	Wetting tension (mN m^{-1})
	Films made with sorbitol	
Polydispersity index for 24 hours of stirring	0.838**	-0.849**
Molecular weight for 24 hours of stirring	0.286	-0.289
Polydispersity index for heated solutions	-0.041	0.042
Molecular weight for heated solutions	0.867**	-0.878**
Polydispersity index for solutions with plasticizer	-0.370	0.371
Molecular weights for solutions with plasticizer	0.462	-0.470
Polydispersity index for films	-0.620	0.615
Molecular weight for films	0.550	-0.560
R1	-0.411	0.417
R2	-0.548	0.555
R3	-0.676*	0.684*
R4	-0.545	0.552
R5	-0.678*	0.686*
R6	-0.535	0.542
R7	0.524	-0.530
Film moisture content (g kg^{-1})	0.661	-0.672*
	Films made with glycerol	
Polydispersity index for 24 hours of stirring	0.600	-0.599
Molecular weight for 24 hours of stirring	0.623	-0.619
Polydispersity index for heated solutions	-0.516	0.513
Molecular weight for heated solutions	0.400	-0.400
Polydispersity index for solutions with plasticizer	0.058	-0.068
Molecular weights for solutions with plasticizer	-0.113	0.107
Polydispersity index for films	0.232	-0.241
Molecular weight for films	0.462	-0.467
R1	-0.660	0.657
R2	-0.684*	0.681*
R3	-0.682*	0.680*
R4	-0.683*	0.681*
R5	-0.682*	0.680*
R6	-0.685*	0.683*
R7	0.681*	-0.678*
Film moisture content (g kg^{-1})	-0.148	0.132

* Significant at $P \leq 0.05$

** Significant at $P \leq 0.01$

R1 = Arabinose C-(O)-3 Linked to Anomeric Xylose, R2 = Anomeric Proton of Arabinose C-(O)-3 to Disubstituted Xylose, R3 = Anomeric Proton of Arabinose C-(O)-2 to Disubstituted Xylose with Adjoining Disubstituted Xylose, R4 = Anomeric Proton of Arabinose C-(O)-2 to Disubstituted Xylose, R5 = Anomeric Proton of Disubstituted Xylose, R6 = Anomeric Proton of Monosubstituted Xylose, R7 = Anomeric Proton of Xylopyranosyl Unit

Similar to determining hydrophilicity using the contact angle and wetting tension of between an AX film and water, the hydrophobicity of AX films can be determined using mineral oil. Figure 3.27 provides an example of the image produced when the contact angle between AX film and mineral oil was measured. Utilization of mineral oil instead of water decreased the risk of having the AX films dissolve. Table 3.40 provides the contact angles and wetting tensions between all AX films and mineral oil. These hydrophobicity parameters were determined for both the smooth side and rough sides of the films. When mineral oil is used for contact angle determination, a decrease in the contact angle and an increase in wetting ability is indicative of a hydrophobic film.

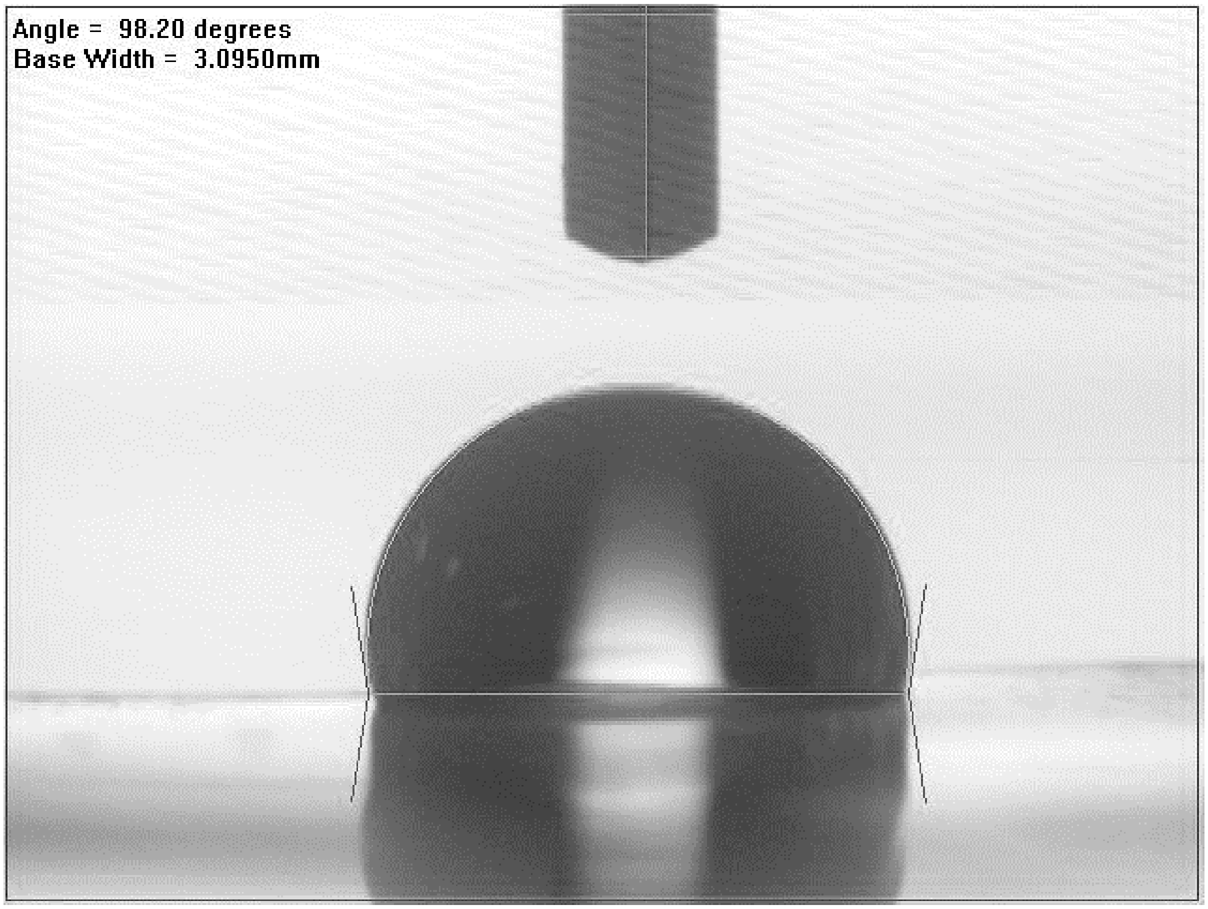

Figure 3.27. Contact angle with mineral oil of a maize bran arabinoxylan film plasticized with 100 g kg^{-1} glycerol.

Table 3.40. Contact angles and wetting tensions of arabinoxylan films with mineral oil.

Film Composition	Contact angle (deg)	Wetting tension (mN m^{-1})
	Smooth side of films	
WB AX[a] + 100 g kg^{-1} Sorbitol	13.82 ± 1.18	70.68 ± 0.36
WB AX + 250 g kg^{-1} Sorbitol	10.85 ± 0.28	71.50 ± 0.06
WB AX + 500 g kg^{-1} Sorbitol	18.00 ± 1.91	69.21 ± 0.75
WB AX + 100 g kg^{-1} Glycerol	20.13 ± 0.54	68.36 ± 0.23
WB AX + 250 g kg^{-1} Glycerol	21.10 ± 0.89	67.92 ± 0.40
WB AX + 500 g kg^{-1} Glycerol	29.87 ± 0.30	63.13 ± 0.19
MB AX[b] + 100 g kg^{-1} Sorbitol	21.61 ± 0.55	67.68 ± 0.26
MB AX + 250 g kg^{-1} Sorbitol	17.68 ± 0.40	69.36 ± 0.16
MB AX + 500 g kg^{-1} Sorbitol	19.76 ± 0.40	68.85 ± 0.45
MB AX + 100 g kg^{-1} Glycerol	24.81 ± 0.83	66.07 ± 0.44
MB AX + 250 g kg^{-1} Glycerol	23.17 ± 0.46	66.93 ± 0.23
MB AX + 500 g kg^{-1} Glycerol	24.44 ± 0.49	66.27 ± 0.26
DDG AX[c] + 100 g kg^{-1} Sorbitol	23.10 ± 0.51	66.96 ± 0.26
DDG AX + 250 g kg^{-1} Sorbitol	22.65 ± 0.17	67.19 ± 0.09
DDG AX + 500 g kg^{-1} Sorbitol	27.14 ± 0.70	64.78 ± 0.41
DDG AX + 100 g kg^{-1} Glycerol	17.86 ± 0.32	69.29 ± 0.13
DDG AX + 250 g kg^{-1} Glycerol	22.89 ± 0.73	67.07 ± 0.36
DDG AX + 500 g kg^{-1} Glycerol	32.19 ± 0.49	61.61 ± 0.33
	Rough side of films	
WB AX + 100 g kg^{-1} Sorbitol	18.20 ± 1.44	69.14 ± 0.57
WB AX + 250 g kg^{-1} Sorbitol	18.31 ± 1.20	69.11 ± 0.47
WB AX + 500 g kg^{-1} Sorbitol	18.56 ± 0.93	69.01 ± 0.37
WB AX + 100 g kg^{-1} Glycerol	21.57 ± 0.48	67.70 ± 0.22
WB AX + 250 g kg^{-1} Glycerol	15.82 ± 0.66	70.04 ± 0.23
WB AX + 500 g kg^{-1} Glycerol	24.03 ± 0.03	66.49 ± 0.02
MB AX + 100 g kg^{-1} Sorbitol	19.65 ± 0.70	68.55 ± 0.30
MB AX + 250 g kg^{-1} Sorbitol	19.76 ± 1.00	68.51 ± 0.42
MB AX + 500 g kg^{-1} Sorbitol	21.09 ± 0.31	67.92 ± 0.14
MB AX + 100 g kg^{-1} Glycerol	21.50 ± 0.22	67.74 ± 0.10
MB AX + 250 g kg^{-1} Glycerol	20.32 ± 0.96	68.26 ± 0.42
MB AX + 500 g kg^{-1} Glycerol	23.39 ± 0.17	66.82 ± 0.09
DDG AX + 100 g kg^{-1} Sorbitol	24.13 ± 0.36	66.44 ± 0.19
DDG AX + 250 g kg^{-1} Sorbitol	27.60 ± 1.05	64.51 ± 0.62
DDG AX + 500 g kg^{-1} Sorbitol	21.76 ± 0.48	67.61 ± 0.23
DDG AX + 100 g kg^{-1} Glycerol	17.54 ± 0.24	69.41 ± 0.09
DDG AX + 250 g kg^{-1} Glycerol	24.08 ± 0.47	66.46 ± 0.24
DDG AX + 500 g kg^{-1} Glycerol	23.82 ± 0.70	66.60 ± 0.36

[a] Wheat bran arabinoxylan
[b] Maize bran arabinoxylan
[c] Dried distillers grain arabinoxylan

The separation of the means for the hydrophobicity characteristics all AX films (both the smooth and rough sides) are presented in Table 3.41. In this table, the contact angles and wetting tensions are averaged three ways for each side of the films: by AX type, by plasticizer type, and by plasticizer level. Firstly, when averaged across the type of AX used in the film, there was a significant (*P*≤0.01) decrease in hydrophobicity as the type of AX used switches from WB AX to MB AX to DDG AX. This trend was seen in both the smooth and rough sides of the AX films. The wetting tensions also followed this trend, which demonstrated that WB AX films are the most hydrophobic and the DDG AX films are the least hydrophobic. This also supports the data shown by the contact angles and wetting tensions produced when water was used. Secondly, when averaged by type of plasticizer used, for both sides of the films, the films made with glycerol were less hydrophobic than those made with sorbitol. This difference in hydrophobicity was significant (*P*≤0.01) for the smooth side of the films but not the rough side. This result is opposite of that obtained using water for hydrophilicity determination. When water was used, the films made with sorbitol were more hydrophilic, or less hydrophobic. Thirdly, when averaged across the plasticizer level, there was an observed decrease in hydrophobicity as the level of plasticizer increased on the rough side of the films, but for the smooth side of the films there was no clear trend. This result is also opposite of the result obtained using water. When water was used, the trend observed was that as plasticizer level was increased, there was a decrease in hydrophilicity of the films (or increase in hydrophobicity).

There were multiple two-way interactions that affected the hydrophobicity of the AX films including AX type by plasticizer type, AX type by plasticizer level, and plasticizer type by plasticizer level. The mean separation for these two-way interactions are given in Table 3.42. This data represents that the effects of two of the three factors interacting affected the contact angle and wetting tensions of the AX films. Essentially, when one small change in composition was made, all hydrophobicity characteristics of the film changed.

Table 3.41. Mean separation of contact angles and wetting tensions of arabinoxylan films with mineral oil.

Arabinoxylan type	Plasticizer type	Plasticizer level ($g\ kg^{-1}$)	Contact angle (deg)	Wetting tension ($mN\ m^{-1}$)
			Smooth side of films	
WB[a]			18.96	68.47
MB[b]			21.91	67.53
DDG[c]			24.3	66.15
LSD ($P≤0.05$)[d]			0.50	0.23
LSD ($P≤0.01$)			0.67	0.31
	Glycerol		24.05	66.29
	Sorbitol		19.40	68.47
	LSD ($P≤0.05$)		0.41	0.19
	LSD ($P≤0.01$)		0.54	0.25
		10	20.22	66.17
		25	19.72	68.33
		50	25.23	65.64
		LSD ($P≤0.05$)	0.50	0.23
		LSD ($P≤0.01$)	0.67	0.31
			Rough side of films	
WB			19.41	68.58
MB			20.95	67.97
DDG			23.15	66.84
LSD ($P≤0.05$)			0.50	0.23
LSD ($P≤0.01$)			0.67	0.31
	Glycerol		21.34	67.72
	Sorbitol		21.01	67.87
	LSD ($P≤0.05$)		0.41	0.18
	LSD ($P≤0.01$)		0.55	0.24
		10	20.43	68.16
		25	20.98	67.82
		50	22.11	67.41
		LSD ($P≤0.05$)	0.50	0.23
		LSD ($P≤0.01$)	0.67	0.31

[a] Wheat bran
[b] Maize bran
[c] Dried distillers grain
[d] Least significant difference

Table 3.42. Mean separation for the significant ($P \le 0.05$) two-way interactions affecting the hydrophilicity of the arabinoxylan films measured with mineral oil.

Arabinoxylan type	Plasticizer type	Plasticizer level ($g\ kg^{-1}$)	Contact angle (deg)	Wetting tension ($mN\ m^{-1}$)
			Smooth side of films	
WB[a]	Glycerol		23.70	66.47
WB	Sorbitol		14.22	70.46
MB[b]	Glycerol		24.14	66.43
MB	Sorbitol		19.68	68.63
DDG[c]	Glycerol		24.31	65.99
DDG	Sorbitol		24.30	66.31
LSD ($P \le 0.05$)[d]			0.70	0.32
LSD ($P \le 0.01$)			0.94	0.43
WB		100	16.98	69.52
WB		250	15.97	69.71
WB		500	23.94	66.17
MB		100	23.21	66.88
MB		250	20.42	68.15
MB		500	22.10	67.56
DDG		100	20.48	68.12
DDG		250	22.77	67.13
DDG		500	29.66	63.20
LSD ($P \le 0.05$)			0.86	0.39
LSD ($P \le 0.01$)			1.15	0.53
	Glycerol	100	20.94	67.91
	Glycerol	250	22.38	67.31
	Glycerol	500	28.83	63.67
	Sorbitol	100	19.51	68.44
	Sorbitol	250	17.06	69.35
	Sorbitol	500	21.63	67.61
	LSD ($P \le 0.05$)		0.70	0.32
	LSD ($P \le 0.01$)		0.94	0.43
			Rough side of films	
WB	Glycerol		20.47	68.08
WB	Sorbitol		18.36	69.09
MB	Glycerol		21.74	67.61
MB	Sorbitol		20.17	68.33
DDG	Glycerol		21.81	67.49
DDG	Sorbitol		24.50	66.19
LSD ($P \le 0.05$)			0.71	0.31
LSD ($P \le 0.01$)			0.95	0.42
WB		100	19.89	68.42
WB		250	17.06	69.57
WB		500	21.30	67.75
MB		100	20.58	68.15
MB		250	20.04	68.39
MB		500	22.24	67.37
DDG		100	20.83	67.93
DDG		250	25.84	65.49
DDG		500	22.79	67.10
LSD ($P \le 0.05$)			0.87	0.38
LSD ($P \le 0.01$)			1.16	0.52
	Glycerol	100	20.20	68.28
	Glycerol	250	20.07	68.26
	Glycerol	500	23.75	66.64
	Sorbitol	100	20.66	68.04
	Sorbitol	250	21.89	67.37
	Sorbitol	500	20.47	68.18
	LSD ($P \le 0.05$)		0.71	0.31
	LSD ($P \le 0.01$)		0.95	0.42

[a] Wheat bran

[b] Maize bran

[c] Dried distillers grain

[d] Least significant difference

The three-way interaction between AX type, plasticizer type, and plasticizer level was also significant (*P*≤0.05). The mean separation for all combinations of the three factors is given in Table 3.43. In addition, Figures 3.28 through 3.35 provide a graphic representation of this data, which is more useful in describing the responses of the three-way interactions. Firstly, for the smooth side of the films there were multiple interactions for the contact angles of the films. The three-way interaction between AX type and plasticizer level for all films made with glycerol, showed a crossover response for all three types of AX. For WB AX and DDG AX films, there was an increase in the contact angle as plasticizer level increases for films made with glycerol. The three-way interaction for the contact angles of the smooth side of films made with sorbitol had a different set of responses for the AX type used and level of plasticizer. The MB AX films and DDG AX films showed a diverging response as plasticizer level increased for films made with sorbitol. Conversely, films made with MB AX and those made with WB AX showed a converging response as the level of plasticizer increased for films made with sorbitol. Secondly, the interactions for the rough side of the films resulted in a similar trend for contact angle. For films made with glycerol, all the responses were the same as for the smooth side of the film. However, for films made with sorbitol, the response of DDG AX films and MB AX films over increasing levels of plasticizer was convergence instead of divergence. Thirdly, the responses of the three-way interactions for the wetting tensions were the inverse of the interactions for the contact angles for each set of corresponding factors. This trend was also seen in the three-way interactions of the contact angle and wetting tensions determined with water.

Table 3.43. Mean separation for the significant ($P \leq 0.05$) three-way interaction between arabinoxylan type, plasticizer type, and plasticizer level affecting the hydrophilicity of the arabinoxylan films measured with water.

Arabinoxylan type	Plasticizer type	Plasticizer level (g kg^{-1})	Contact angle (deg)	Wetting tension (mN m^{-1})
			Smooth side of films	
WB[a]	Glycerol	100	20.13	68.36
WB	Glycerol	250	21.10	67.92
WB	Glycerol	500	29.87	63.13
MB[b]	Glycerol	100	24.81	66.07
MB	Glycerol	250	23.17	66.93
MB	Glycerol	500	24.44	66.27
DDG[c]	Glycerol	100	17.86	69.29
DDG	Glycerol	250	22.89	67.07
DDG	Glycerol	500	32.19	61.61
WB	Sorbitol	100	13.82	70.68
WB	Sorbitol	250	10.85	71.50
WB	Sorbitol	500	18.00	69.21
MB	Sorbitol	100	21.61	67.68
MB	Sorbitol	250	17.68	69.36
MB	Sorbitol	500	19.76	68.85
DDG	Sorbitol	100	23.10	66.96
DDG	Sorbitol	250	22.65	67.19
DDG	Sorbitol	500	27.14	64.78
LSD ($P \leq 0.05$)[d]			1.22	0.56
LSD ($P \leq 0.01$)			1.63	0.75
			Rough side of films	
WB	Glycerol	100	21.57	67.70
WB	Glycerol	250	15.82	70.04
WB	Glycerol	500	24.03	66.49
MB	Glycerol	100	21.50	67.74
MB	Glycerol	250	20.32	68.26
MB	Glycerol	500	23.39	66.82
DDG	Glycerol	100	17.54	69.41
DDG	Glycerol	250	24.08	66.46
DDG	Glycerol	500	23.82	66.60
WB	Sorbitol	100	18.20	69.14
WB	Sorbitol	250	18.31	69.11
WB	Sorbitol	500	18.56	69.01
MB	Sorbitol	100	19.65	68.55
MB	Sorbitol	250	19.76	68.51
MB	Sorbitol	500	21.09	67.92
DDG	Sorbitol	100	24.13	66.44
DDG	Sorbitol	250	27.60	64.51
DDG	Sorbitol	500	21.76	67.61
LSD ($P \leq 0.05$)			1.22	0.54
LSD ($P \leq 0.01$)			1.64	0.73

[a] Wheat bran

[b] Maize bran

[c] Dried distillers grain

[d] Least significant difference

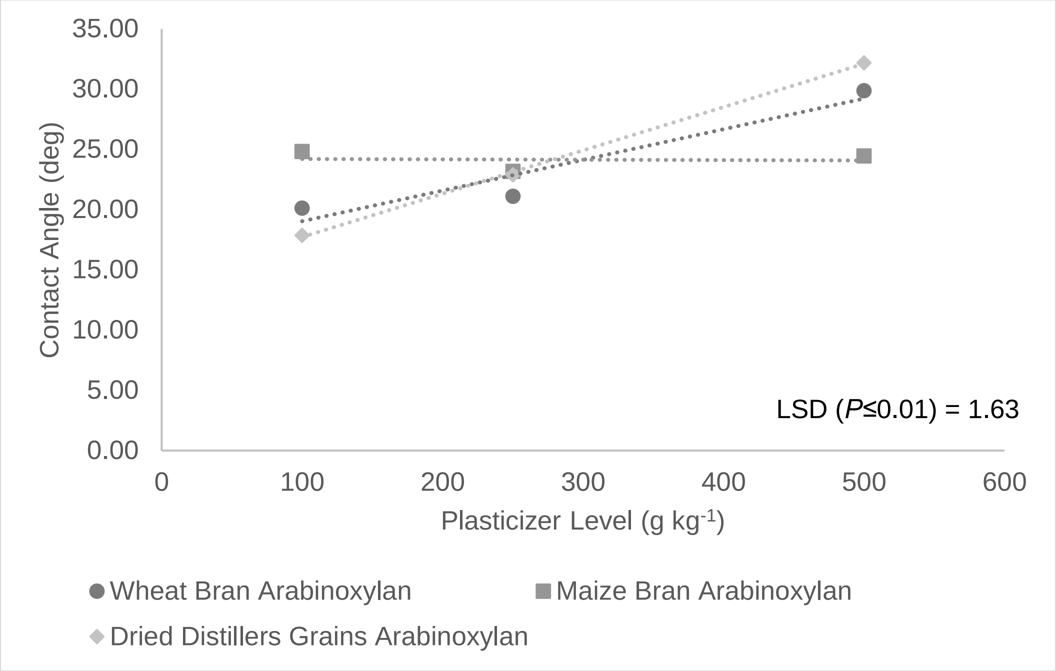

Figure 3.28. Three-way interaction for contact angle with mineral oil of the smooth side of films made with glycerol.

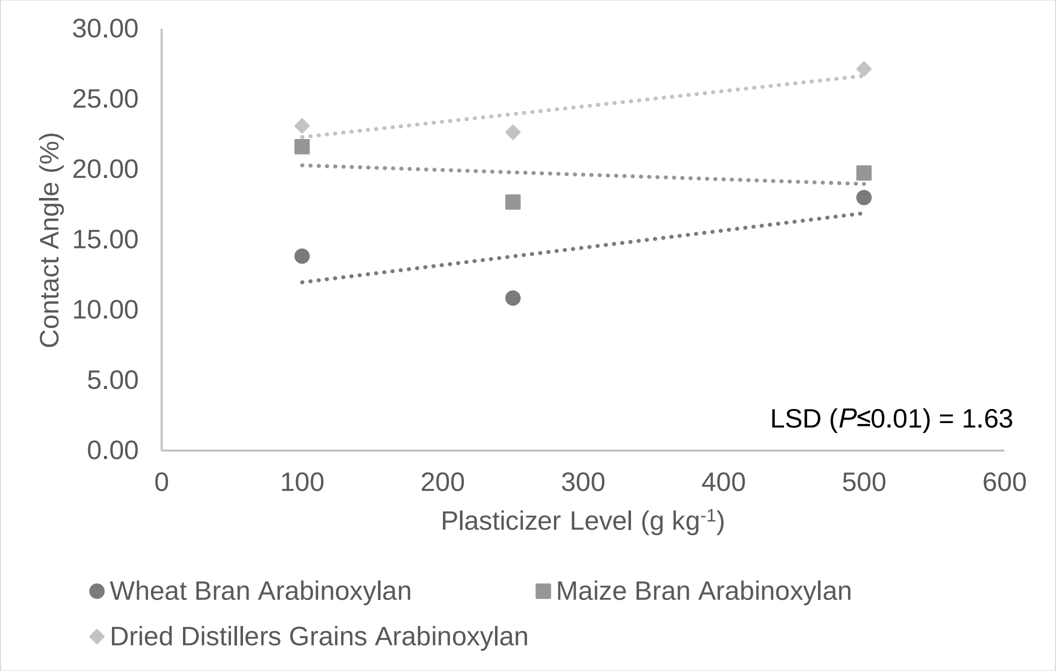

Figure 3.29. Three-way interaction for contact angle with mineral oil of the smooth side of films made with sorbitol.

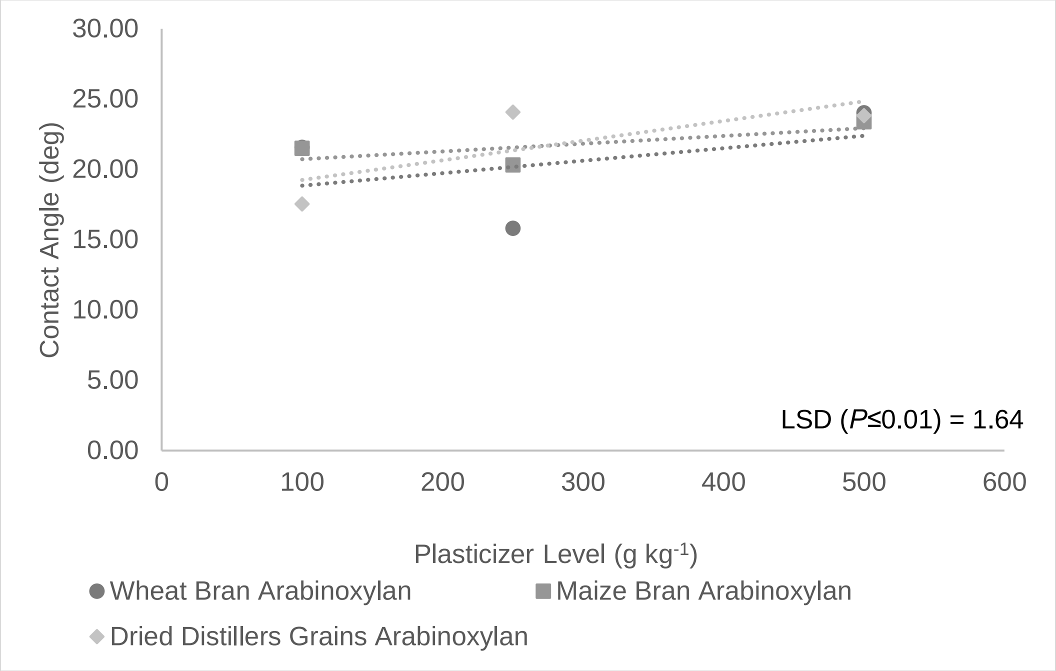

Figure 3.30. Three-way interaction for contact angle with mineral oil of the rough side of films made with glycerol.

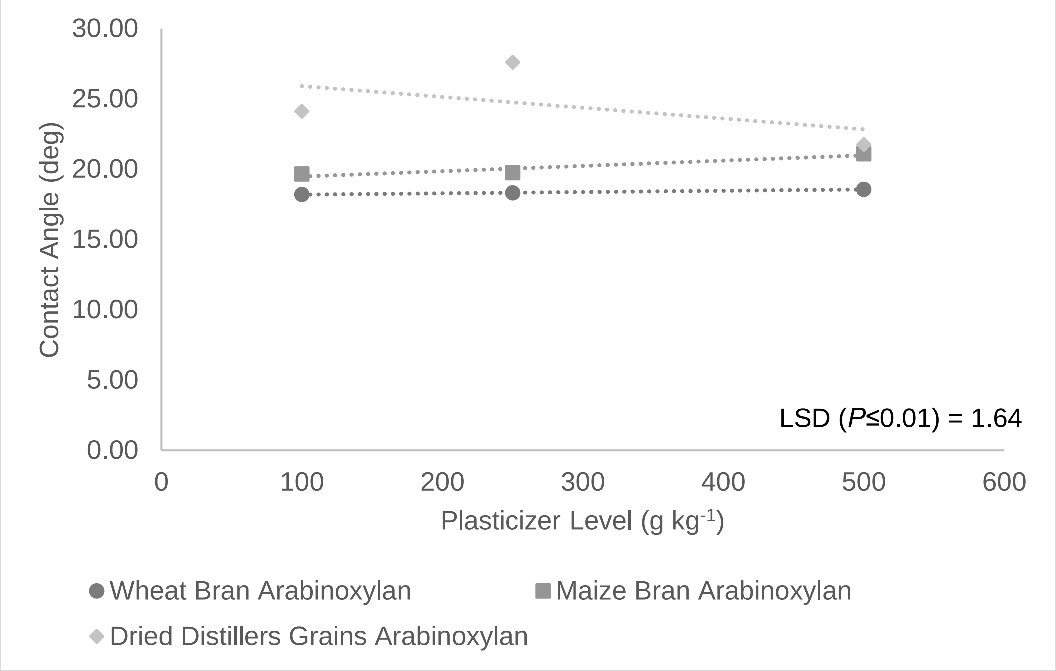

Figure 3.31. Three-way interaction for contact angle with mineral oil of the rough side of films made with sorbitol.

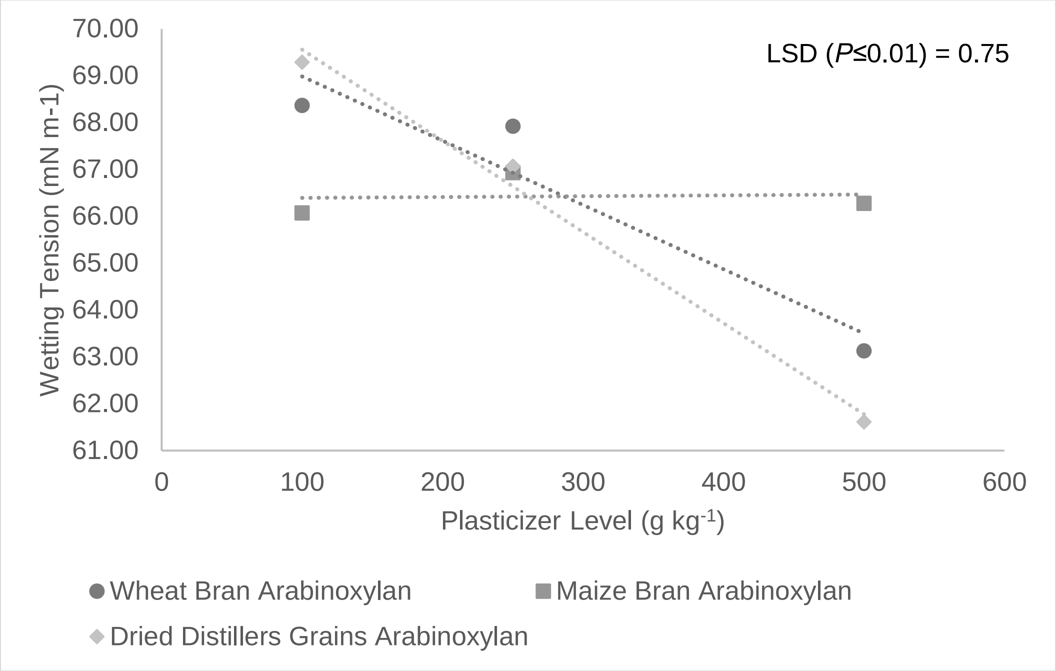

Figure 3.32. Three-way interaction for wetting tension with mineral oil of the smooth side of films made with glycerol.

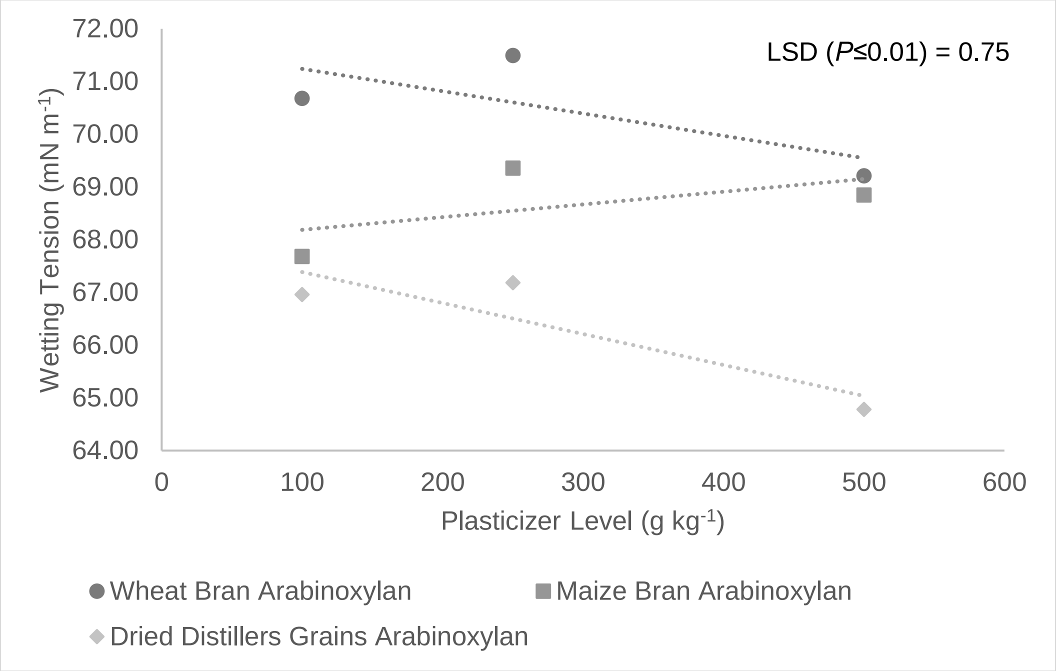

Figure 3.33. Three-way interaction for wetting tension with mineral oil of the smooth side of films made with sorbitol.

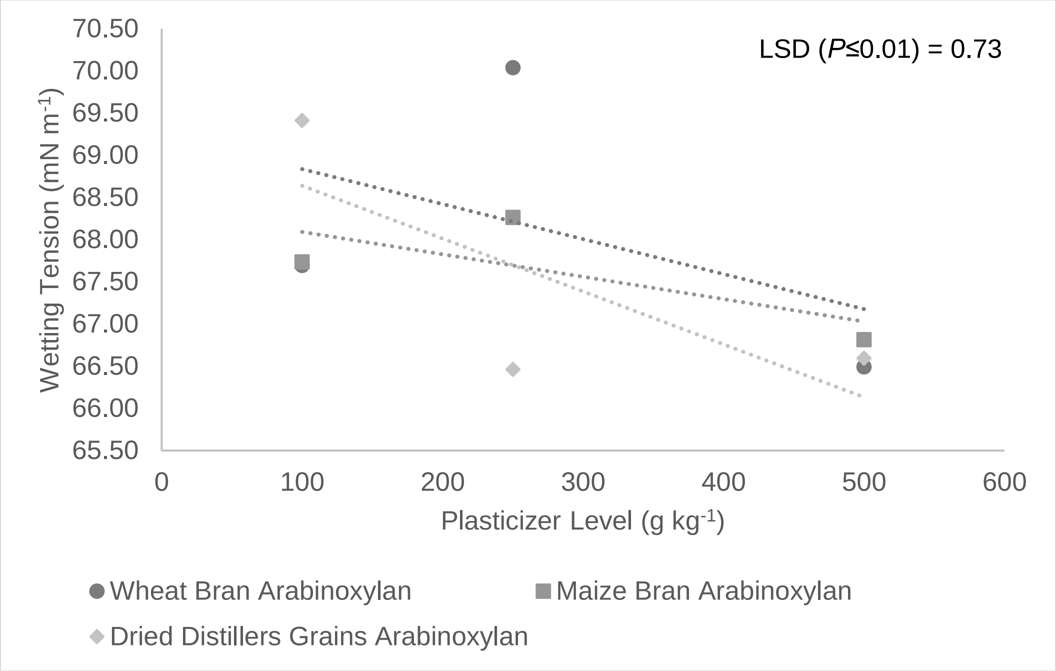

Figure 3.34. Three-way interaction for wetting tension with mineral oil of the rough side of films made with glycerol.

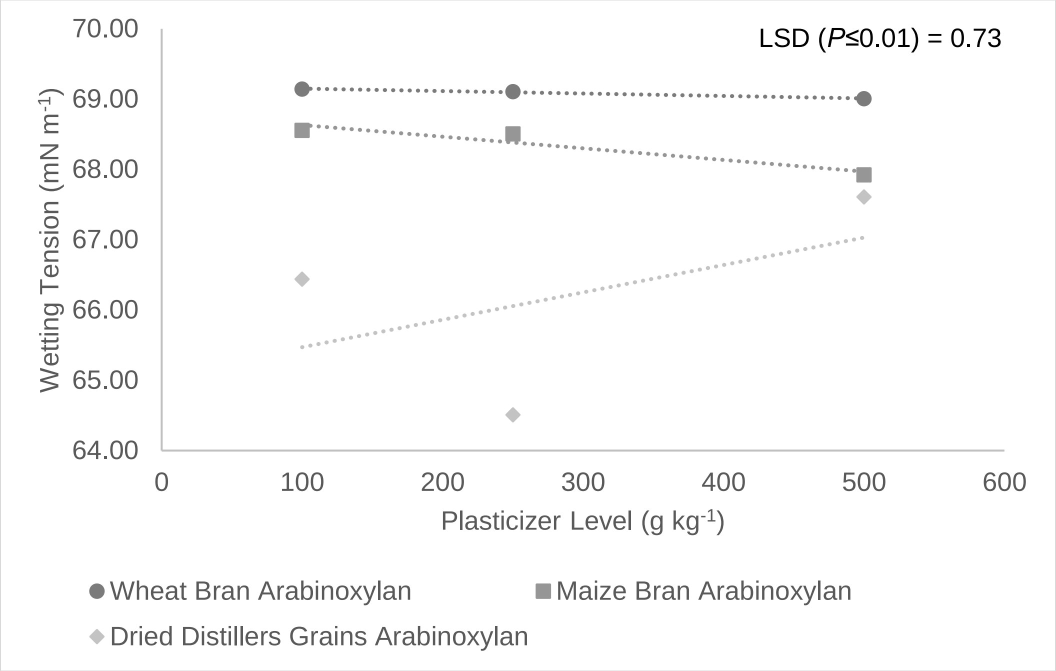

Figure 3.35. Three-way interaction for wetting tension with mineral oil of the rough side of films made with sorbitol.

Notable correlations between the hydrophobicity of the AX films and their chemical properties for the smooth sides of the films are given in Table 3.44. Firstly, there was a significant ($P \leq 0.01$) correlation between an increase in the PI of the film solutions after 24 hours of stirring and an increase in hydrophobicity of the films. Secondly, there was a significant (*P*≤0.05) correlation between the Mw of the film solutions made with sorbitol after heating and the hydrophobicity of the films after curing. This correlation indicates that as the Mw increased, so did the hydrophobicity of the films. Thirdly, there was a significant (*P*≤0.05) correlation between the level of plasticizer and the hydrophobicity of the films made with glycerol. This correlation was positive, which means that as the plasticizer level increased, the contact angle increased and the wetting tension decreased, indicating a decrease in hydrophobicity. Fourthly, there was a significant (*P*≤0.05) correlation between the PI for the films made with glycerol and their hydrophobicity. The correlation was positive, which is indicative of a decrease in hydrophobicity as the PI increased. Fifthly, there was a significant (*P*≤0.01) correlation between an increase in the film moisture content and a decrease in the hydrophobicity of films made with glycerol. This indicates that when there is more water present in the films, they were more hydrophilic. The correlations between the substitution pattern of the AX polymer and the hydrophobicity for the AX films were also analyzed. These correlations were significant (*P*≤0.05 or *P*≤0.01) for films made with sorbitol, but not those made with glycerol. The first group of significant (*P*≤0.05 or *P*≤0.01) correlations show that as the AX was more substituted, the hydrophobicity of the films decreased. Similarly, there was a significant (*P*≤0.01) correlation between an increase in the presence of unsubstituted xylose and an increase in the hydrophobicity of the films. These things indicate that the films that were the most hydrophobic had the least substituted AX, and by extension, the most ordered overall film structure.

Correlations between the chemical properties of AX films and the hydrophobicity of the rough side of the film are given in Table 3.45. Firstly, the PI and Mw of the film solutions after 24 hours of stirring were significantly (*P*≤0.05) correlated to the hydrophobicity of films made with sorbitol. The correlation shows that as the PI and Mw increased, there was an increase in the hydrophobicity of the rough sides of the films. Secondly, there was a significant (*P*≤0.05) correlation between the PI of the solutions after the addition of sorbitol and their hydrophobicity. As the PI of this solution increased, the hydrophobicity of the rough side of the films made with sorbitol decreased. There were no notable correlations between the hydrophobicity of the rough side of AX films made with glycerol and the chemical characteristics of the films. In addition, similar correlations between linkages and hydrophobicity of the AX films were seen for the rough side of the film as the smooth side of the films.

Table 3.44. Correlations between hydrophobicity (measured with mineral oil) of the smooth side of arabinoxylan films and other chemical characteristics.

	Contact Angle (deg)	Wetting Tension ($mN\ m^{-1}$)
	Films made with sorbitol	
Plasticizer level ($g\ kg^{-1}$)	0.233	-0.219
Polydispersity index for 24 hours of stirring	-0.876**	0.867**
Molecular weight for 24 hours of stirring	-0.548	0.624
Polydispersity index for heated solutions	0.333	-0.425
Molecular weight for heated solutions	-0.775*	0.723*
Polydispersity index for solutions with plasticizer	0.528	-0.548
Molecular weights for solutions with plasticizer	-0.109	0.004
Polydispersity index for films	0.542	-0.425
Molecular weight for films	-0.388	0.380
R1	0.648	-0.713*
R2	0.746*	-0.796*
R3	0.824**	-0.855**
R4	0.744*	-0.794*
R5	0.825**	-0.856**
R6	0.760*	-0.807**
R7	-0.730*	0.782*
Film moisture content ($g\ kg^{-1}$)	-0.313	0.260
	Films made with glycerol	
Plasticizer level ($g\ kg^{-1}$)	0.784*	-0.778*
Polydispersity index for 24 hours of stirring	-0.060	0.080
Molecular weight for 24 hours of stirring	-0.027	0.085
Polydispersity index for heated solutions	0.011	-0.072
Molecular weight for heated solutions	-0.058	0.052
Polydispersity index for solutions with plasticizer	0.547	-0.568
Molecular weights for solutions with plasticizer	0.181	-0.228
Polydispersity index for films	0.724*	-0.725*
Molecular weight for films	0.346	-0.343
R1	0.035	-0.090
R2	0.044	-0.093
R3	0.051	-0.092
R4	0.044	-0.093
R5	0.052	-0.092
R6	0.045	-0.093
R7	-0.042	0.093
Film moisture content ($g\ kg^{-1}$)	0.851**	-0.876**

* Significant at $P \leq 0.05$

** Significant at $P \leq 0.01$

R1 = Arabinose C-(O)-3 Linked to Anomeric Xylose, R2 = Anomeric Proton of Arabinose C-(O)-3 to Disubstituted Xylose, R3 = Anomeric Proton of Arabinose C-(O)-2 to Disubstituted Xylose with Adjoining Disubstituted Xylose, R4 = Anomeric Proton of Arabinose C-(O)-2 to Disubstituted Xylose, R5 = Anomeric Proton of Disubstituted Xylose, R6 = Anomeric Proton of Monosubstituted Xylose, R7 = Anomeric Proton of Xylopyranosyl Unit

Table 3.45. Correlations between hydrophobicity (measured with mineral oil) of the rough side of arabinoxylan films and other chemical characteristics.

	Contact Angle (deg)	Wetting Tension (mN m^{-1})
	Films made with sorbitol	
Plasticizer level (g kg^{-1})	-0.056	0.072
Polydispersity index for 24 hours of stirring	-0.827**	0.796*
Molecular weight for 24 hours of stirring	-0.713*	0.712*
Polydispersity index for heated solutions	0.542	-0.553
Molecular weight for heated solutions	-0.628	0.590
Polydispersity index for solutions with plasticizer	0.741*	-0.732*
Molecular weights for solutions with plasticizer	-0.064	0.036
Polydispersity index for films	0.104	-0.067
Molecular weight for films	-0.610	0.601
R1	0.783*	-0.776*
R2	0.841**	-0.828**
R3	0.871**	-0.851**
R4	0.840**	-0.827**
R5	0.871**	-0.851**
R6	0.848**	-0.834**
R7	-0.832**	0.820**
	Films made with glycerol	
Plasticizer level (g kg^{-1})	0.555	-0.583
Polydispersity index for 24 hours of stirring	-0.203	0.199
Molecular weight for 24 hours of stirring	-0.054	0.077
Polydispersity index for heated solutions	-0.008	-0.020
Molecular weight for heated solutions	-0.218	0.201
Polydispersity index for solutions with plasticizer	0.334	-0.363
Molecular weights for solutions with plasticizer	-0.024	-0.009
Polydispersity index for films	0.457	-0.485
Molecular weight for films	0.081	-0.107
R1	0.086	-0.106
R2	0.122	-0.137
R3	0.157	-0.165
R4	0.121	-0.136
R5	0.157	-0.165
R6	0.128	-0.141
R7	-0.116	0.131

* Significant at $P \leq 0.05$

** Significant at $P \leq 0.01$

R1 = Arabinose C-(O)-3 Linked to Anomeric Xylose, R2 = Anomeric Proton of Arabinose C-(O)-3 to Disubstituted Xylose, R3 = Anomeric Proton of Arabinose C-(O)-2 to Disubstituted Xylose with Adjoining Disubstituted Xylose, R4 = Anomeric Proton of Arabinose C-(O)-2 to Disubstituted Xylose, R5 = Anomeric Proton of Disubstituted Xylose, R6 = Anomeric Proton of Monosubstituted Xylose, R7 = Anomeric Proton of Xylopyranosyl Unit

3.4.5.7. Water Vapor Permeability

Characterization of the water vapor permeability of a material such as a film is of the upmost importance if the material is going to be used as a food packaging material. Water vapor permeability is explained using two parameters: WVTR and permeance. The WVP of all of the AX films analyzed in this research are given in Table 3.46. Water vapor permeability of an AX film depends upon a number of factors including the type of AX used in the film, type of plasticizer used, and the amount of the plasticizer used.

Table 3.46. Water vapor permeability of arabinoxylan films.

Film composition	Water vapor transmission rate ($g\ h^{-1}\ m^{-2}$)	Permeance (perms)
WB AX[a] + 100 $g\ kg^{-1}$ Sorbitol	44.79 ± 3.07	3.29 ± 0.23
WB AX + 250 $g\ kg^{-1}$ Sorbitol	45.45 ± 0.88	3.34 ± 0.06
WB AX + 500 $g\ kg^{-1}$ Sorbitol	47.34 ± 1.85	3.47 ± 0.14
WB AX + 100 $g\ kg^{-1}$ Glycerol	56.57 ± 3.82	4.15 ± 0.28
WB AX + 250 $g\ kg^{-1}$ Glycerol	60.59 ± 7.00	4.45 ± 0.51
WB AX + 500 $g\ kg^{-1}$ Glycerol	73.68 ± 3.55	5.41 ± 0.26
MB AX[b] + 100 $g\ kg^{-1}$ Sorbitol	50.55 ± 1.85	3.70 ± 0.14
MB AX + 250 $g\ kg^{-1}$ Sorbitol	53.18 ± 2.92	3.90 ± 0.21
MB AX + 500 $g\ kg^{-1}$ Sorbitol	54.09 ± 0.78	3.96 ± 0.06
MB AX + 100 $g\ kg^{-1}$ Glycerol	60.12 ± 1.09	4.41 ± 0.08
MB AX + 250 $g\ kg^{-1}$ Glycerol	78.92 ± 3.64	5.78 ± 0.27
MB AX + 500 $g\ kg^{-1}$ Glycerol	90.84 ± 1.62	6.66 ± 0.12
DDG AX[c] + 100 $g\ kg^{-1}$ Sorbitol	48.54 ± 3.07	3.56 ± 0.22
DDG AX + 250 $g\ kg^{-1}$ Sorbitol	49.81 ± 0.79	3.65 ± 0.06
DDG AX + 500 $g\ kg^{-1}$ Sorbitol	55.16 ± 4.45	4.04 ± 0.33
DDG AX + 100 $g\ kg^{-1}$ Glycerol	51.52 ± 1.60	3.78 ± 0.12
DDG AX + 250 $g\ kg^{-1}$ Glycerol	60.95 ± 2.72	4.47 ± 0.20
DDG AX + 500 $g\ kg^{-1}$ Glycerol	78.04 ± 2.46	5.73 ± 0.18

[a] Wheat bran arabinoxylan
[b] Maize bran arabinoxylan
[c] Dried distillers grain arabinoxylan

To properly analyze the water vapor permeability trends of the AX films, separation of the means for each treatment and water vapor permeability parameter were determined as seen in Table 3.47. The first way this was analyzed was by averaging across the type of AX used to make the films. The trend seen in this analysis was that the WB AX films had the lowest water vapor permeability, followed by the DDG AX films, and lastly the MB AX films. These differences were all significantly ($P \leq 0.05$) different.

Heikkinen et al. (2013) found that MB AX films have a lower water vapor permeability than WB AX films, however there were several differences between the testing conditions. Two of the main differences include, the RH difference used during testing and the processing of the AX used. The second way the variation in water vapor permeability was analyzed was by averaging the permeability of the AX films by the type of plasticizer used. The trend observed in this research was that the films made with sorbitol had a significantly ($P \leq 0.01$) lower water vapor permeability than those made with sorbitol. Zhang and Whistler (2004) also observed this trend in the water vapor permeability of their MB AX films. Lastly, the water vapor permeability of the AX films was analyzed by averaging the permeability across the level of plasticizer used in the film. In this research, it was observed that as the level of plasticizer increased, the water vapor permeability also significantly ($P \leq 0.01$) increased. This is the same trend observed by Nazan Turhan and Şahbaz (2004) in their research on water vapor permeability of films at varying plasticizer levels.

Table 3.47. Mean separation of water vapor permeability of arabinoxylan films.

Arabinoxylan type	Plasticizer type	Plasticizer level ($g\ kg^{-1}$)	Water vapor transmission rate ($g\ h^{-1} m^{-2}$)	Permeance (perms)
WB[a]			54.74	4.02
MB[b]			64.62	4.74
DDG[c]			57.34	4.21
LSD ($P \leq 0.05$)[d]			2.05	0.15
LSD ($P \leq 0.01$)			2.75	0.20
	Glycerol		67.92	4.98
	Sorbitol		49.89	3.66
	LSD ($P \leq 0.05$)		1.67	0.12
	LSD ($P \leq 0.01$)		2.24	0.16
		100	52.02	3.81
		250	58.15	4.26
		500	66.53	4.88
		LSD ($P \leq 0.05$)	2.05	0.15
		LSD ($P \leq 0.01$)	2.75	0.20

[a] Wheat bran
[b] Maize bran
[c] Dried distillers grain
[d] Least significant difference

There were multiple significant ($P \leq 0.05$) two-way interactions affecting the water vapor permeability for the AX films. The three two-way interactions between the AX type, plasticizer type, and plasticizer level were significant ($P \leq 0.05$) for both the WVTR and the permeance. The mean separation for these interactions is given in Table 3.48. These two-way interactions demonstrate that the combined effect of two of the three factors affected the water vapor permeability of the AX films. There were multiple notable things about these interactions. Firstly, in the two-way interaction between AX type and plasticizer type, there was a converging response between the effects of MB AX and DDG AX as the plasticizer type was switched from glycerol to sorbitol. The overall result of these interactions was that for all three types of AX, as the plasticizer type was switched from glycerol to sorbitol, the WVTR decreased. Secondly, in the two-way interaction between AX type and plasticizer level, there was a crossover response between DDG AX and WB AX as the plasticizer type was switched from glycerol to sorbitol. Overall, as the AX type and plasticizer level interaction took place, the WVP increased as the level of plasticizer was increased. Thirdly, the effect of the interaction between plasticizer type and plasticizer level was an increase in water vapor transmission rate. The response of the two types of plasticizers over increasing plasticizer level was divergence.

Table 3.48. Mean separation for two-way interactions affecting the water vapor permeability of arabinoxylan films.

Arabinoxylan type	Plasticizer type	Plasticizer level (g kg^{-1})	Water vapor transmission rate (g h^{-1}m^{-2})	Permeance (perms)
WB[a]	Glycerol		63.61	4.67
WB	Sorbitol		45.86	3.37
MB[b]	Glycerol		76.63	5.61
MB	Sorbitol		52.60	3.85
DDG[c]	Glycerol		63.50	4.66
DDG	Sorbitol		51.17	3.75
LSD ($P \leq 0.05$)[d]			2.90	0.21
LSD ($P \leq 0.01$)			3.89	0.29
WB		100	50.68	3.72
WB		250	53.02	3.89
WB		500	60.51	4.44
MB		100	55.34	4.05
MB		250	66.05	4.84
MB		500	72.47	5.31
DDG		100	50.03	3.67
DDG		250	55.38	4.06
DDG		500	66.60	4.88
LSD ($P \leq 0.05$)			3.55	0.26
LSD ($P \leq 0.01$)			4.76	0.35
	Glycerol	10	56.07	4.11
	Glycerol	25	66.82	4.90
	Glycerol	50	80.86	5.93
	Sorbitol	10	47.96	3.52
	Sorbitol	25	49.48	3.63
	Sorbitol	50	52.19	3.83
	LSD ($P \leq 0.05$)		2.90	0.21
	LSD ($P \leq 0.01$)		3.89	0.29

[a] Wheat bran
[b] Maize bran
[c] Dried distillers grain
[d] Least significant difference

There were correlations between the chemical characteristics of the AX films and their water vapor permeability as seen in Table 3.49. Firstly, there was a significant (*P*≤0.05) correlation between the PI of the film solutions after stirring for 24 hours and the WVTR of films made with sorbitol. This correlation was negative, which indicates that as the PI decreased, the WVTR increased. Secondly, there was a significant (*P*≤0.01) negative correlation between the Mw of the film solutions after heating and both the water vapor transmission rate and permeance of the films made with sorbitol. This means that as the Mw increased, the water vapor permeability decreased. This could be due to a decrease in the intermolecular spaces present in the AX film due to an increase in Mw. Thirdly, there was a significant (*P*≤0.05) correlation between an increase in the PI of the films made with sorbitol and an increase in their WVTR and water permeance. Fourthly, there was a significant (*P*≤0.01) correlation between an increase in the level of glycerol used and an increase in both the WVTR and water vapor permeance.

Table 3.49. Correlations between water vapor permeability and chemical characteristics of arabinoxylan films.

	Water vapor transmission rate ($g\ h^{-1}m^{-2}$)	Permeance (perms)
	Films made with sorbitol	
Plasticizer level ($g\ kg^{-1}$)	0.499	0.504
Polydispersity index for 24 hours of stirring	-0.667*	-0.663
Molecular weight for 24 hours of stirring	0.004	0.006
Polydispersity index for heated solutions	-0.236	-0.237
Molecular weight for heated solutions	-0.812**	-0.809**
Polydispersity index for solutions with plasticizer	0.012	0.010
Molecular weights for solutions with plasticizer	-0.526	-0.524
Polydispersity index for films	0.679*	0.679*
Molecular weight for films	-0.165	-0.159
	Films made with glycerol	
Plasticizer level ($g\ kg^{-1}$)	0.829**	0.831**
Polydispersity index for 24 hours of stirring	-0.044	-0.043
Molecular weight for 24 hours of stirring	0.381	0.378
Polydispersity index for heated solutions	-0.458	-0.455
Molecular weight for heated solutions	-0.255	-0.253
Polydispersity index for solutions with plasticizer	-0.097	-0.094
Molecular weights for solutions with plasticizer	-0.352	-0.348
Polydispersity index for films	0.528	0.531
Molecular weight for films	0.215	0.217

* Significant at *P*≤0.05
** Significant at *P*≤0.01

Polyvinyl chloride has a WVP around 0.000005 cm^2 s^{-1} cm^{-2} cm Hg^{-1} (Ulutan and Balkose 1996). This WVP is very low, which makes is very good for packaging certain types of food. In addition, it is much lower than the WVP of the AX films developed in this research. The WVP of chitosan films is also lower than that of the AX films developed in this research. Butler et al. (1996) developed chitosan films with a WVP of 0.22 g m^{-1} day^{-1} atm^{-1}. The difference in WVP between all of these materials can be utilized to provide a packaging material with the most appropriate WVP for the food being stored.

3.5. Summary and Conclusions

This research demonstrated numerous trends in AX film mechanical characteristics, including the relationships between those characteristics and the chemical structure of the AX used as the basis of the films. The PI and Mw of the film solutions as well as the AX film were correlated to the mechanical characteristics of the films. Similarly, the linkages present in each type of AX were correlated to these mechanical strengths. Overall, the WB AX films were the most hydrophobic and have the highest mechanical strengths including puncture resistance, tensile strength, and tear resistance. The DDG AX films were the most hydrophilic and as such have the highest moisture contents and water solubility.

There were also many trends observed in AX films depending upon the type and level of plasticizer used to plasticize the AX films. These chemical species impact several of the mechanical characteristics of the films including moisture content, water solubility, tensile strength, hydrophilicity, and water vapor permeability. Firstly, Glycerol is more hydrophilic than sorbitol, and the moisture contents of AX films made with these two plasticizers demonstrated this fact. In addition, as the level of plasticizer increased, so did the moisture content of the films. Secondly, the disruption of polymer chain by sorbitol or glycerol impacted the water solubility of the film because it caused a decrease in order resulting in increased solubility. Thirdly, films made with sorbitol had higher puncture resistances but lower tensile strengths and tear resistances. Fourthly, films made with glycerol had larger elongations due to the higher level of plasticization imparted upon the film by glycerol instead of sorbitol. Fifthly, utilization of glycerol in an AX film resulted in a higher WVTR due to the hydrophilic nature of glycerol.

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CHAPTER 4. BIODEGRADABILITY AND PHYSICAL PROPERTIES OF ARABINOXYLAN FILMS

4.1. Abstract

The biodegradability and physical properties of arabinoxylan (AX) films are related to their composition. The main purpose of this research was to determine the differences between the amounts of biodegradable material present in films made from wheat (*Triticum aestivum* L.) bran (WB) AX, maize (*Zea mays* L.) bran (MB) AX, and dried distillers grains (DDG) AX. Secondary purposes of this research included analysis of both the color and surface roughness of the same films to determine how they vary with film composition. All films were made with either glycerol or sorbitol at varying levels (100, 250, or 500 g kg^{-1}). The chemical compositions of the three types of AX used to make the films were determined using standard methods from the American Association of Cereal Chemists International (AACCI). These chemical compositions were then analyzed in conjunction with the biodegradability and physical properties of the films. The three types of AX used were alkaline extracted and had the following purities: 730 g kg^{-1} for WB AX, 850 g kg^{-1} for MB AX, and 580 g kg^{-1} for DDG AX. The biodegradability of all films was at least 490 g kg^{-1}, and in some cases as high as 680 g kg^{-1}. In addition, as the level of plasticizer increased, the biodegradability of the films increased. The WB AX films were the darkest, while the MB AX films were the lightest. The colors of the films demonstrated the presence of impurities including N and phenolic acids. The surface topography of all films was analyzed using atomic surface microscopy (AFM), and the WB AX films were the roughest. Overall, this research showed that the composition of AX films affected their biodegradability and physical properties including color and surface topography.

4.2. Introduction

Cereals including wheat and maize are in the grass (*Gramineae*) family (Delcour and Hoseney 2010b). These are two of the top three most commonly produced cereals (Heikkinen et al. 2013). Both of these crops produce a fruit that is called a caryopsis, also known as a kernel (Delcour and Hoseney 2010b). This fruit is made up of starch (600 to 700 g kg^{-1}), protein (100 to 150 g kg^{-1}), and non-starch polysaccharides (30 to 80 g kg^{-1}) (Saulnier et al. 2007).

In the U.S., wheat is divided into six classes including hard red winter, hard red spring, soft red winter, soft white, hard white, and durum wheat (Delcour and Hoseney 2010b). Wheat kernels are about

8 mm long and have a mass around 35 mg, but exact size depends upon cultivar and location in the wheat spike. The four main parts of a wheat kernel are the pericarp, seed coat, endosperm, and germ. The pericarp and seed coat collectively are called the wheat bran (WB). This portion is often used for animal feed because it is undesirable for patent flours (Apprich et al. 2014; Swennen et al. 2006).

Wheat bran is primarily made up of the outer pericarp, inner pericarp, testa, nuclear epidermis, and aleurone layer (Apprich et al. 2014; Anson et al. 2012). These layers collectively make up 140 to 190 g kg^{-1} of the total mass of the kernel (Maes and Delcour 2002). Wheat bran is mainly non-starch polysaccharides (460 g kg^{-1}) and starch (100 to 200 g kg^{-1}) (Zhang et al. 2011), but protein (150 to 220 g kg^{-1}) and lignin (40 to 80 g kg^{-1}) are also present. The main non-starch polysaccharides in WB include AX (700 g kg^{-1}), cellulose (240 g kg^{-1}), and β-glucan (60 g kg^{-1}) (Maes and Delcour 2002). In addition, glucomannan and arabinogalactan are in the aleurone layer in very low amounts

Maize kernels are larger than wheat kernels and are very flat and cuboidal with an average mass of 350 mg (Delcour and Hoseney 2010b). The four main parts of a maize kernel include the bran (50 to 60 g kg^{-1}), germ (100 to 140 g kg^{-1}), endosperm (800 to 850 g kg^{-1}), and tip cap (<10 g kg^{-1}). When maize is milled, the bran is one of the main byproducts because it is undesirable for use in most products made from maize (Agger et al. 2010). The layers present in MB include pericarp, epidermis, and seed coat. The main components present in MB include heteroxylans (500 g kg^{-1}), cellulose (200 g kg^{-1}), protein (100 to 130 g kg^{-1}), starch (90 to 230 g kg^{-1}), phenolic acids (40 g kg^{-1}), lipid (20 to 30 g kg^{-1}), and ash (20 g kg^{-1}) (Carvajal-Millan et al. 2007). The main type of heteroxylan in MB is AX, which is found in the cell walls of the MB (Agger et al. 2010; Carvajal-Millan et al. 2007).

Dried distillers grain is a second byproduct of processing maize, but it comes from the ethanol industry instead of the milling industry (Zarrinbakhsh et al. 2013). DDG remain after maize has been fermented and distilled during the production of ethanol (U.S. Grains Council 2012). The DDG are then centrifuged and dried to remove excess water so they can be used as animal feed. The composition of DDG varies greatly with production and the type of maize used initially during the ethanol production. However, the main components include protein (about 300 g kg^{-1}), hemicellulose (about 200 g kg^{-1}), and cellulose (about 150 g kg^{-1}) (Xiang et al. 2014). Out of all the hemicellulose present in DDG, about half is AX, which give DDG an AX content around 100 g kg^{-1} (Zarrinbakhsh et al. 2013).

Arabinoxylan is present in in both wheat and maize cell walls, and it is one of the most common non-starch polysaccharides found on earth (Zhang et al. 2011). AX is mainly present in the cell walls of the cells in the bran and endosperm (Zhang et al. 2014; Saeed et al. 2011; BeMiller 2007b). AX is made up of a backbone of β-1,4 linked xylose units that has O-2 and/or O-3 linked arabinose substituents (Reis et al. 2015; Kiszonas et al. 2013; Saeed et al. 2011). Two other species commonly present in AX are glucose (Zhang et al. 2014; BeMiller 2007a) and ferulic acid (Anson et al. 2012). Ferulic acid can be substituted on the xylose backbone in the O-3 and/or O-2 locations (Kiszonas et al. 2013; Saeed et al. 2011; Delcour and Hoseney 2010a). AX cross linkages are often formed in the presence of ferulic acid because ferulic acid can couple at the O-5 location via an ester linkage.

Arabinoxylan can be extracted from WB, MB, and DDG through a variety of methods including alkaline extraction (Aguedo et al. 2014). This AX can then be used to make films, which are utilized as a prototype of food packaging when testing mechanical properties. Materials to be used for food packaging must be both mechanically sound to extend the shelf-life of the food being stored and to facilitate sales of said food. Due to this, AX is often combined with a plasticizer, such as glycerol or sorbitol, to increase both flexibility and strength (Zhang and Whistler 2004; Phan The et al. 2002).

Food packaging has historically been created from synthetic materials including polyoefins, polyamids, and polyesters (Casariego et al. 2009; Tharanathan 2003). These materials have been used because of their desirable barrier properties that assist in elongating the shelf life of the food(s) they were packaging. However, the use of these materials has resulted in many ecological problems because they are not biodegradable or recyclable. When developing food packaging for future use, biodegradability is an important factor to consider. Utilization of AX from WB, MB, and DDG for use as the basis in food packaging materials will increase the overall sustainability of the food packaging industry because these materials are biodegradable (Tharanathan 2003).

There were three objectives throughout this research. Firstly, to obtain AX of the highest purity possible and utilize it for use as the basis of films. Secondly, to determine the biodegradability and physical properties of AX films. Thirdly, to determine if there are any correlations between the chemical and physical properties or biodegradability of the AX films.

4.3. Materials and Methods

4.3.1. Procurement and Milling

All starting materials used were donated by commercial mills and a commercial ethanol plant. These materials are the same as those described in chapter two on page 47, however they have been briefly described here for reference. The WB was from the North Dakota State Mill in Grand Forks, ND. Agricor, Incorporated provided the MB from a facility in Marion, IN. The Casselton, ND ethanol plant owned by Tharaldson Ethanol provided the DDG. Once obtained, all materials except the MB were milled on a Falling Number Hammer Mill (Type KT-12; Numer 10071). A Perten Instrument Mill Feeder 3170 (Perten Instruments North America, Incorporated) was used in conjunction with the mill. The MB was milled on a commercial scale by Agricor, Incorportated, so it was not milled again. To determine particle size range for the starting materials, the AACCI method for particle size range was used (AACC International 2011).

4.3.2. Arabinoxylan Extraction and Purification

The extraction and purification method utilized in described in detail in chapter two on page 48, but described here concisely. The first step of the extraction performed was defatting with hexane. This was done with a 1:3 (w v^{-1}) ratio of starting material (WB, MB, or DDG) to hexane. Next, an alkaline extraction method proposed by Xiang et al. (2014) was modified for this experiment. The extraction process began by individually treating the starting materials (WB, MB, and DDG) with 30 g kg^{-1} sodium hydroxide for three hours at 50 °C while continuously stirring. The ratio of starting material to sodium hydroxide was 1:2 (w v^{-1}). After stirring for three hours, all solutions were centrifuged for 20 minutes at 3,000 rpm. The supernatant was neutralized to a pH of seven using hydrochloric acid and de-starched using using α-amylase from *Bacillus lichenformis* (Sigma-Aldrich Incorporated; Saint Louis, MO; Termamyl 120; 1186 Units mg^{-1} protein; 19.8 mg protein mL^{-1}; A-3403-1MU). For every liter of AX solution, 0.25 mL of α-amylase were used. Next, the protein was removed from the AX solutions using *Bacillus amyloliquefaciens* (Sigma-Aldrich Incorporated; Saint Louis, MO; P1236-50 mL; Lot No. SLBG5002V, PC Code:1001778189; ≥0.8 units g^{-1}). Fractionation of the polysaccharide fraction was then performed using 950 g kg^{-1} ethanol followed by dialysis to remove all chemical species with molecular weights below 12 kDa. The dialysis membranes that were utilized were Spectra Por 2 Dialysis

Membranes (Spectrum Laboratories Incorporated; 12 to 14 kDa; Lot No. 9200228). All samples were dialyzed for 72 hours and subsequently freeze-dried in a VirTis Freeze Dryer (Model No. 10-MRSM; Serial No. SM-1028).

4.3.3. Chemical Characterization of Arabinoxylan

The methods used for chemical characterization of all materials is provided in detail in chapter two on page 50, but shortly described here for reference. Proximate compositions were determined for the starting materials, defatted materials, and extracted AX. These analyses included moisture content determined in duplicate (AACC International 1999a), total ash determined in duplicate (AACC International 1999d), total N determined in at least duplicate (AACC International 1999c), total lipid determined in duplicate for each milled and defatted material (AOCS 2009), and total starch determined in at least duplicate (AACC International 1999b). To determine the AX content, arabinose to xylose ratio (A:X), and sugars present in each sample, gas chromatography with a flame ionization detector (GC-FID) was used. The method developed by Blakeney et al. in (1983) was followed for these determination of sugar profile and A:X.

The gas chromatograph used was a Hewlett Packard 5890 Series II GC system with a flame ionization detector (Agilent Technologies, Incorporated Santa Clara, CA). The column used was a SupelcoSP-2380 fused silica capillary column (30 m x 0.25 mm x 2 µm) (Supelco Bellefonte, PA). The following parameters were used for analysis: flow pressure of 827372 Pa, oven temperature of 100 °C, flow rate of 0.8 mL min^{-1}, detector temperature of 250 °C, injector temperature of 230 °C, and helium was the mobile phase.

High performance size exclusion chromatography with a multi-angle light scattering detector and refractive index detector (HPSEC-MALS-RI) was used to determine the weight average molecular weight (Mw) and polydispersity index (PI) of all AX, film solutions, and finished films. The method utilized was that of Mendis and Simsek (2015). This process involved dissolving a small amount of sample in deionized water and stirring continuously for 60 minutes at 40 °C before filtering all samples through 5 µm filter paper and performing the analysis. The high performance liquid chromatograph (HPLC) used was an Agilent 1200 with a Wyatt Dawn Helios-II multi- angle light scattering detector (MALS) and a refractive index detector (RI). Two columns were used including a Shodex OHpak guard and an SB 806-HQ column. Pullulan (Mw = 300 kDa) was utilized to normalize the MALS detector, and water with a 0.5 mL min^{-1} flow rate was used as the mobile phase for all analyses. To calculate the Mw and PI for all samples, a 3rd order Debye plot with second-order polynomial fit was used in conjunction with Astra 6.0.5 software (Wyatt Technology 2016). The proportional change used was 0.146 in the refractive index as the AX concentration changed (Dervilly et al. 2000).

To determine the nuclear magnetic resonance spectroscopy (NMR) spectrum for each type of AX extracted, the method of Mendis and Simsek (2015) was used. In preparation for analysis, all samples were dissolved in deuterium oxide and freeze dried. This was completed a total of three times. The NMR analysis of each sample was completed with the sample dissolved in deuterium oxide. The samples were analyzed at a temperature of 80 °C on a 400 MHz spectrometer (Bruker AV3 HD 400 MHZ NMR that had a 5 mm PABBO BB/19F-!H/D Z-GRD Z probe). All NMR results were analyzed using TopSpin 3.2 software (Bruker BioSpin Corporation 2015).

4.3.4. Film Development

The method utilized for film solution and casting are described in detail in chapter three on page 74, but a brief discussion is provided here as well. To begin the development of the film solutions, each sample began by creating a 26.7 g kg^{-1} AX solution in deionized water. These solutions were stirred for 24 hours on a Lab Line Multi Magnestir (Model No. 1278). Next, the solutions were heated for 15 minutes at 90 °C in a Thermo Scientific Reacti-Therm III (Model No. TS-18823). The appropriate type (glycerol or sorbitol) and amount (100, 250, or 500 g kg^{-1}) of plasticizer was then added to the solution and vortexed on a vortex mixer. The glycerol (ACS Reagent Grade; ≥ 99.5 g kg^{-1}) and sorbitol (BioUltra Grade; ≥ 99.5 g kg^{-1}) were purchased from Sigma-Aldrich Incorporated (Saint Louis, MO). These solutions were then heated for 10 minutes at 90 °C. All film solutions were cast onto polystyrene petri dishes (VWR; 100 X 15mm; Cat No. 25384-094) and dried for eight hours at 60 °C. After curing, the films were stored at 49 % relative humidity (RH) in a Dry Keeper (Sanplatec Corporation; Catalog No. H42056-0001). Boveda 49 % RH packs were used to maintain the humidity in the dry keeper (Item No. B49-60-48).

4.3.5. Biodegradability Testing

The carbon content of all films were determined (in singlet) on a Primacs TOC Analyzer (Model CS22). The samples were heated to 1050 °C in a stream of pure oxygen and the presence of a cobalt oxide catalyst. The result of this was decomposition of all organically and inorganically bound carbon. The gas was then passed through a 2 °C peltier cooler to remove all moisture. Next, the gas flowed through a copper mesh chlorine scrubber. After that, three delay vessels evened out the gas flow. Next, a silver wool scrubber removed the acid sulfates. Finally, the carbon dioxide was measured by infrared detection at 4.2 μm. The carbon present in the films was determined by calculation using a standard curve made with carbon standards.

The biodegradability analysis of the AX films was completed according to a combination of the method of Colussi et al. (2017) and ASTM method D5988-12 with some modifications (ASTM International 2012a). Soil collected from Foster County, ND (100 g) was mixed with enough water to satisfy 600 g kg^{-1} of the moisture holding capacity (MHC) of the soil, and half of the wetted soil was added to a 1-L airtight glass jar. Then, 400 mg sample of a film was placed on top of the soil and the film was covered with the remaining soil. A polystyrene cup (50 mL) with 20 mL of 1 M sodium hydroxide was placed on top of the soil and the jar was sealed tightly. A control sample of soil only was used as a blank and all samples were stored at room temperature (23 to 26 °C). The carbon dioxide production was measured over days. The cups containing sodium hydroxide were removed from the jars on days 19, 40, 55, 82, 103, 119 and 145 and replaced with new cups containing fresh sodium hydroxide solution. The sodium hydroxide was titrated to determine the carbon dioxide produced. This titration was conducted by transferring the sodium hydroxide that had been stored with each film sample to a flask and adding 5 mL of barium chloride (250 g kg^{-1}). Three drops of 1 g kg^{-1} phenolphthalein were added and 1 M hydrochloric acid was used to titrate the solution until it turned from pink to white. Equation 4.1 was used to determine the amount of carbon released throughout the biodegradation process. Where A is the amount of hydrochloric acid spent in reagent blank in mL, B is the amount of hydrochloric acid spent in the sample in mL, Acid Molarity is the molarity of the hydrochloric acid in mol L^{-1}, and Eq.g. C-CO2 is the equivalent gram C-CO2. Equation 4.2 was then used to determine the percentage of the film that had been biodegraded.

$$Release\ of\ Carbon\ per\ 100\ g\ Carbon\ Dioxide = \frac{1}{mg\ CO_2} - (A\ B) * (Acid\ Molarity) * Eq.g.C - CO_2 \qquad (4.1)$$

$$Percentage\ of\ Film\ Biodegraded\ = \frac{CO_2\ \ soil\ with\ film - CO_2\ \ soil\ without\ film}{mg\ of\ Carbon\ in\ film} * 100 \qquad (4.2)$$

The soil (silt loam: 258 g kg^{-1} sand, 578 g kg^{-1} silt, 164 g kg^{-1} clay) used for testing was collected from Foster County, ND in June of 2015. The depth of the soil collected was 0 to 6 inches. It was stored at 23 °C in a closed container. It was handled with gloved hands and clean tools at all times. The particle size was of the soil was less than 2 mm. The MHC of the soil was 22 g water per 100 g soil (600 g kg^{-1} MHC). The carbon to nitrogen ratio of the soil was 11:1. The pH of the soil was 6.2. The Soil Testing Lab at North Dakota State University determined the characteristics of the soil (Fargo, ND).

4.3.6. Color Analysis

To determine the color of both the smooth and rough sides of each film, a MacBeth Color Eye 7000 Spectrophotometer was used. Each treatment was tested in triplicate and analyzed using Pro Palette 5.0 software. The analysis of the color of both sides of all samples produced XYZ values, CIELab, and Hunter Lab. ASTM E313-15e1 was used to determine the yellowness and whiteness indices for each sample (ASTM International 2015).

4.3.7. Atomic Force Microscopy

Atomic force microscopy (AFM) was used to determine the Z range, root mean square (RMS), and roughness average (Ra) for the height, phase, and amplitudes of all treatments. A modified version of ASTM E2382-04(2012) was used (ASTM International 2012b). This was done in triplicate for both sides of each type of film. The specimen disks used had 12 mm diameters and were made by Ted Pella, Incorporated (Product No. 16208). The cantilever used for all treatments except for films made with MB AX and 50 % glycerol or DDG AX and 50 % glycerol were Golden Silicon Probes NSG03 (Reflective side: Au; Tip Height: 14 to 16 µm; Tip Curve Radius: 10 nm; Chip Size: 3.4 x 1.6 x 0.3 mm, Cantilever Length: 135 ± 5 µm; Cantilever Width: 30 ± 5 µm; Cantilever Thickness: 1 to 2 µm). For films made with MB AX and 50 % glycerol or DDG AX and 50 % glycerol, the cantilever used was the B Tip of an SPM probe by µMasch (HQ: CSC37/A1 BS; Cantilever Length: 350 ± 5 µm; Cantilever Width: 35 ± 3 µm; Cantilever Thickness: 2.0 ± 0.5 µm; Frequency: 15 to 30 Hz; Force Constant: 0.3 N m^{-1}; Tip Radius: 8nm, Tip Cone Angle: 40 °; Tip Side Coating: None; Backside Coating: Al 3D; Chip Dimensions: 3.4 x 1.6 x 0.315 mm).

4.3.8. Statistical Analysis

This experiment utilized completely random design with a factorial arrangement. The AX source was the first factor, the type of plasticizer was the second factor, and the plasticizer level was the third factor in the arrangement. The analysis of all data was completed using Statistical Analysis Software (SAS) version 9.3 (SAS Institute Inc. 2011). SAS was used to produce the analysis of variance for each data set. The data was then further analyzed using Fischer's protected least significant difference to analyze the separation of the means and correlations.

4.4. Results and Discussion

4.4.1. Particle Size Range and Composition

For the WB, MB, and DDG, most the particle size range was between 177 and 250 microns. The majority of the MB (97.1 g kg^{-1}) fell onto screen 60 (250 microns), while the WB and DDG were evenly divided between screen 60 (250 microns) and screen 80 (177 microns). 45.9 g kg^{-1} of the milled WB fell on screen 60 and 46.9 g kg^{-1} fell on screen 80. For the DDG, 39.6 g kg^{-1} fell on screen 60 and 44.3 g kg^{-1} fell on screen 80. The compositions of the starting materials and AX are given in Table 4.1. Most notable is the purity of the AX extracted. After extraction and purification, the total AX in each material was greatly increased. The WB AX had a purity of 730 g kg^{-1}, the MB AX had a purity of 850 g kg^{-1}, and the DDG AX had a purity of 580 g kg^{-1}. These purities are all significantly ($P \le 0.01$) different from one another. In addition, these purities are all higher than the purities of previously published work. For example, Maes and Delcour obtained WB AX with a purity of only 450 g kg^{-1} using alkaline extraction (2002).

Table 4.1. Proximate composition of wheat bran, maize bran, dried distillers grains, and arabinoxylan extracted from all three materials.

	Composition (g kg^{-1})								
Material	Moisture	Ash	N	Lipid	Starch	Arabinoxylan	Arabinose	Xylose	A:X[e]
WB[a]	84.5	67.9	179.4	40.7	126.2	409.2	190.8	274.2	0.70
MB[b]	96.1	7.6	49.1	24.3	128.5	683.7	263.2	513.7	0.51
DDG[c]	93.3	68.4	286.8	85.6	39.8	179.2	84.7	118.9	0.71
LSD ($P \le 0.05$)[d]	-	0.1	7.4	9.8	3.3	14.1	6.5	9.5	0.01
LSD ($P \le 0.01$)	-	0.3	13.5	18.0	4.8	25.8	11.8	17.5	0.02
WB arabinoxylan	62.2	85.5	136.2	-	15.0	729.4	280.0	548.9	0.51
MB arabinoxylan	111.1	12.2	38.9	-	3.3	847.1	325.1	637.5	0.51
DDG arabinoxylan	63.1	18.9	149.5	-	3.1	580.5	223.5	436.2	0.51
LSD ($P \le 0.05$)	13.9	0.6	13.1	-	1.1	29.7	10.2	23.7	-
LSD ($P \le 0.01$)	25.6	1.1	24.0	-	1.5	54.5	18.8	43.6	-

[a] Wheat bran
[b] Maize bran
[c] Dried distillers grains
[d] Least significant difference
[e] Arabinose to xylose ratio

4.4.2. High Performance Size Exclusion Chromatography-Multi Angle Light Scattering-Refractive Index

The Mw and PI for all materials (WB, MB, and DDG) during all steps of the film making process (after stirring with water for 24 hours, after heating, after the addition of plasticizer, and after the films were dried) were determined using HPSEC-MALS-RI. Table 4.2 provides this comparison of this data by material type (WB, MB, or DDG). Throughout the film solution development process, many chemical changes took place. The decrease in Mw after heating the film solutions indicates a hydrolysis reaction. After the films were completely dried, their Mw increased. This indicates that there were intermolecular interactions such as hydrogen bonding or the formation of cross-linkages taking place.

Table 4.2. Molecular weight and polydispersity index for arabinoxylan film solutions and films averaged across overall treatment.

Type	Treatment	Molecular weight (Da)	Polydispersity index
Solution	24 hr stirring	6906667	1.30
Solution	90° C	4985556	1.52
Solution	100 g kg^{-1} Glycerol	4618889	1.59
Solution	250 g kg^{-1} Glycerol	4811111	1.77
Solution	500 g kg^{-1} Glycerol	5543333	1.78
Solution	100 g kg^{-1} Sorbitol	4838889	1.55
Solution	250 g kg^{-1} Sorbitol	5136667	1.45
Solution	500 g kg^{-1} Sorbitol	5700000	1.59
Film	100 g kg^{-1} Glycerol	11195556	1.48
Film	250 g kg^{-1} Glycerol	12733333	1.72
Film	500 g kg^{-1} Glycerol	15066667	2.22
Film	100 g kg^{-1} Sorbitol	9913333	1.41
Film	250 g kg^{-1} Sorbitol	11316667	1.38
Film	500 g kg^{-1} Sorbitol	15555556	1.49
LSD (P≤0.05)[a]		34373	0.13
LSD (P≤0.01)		45557	0.18

[a] Least significant difference

4.4.3. ^{1}H Nuclear Magnetic Resonance Spectroscopy

Figure 4.1 provides a graphic representation of the abundances of each type of anomeric proton in AX for WB, MB, and DDG. All relative abundances were determined relative to the abundance of unsubstituted xylose as shown by the relative integral equal to one. The most abundant types of anomeric protons in WB AX include the anomeric proton of the unsubstituted xylose, and the anomeric proton of arabinose that is C-(O)-3 linked to disubstituted xylose, and the anomeric proton of arabinose C-(O)-2 linked to disubstituted xylose that has an adjoining disubstituted xylose. In MB AX the most abundant anomeric protons include the anomeric proton of monosubstituted xylose, unsubstituted xylose, the anomeric proton of arabinose that is C-(O)-3 linked to disubstituted xylose. DDG has the following three most abundant types of anomeric protons: the anomeric proton of disubstituted xylose, the anomeric proton of the monosubstituted xylose, and the anomeric proton of arabinose that is C-(O)-2 linked to disubstituted xylose that has an adjoining disubstituted xylose.

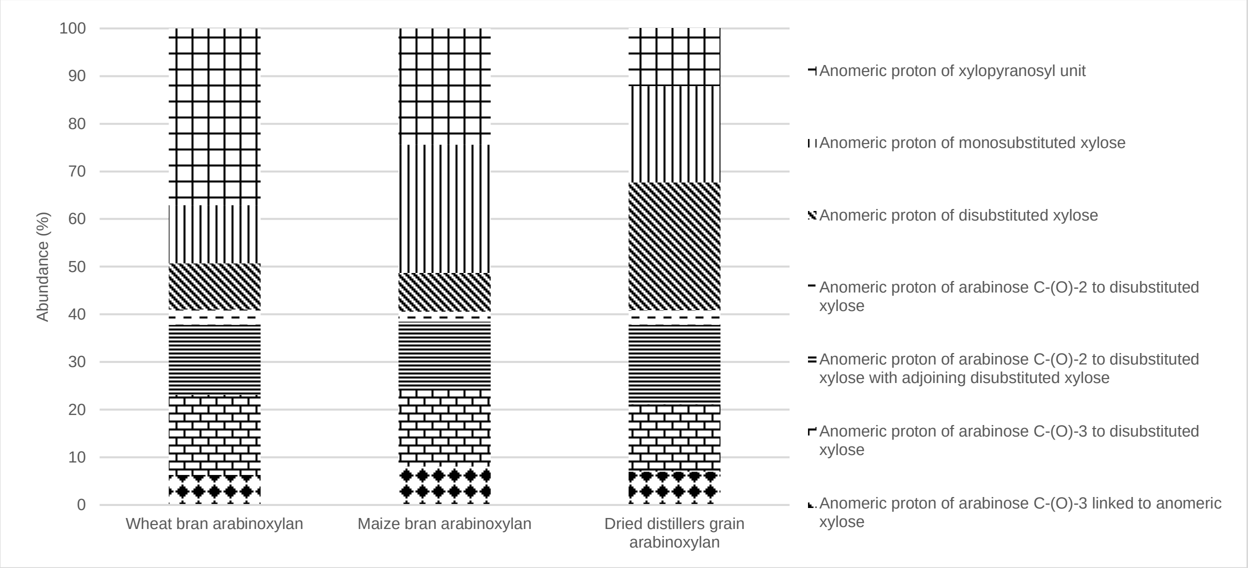

Figure 4.1. Relative abundances of each linkage in arabinoxylan extracted from wheat bran, maize bran, and dried distillers grain.

4.4.4. Arabinoxylan Film Biodegradability

Biodegradability of the AX films was analyzed every 14 to 27 days to determine the overall biodegradability of each type of film as well as the biodegradability profile for each type of film. The carbon content of all films is provided in Table 4.3. Table 4.4 provides the percentage of material biodegraded in each film on each day measurement took place. Figure 4.2 provides an example biodegradability profile for the films made with WB AX. Over time, the amount of total biodegradable material within each treatment increased significantly (*P*≤0.05) with almost every single measurement taken. The exception to this is when the biodegradability profile began to plateau at the end and the total biodegradable material present in each type of film increased more slowly with time. On average, the total amount of biodegradable material in each type of films was as follows: 550 g kg^{-1} in WB AX films, 630 g kg^{-1} in MB AX films, and 530 g kg^{-1} in DDG AX films. When comparing the biodegradability of these films, the general trend was that as the plasticizer level increased so did their biodegradability. In addition, utilization of glycerol instead of sorbitol increased the total biodegradability of the film by about 30 g kg^{-1}.

Table 4.3. Carbon contents of arabinoxylan films made with arabinoxylan extracted from wheat bran, maize bran, or dried distillers grain.

Arabinoxylan type	Plasticizer type	Plasticizer level (g kg^{-1})	Carbon content (mg)
WB[a]	Sorbitol	100	140.6
WB	Sorbitol	250	140.6
WB	Sorbitol	500	141.5
WB	Glycerol	100	141.4
WB	Glycerol	250	142.2
WB	Glycerol	500	135.8
MB[b]	Sorbitol	100	154.3
MB	Sorbitol	250	152.1
MB	Sorbitol	500	145.6
MB	Glycerol	100	148.3
MB	Glycerol	250	146.6
MB	Glycerol	500	134.7
DDG[c]	Sorbitol	100	161.3
DDG	Sorbitol	250	159.4
DDG	Sorbitol	500	157.4
DDG	Glycerol	100	153.9
DDG	Glycerol	250	153.6
DDG	Glycerol	500	143.4

[a] Wheat bran
[b] Maize bran
[c] Dried distillers grain

When compared to other materials, the AX films developed in this research are less aerobically biodegradable than other biopolymer-based films, but more biodegradable than films made from polyvinyl chloride. Chitosan films modified via acetylation are 100 % biodegradable, while unmodified chitosan films are not biodegradable (Xu et al. 1996). Polynivyl chloride is not biodegradable unless modified with cassava starch (Ming et al. 1992). The AX films in this research were biodegradable without modification of the substrate, which is desirable in food packaging.

Table 4.4. Biodegradability of arabinoxylan films made from wheat bran arabinoxylan, maize bran arabinoxylan, and dried distillers grain arabinoxylan and varying levels of either sorbitol or glycerol by measurement day.

	Measurement day						
	19	40	55	82	104	118	145
Film composition	Total biodegradable material (g kg^{-1})						
WB AX[a] + 10 g kg^{-1} Sorbitol	341	453	476	500	513	517	528
WB AX + 25 g kg^{-1} Sorbitol	343	459	486	507	518	528	534
WB AX + 50 g kg^{-1} Sorbitol	345	467	496	516	522	528	534
WB AX + 10 g kg^{-1} Glycerol	354	470	496	515	525	535	545
WB AX + 25 g kg^{-1} Glycerol	337	455	481	507	522	537	554
WB AX + 50 g kg^{-1} Glycerol	343	489	521	557	570	580	595
MB AX[b] + 10 g kg^{-1} Sorbitol	356	464	493	514	521	528	530
MB AX + 25 g kg^{-1} Sorbitol	353	538	575	611	628	644	652
MB AX + 50 g kg^{-1} Sorbitol	338	551	591	619	638	659	673
MB AX + 10 g kg^{-1} Glycerol	334	524	545	587	599	608	634
MB AX + 25 g kg^{-1} Glycerol	337	532	544	576	593	603	624
MB AX + 50 g kg^{-1} Glycerol	314	581	599	631	640	653	677
DDG AX[c] + 10 g kg^{-1} Sorbitol	395	420	431	452	464	475	494
DDG AX + 25 g kg^{-1} Sorbitol	389	430	445	469	478	491	505
DDG AX + 50 g kg^{-1} Sorbitol	362	439	454	488	507	522	541
DDG AX + 10 g kg^{-1} Glycerol	386	447	467	503	513	518	537
DDG AX + 25 g kg^{-1} Glycerol	375	445	454	485	497	506	526
DDG AX + 50 g kg^{-1} Glycerol	353	475	493	532	545	559	580
LSD ($P \leq 0.05$)[d] between mean values within a treatment is 6							
LSD ($P \leq 0.05$) between mean values for different treatments is 14							

[a] Wheat bran arabinoxylan

[b] Maize bran arabinoxylan

[c] Dried distillers grain arabinoxylan

[d] Least significant difference

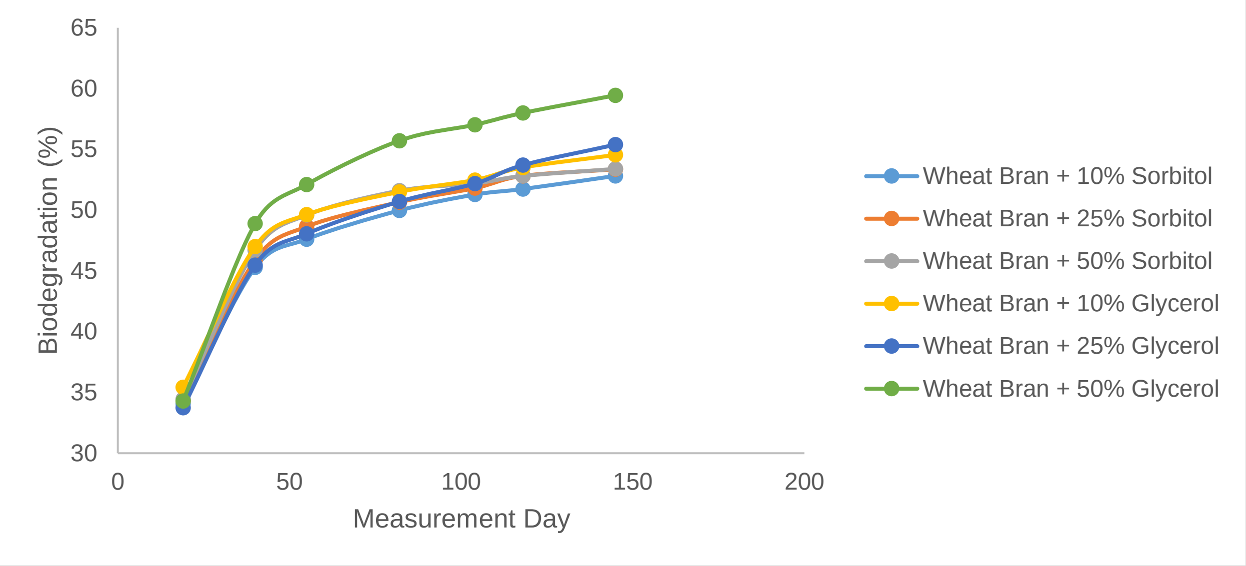

Figure 4.2. Biodegradability profile for films made with wheat bran arabinoxylan.

In Table 4.5, the correlations between total biodegradable material on each measurement day and either Mw or PI are given for films made with sorbitol. There were three main trends in correlations between these factors. Firstly, there was a significant ($P \leq 0.05$) negative correlation between the PI of the film and the biodegradability of the film. This shows that as the PI increased, the biodegradability decreased (and vice versa). Secondly, there was a negative correlation between the Mw of the film solution when it was heated and the biodegradability of the film made with sorbitol. This shows that as the Mw increased during heating, the biodegradability of the film decreased. Thirdly, there was a significant ($P \leq 0.01$) positive correlation between the PI during film solution heating and the biodegradability of the films made with sorbitol. The significance of this is that as the PI of the film solution increased during heating, the biodegradability of the film also increased. The same three significant ($P \leq 0.05$ or $P \leq 0.01$) correlations were present for films made with glycerol as those made with sorbitol.

Table 4.5. Correlations between molecular weight and biodegradability (by measurement day) or polydispersity index and biodegradability (by measurement day) of arabinoxylan films.

	Biodegradability measurement day						
	19	40	55	82	104	118	145
				Films made with sorbitol			
PI[a] for 24 hours of stirring	-0.343	0.362	0.349	0.371	0.381	0.405	0.427
Mw[b] for 24 hours of stirring	-0.785 *	0.207	0.260	0.206	0.172	0.119	0.054
PI for heated solutions	-0.777 *	0.785 *	0.824 **	0.785 *	0.754 *	0.715 *	0.651
Mw for heated solutions	0.631	-0.842 **	-0.869 **	-0.843 **	-0.818 **	-0.791 *	-0.739 *
PI for solutions with plasticizer	-0.543	-0.164	-0.120	-0.165	-0.190	-0.234	-0.278
Mw for solutions with plasticizer	0.737 *	-0.587	-0.633	-0.611	-0.583	-0.542	-0.466
PI for films	0.061	-0.695 *	-0.680 *	-0.682 *	-0.685 *	-0.694 *	-0.696 *
Mw for films	-0.036	0.510	0.494	0.499	0.489	0.500	0.513
				Films made with glycerol			
PI for 24 hours of stirring	-0.429	0.357	0.372	0.389	0.401	0.426	0.413
Mw for 24 hours of stirring	-0.456	0.062	0.184	0.101	0.106	0.126	0.064
PI for heated solutions	-0.853 **	0.767 *	0.808 **	0.762 *	0.770 *	0.775 *	0.751 *
Mw for heated solutions	0.834 **	-0.871 **	-0.877 **	-0.853 **	-0.860 **	-0.858 **	-0.852 **
PI for solutions with plasticizer	-0.103	-0.330	-0.204	-0.281	-0.279	-0.257	-0.319
Mw for solutions with plasticizer	0.374	-0.600	-0.582	-0.549	-0.526	-0.513	-0.522
PI for films	0.442	-0.768 *	-0.689 *	-0.708 *	-0.707 *	-0.687 *	-0.722 *
Mw for films	-0.321	0.083	0.163	0.157	0.171	0.194	0.151

[a] Polydispersity index
[b] Molecular weight
* Significant at $P\leq0.05$
** Significant at $P\leq0.01$

When analyzing the relationship between the linkages present in the films made with sorbitol and their biodegradability, there were two clear correlations, as seen in Table 4.6. The first significant ($P\leq0.05$) correlation was between the relative abundance of arabinose C-(O)-3 linked to xylose and film biodegradability. This relationship was negatively correlated, indicating that as the relative abundance of this linkage increased, the biodegradability of the films made with sorbitol decreased. The second significant ($P\leq0.05$) correlation present was between the relative abundance of arabinose C-(O)-3 linked to disubstituted xylose and the biodegradability of the AX films, and once again, this correlation was negative. Both of these correlations were only present during the initial stage of biodegradability. The same two significant (*P*≤0.05) correlations are present in films made with glycerol. However, for films made with glycerol, these correlations remain significant (*P*≤0.05) throughout all stages of biodegradation. Overall, for all films an increase in substitution resulted in a decrease in the order of the AX films, which results in increased ease of breakdown.

Table 4.6. Correlations between the total biodegradability (by measurement day) and the relative abundance of each anomeric proton present in arabinoxylan films.

Measurement Day	R1	R2	R3	R4	R5	R6	R7
Films made with sorbitol							
Day 19	0.830 **	0.868 **	0.874 **	0.867 **	0.874 **	0.871 **	-0.863 **
Day 40	-0.727 *	-0.633	-0.506	-0.636	-0.504	-0.615	0.653
Day 55	-0.771 *	-0.682 *	-0.559	-0.685 *	-0.557	-0.665	0.701 *
Day 82	-0.726 *	-0.633	-0.505	-0.636	-0.503	-0.615	0.652
Day 104	-0.693 *	-0.597	-0.469	-0.600	-0.467	-0.579	0.617
Day 118	-0.648	-0.547	-0.416	-0.550	-0.413	-0.529	0.568
Day 145	-0.580	-0.476	-0.344	-0.479	-0.342	-0.457	0.497
Films made with glycerol							
Day 19	0.834 **	0.784 *	0.699 *	0.786 *	0.697 *	0.773 *	-0.796 *
Day 40	-0.683 *	-0.560	-0.404	-0.564	-0.401	-0.538	0.585
Day 55	-0.742 *	-0.640	-0.502	-0.643	-0.500	-0.620	0.661
Day 82	-0.686 *	-0.573	-0.427	-0.576	-0.424	-0.552	0.596
Day 104	-0.694 *	-0.580	-0.433	-0.584	-0.431	-0.559	0.604
Day 118	-0.702 *	-0.592	-0.449	-0.596	-0.446	-0.572	0.615
Day 145	-0.669 *	-0.550	-0.398	-0.554	-0.395	-0.528	0.574

* Significant at *P*≤0.05
** Significant at *P*≤0.01
R1 = Arabinose C-(O)-3 Linked to Anomeric Xylose, R2 = Anomeric Proton of Arabinose C-(O)-3 to Disubstituted Xylose, R3 = Anomeric Proton of Arabinose C-(O)-2 to Disubstituted Xylose with Adjoining Disubstituted Xylose, R4 = Anomeric Proton of Arabinose C-(O)-2 to Disubstituted Xylose, R5 = Anomeric Proton of Disubstituted Xylose, R6 = Anomeric Proton of Monosubstituted Xylose, R7 = Anomeric Proton of Xylopyranosyl Unit

4.4.5. Color

Aesthetic appeal is of the upmost for the sale of food, and the color of the food packaging is one of the areas where that can be easily modified. This can be done using artificial dyes or printing, however, using a material that is naturally colored is preferable. As seen in Figure 4.3 (photos of films taken with films on white background), there is variation in the color of the AX films. The WB AX films are darker and browner, the MB AX films are almost colorless, and the DDG AX films are lighter and yellower. This darker, brown color seen in the WB AX films was also observed in films made by Aguedo et al. (2014). When analyzing the color of the films, there are three types of light that can be used as well as two types of analyses (CIELab and Hunter Lab) that produce L, a, and B values. Using this method, a quantitative analysis of the color of the AX films can be obtained rather than simply a qualitative description of the colors of the AX films. The L-value is a measure of lightness (100 = white, 0 = black), the a-value is a measure of red to green (+a = red, -a = green), and the b-value is a measure of yellow to blue (+b = yellow, -b = blue) (Perez et al. 2016). The three types of light used in color analysis provided a comprehensive evaluation of the color of the AX films being analyzed. The three different lights used in this research include A (incandescent tungsten light), D65 (noon daylight), and CWF-2 (cool white fluorescent light). CIELab and Hunter Lab both utilize the x,y,z values obtained during measurement of the color of the AX films, however, they analyze them differently to produce the L,a,b values. Hunter Lab uses the square roots of the x,y,z values to obtain the L,a,b values while CIELab uses cubed roots. In Table 4.7, the L,a,b values for all films determined using CIELab are given. In Table 4.8, the L,a,b values for all films determined using Hunter Lab are given. The colors of AX films may be due to a number of chemical species, but is most likely due to the pigments found in the aleurone layer of the bran. In addition, the lignin present in wheat and maize may impart some color into the AX films (Zhang et al. 2011). Extraction method can also impact the color of the AX films. For example, when WB AX is extracted using a hydrothermal process, is it much darker than WB AX that was alkaline extracted (Aguedo et al. 2014).


Wheat bran film

Maize bran film

Dried distillers grain film

Figure 4.3. Comparison of the colors of arabinoxylan films made using wheat bran arabinoxylan, maize bran arabinoxylan, or dried distillers grain arabinoxylan.

Table 4.7. CIELab lightness, redness, and yellowness values of arabinoxylan films using D65 light, A light, and CWF-2 light.

	D65			A			CWF-2		
Film composition	L	a	b	L	a	b	L	a	b
<u>Smooth side of films</u>									
WB AX[a] + 100 g kg^{-1} Sorbitol	28.59 ± 0.00	5.64 ± 0.01	7.49 ± 0.00	29.90 ± 0.24	7.42 ± 0.25	8.95 ± 0.49	29.19 ± 0.26	3.52 ± 0.13	8.15 ± 0.49
WB AX + 250 g kg^{-1} Sorbitol	28.82 ± 0.00	5.29 ± 0.02	6.41 ± 0.02	29.88 ± 0.00	7.07 ± 0.01	8.04 ± 0.02	29.20 ± 0.00	3.37 ± 0.01	7.21 ± 0.01
WB AX + 500 g kg^{-1} Sorbitol	28.02 ± 0.01	5.69 ± 0.03	6.49 ± 0.01	29.13 ± 0.01	7.43 ± 0.02	8.23 ± 0.02	28.41 ± 0.01	3.64 ± 0.02	7.29 ± 0.01
WB AX + 100 g kg^{-1} Glycerol	29.96 ± 0.01	5.43 ± 0.03	7.39 ± 0.03	31.10 ± 0.01	7.36 ± 0.02	9.10 ± 0.02	30.40 ± 0.01	3.45 ± 0.02	8.35 ± 0.02
WB AX + 250 g kg^{-1} Glycerol	28.51 ± 0.01	5.66 ± 0.02	6.77 ± 0.02	29.64 ± 0.00	7.48 ± 0.02	8.51 ± 0.01	28.92 ± 0.01	3.62 ± 0.02	7.59 ± 0.02
WB AX + 500 g kg^{-1} Glycerol	28.43 ± 0.00	6.15 ± 0.01	7.59 ± 0.01	29.66 ± 0.00	8.07 ± 0.01	9.48 ± 0.01	28.90 ± 0.00	3.93 ± 0.01	8.53 ± 0.01
MB AX[b] + 100 g kg^{-1} Sorbitol	41.08 ± 0.00	0.67 ± 0.02	3.32 ± 0.01	41.41 ± 0.00	2.17 ± 0.01	3.53 ± 0.00	41.10 ± 0.00	0.30 ± 0.01	3.59 ± 0.01
MB AX + 250 g kg^{-1} Sorbitol	40.94 ± 0.01	0.66 ± 0.00	3.53 ± 0.01	41.29 ± 0.01	2.22 ± 0.00	3.74 ± 0.01	40.97 ± 0.01	0.29 ± 0.00	3.84 ± 0.02
MB AX + 500 g kg^{-1} Sorbitol	41.06 ± 0.01	0.66 ± 0.01	3.50 ± 0.00	41.40 ± 0.00	2.20 ± 0.01	3.71 ± 0.00	41.09 ± 0.01	0.29 ± 0.01	3.80 ± 0.01
MB AX + 100 g kg^{-1} Glycerol	40.87 ± 0.00	0.70 ± 0.01	3.56 ± 0.00	41.23 ± 0.00	2.25 ± 0.01	3.79 ± 0.00	40.91 ± 0.00	0.31 ± 0.01	3.89 ± 0.00
MB AX + 250 g kg^{-1} Glycerol	41.44 ± 0.00	0.61 ± 0.00	2.32 ± 0.01	41.69 ± 0.00	1.88 ± 0.00	2.48 ± 0.01	41.41 ± 0.00	0.27 ± 0.01	2.46 ± 0.01
MB AX + 500 g kg^{-1} Glycerol	39.15 ± 0.01	0.69 ± 0.01	4.48 ± 0.02	39.56 ± 0.01	2.40 ± 0.01	4.74 ± 0.02	39.24 ± 0.01	0.31 ± 0.00	4.94 ± 0.01
DDG AX[c] + 100 g kg^{-1} Sorbitol	38.45 ± 0.00	1.20 ± 0.00	7.21 ± 0.01	39.09 ± 0.00	3.31 ± 0.00	7.76 ± 0.01	38.71 ± 0.00	0.57 ± 0.01	8.03 ± 0.02
DDG AX + 250 g kg^{-1} Sorbitol	37.95 ± 0.00	1.33 ± 0.02	7.90 ± 0.02	38.65 ± 0.00	3.57 ± 0.01	8.51 ± 0.02	38.25 ± 0.00	0.65 ± 0.01	8.81 ± 0.02
DDG AX + 500 g kg^{-1} Sorbitol	38.88 ± 0.00	1.10 ± 0.01	7.04 ± 0.01	39.50 ± 0.00	3.19 ± 0.01	7.56 ± 0.01	39.13 ± 0.01	0.51 ± 0.01	7.83 ± 0.02
DDG AX + 100 g kg^{-1} Glycerol	37.79 ± 0.01	1.31 ± 0.01	8.02 ± 0.02	38.48 ± 0.01	3.58 ± 0.00	8.61 ± 0.02	38.09 ± 0.01	0.63 ± 0.00	8.93 ± 0.02
DDG AX + 250 g kg^{-1} Glycerol	37.90 ± 0.01	1.25 ± 0.01	7.89 ± 0.01	38.59 ± 0.01	3.50 ± 0.01	8.47 ± 0.01	38.20 ± 0.01	0.59 ± 0.01	8.78 ± 0.01
DDG AX + 500 g kg^{-1} Glycerol	37.55 ± 0.01	1.24 ± 0.01	7.87 ± 0.01	38.22 ± 0.01	3.46 ± 0.01	8.45 ± 0.01	37.85 ± 0.01	0.59 ± 0.01	8.78 ± 0.01
<u>Rough side of films</u>									
WB AX + 100 g kg^{-1} Sorbitol	28.59 ± 0.00	5.64 ± 0.01	7.49 ± 0.00	29.76 ± 0.00	7.56 ± 0.00	9.23 ± 0.00	29.04 ± 0.00	3.60 ± 0.00	8.43 ± 0.02
WB AX + 250 g kg^{-1} Sorbitol	28.10 ± 0.01	5.70 ± 0.01	7.18 ± 0.01	29.25 ± 0.01	7.56 ± 0.00	8.93 ± 0.00	28.53 ± 0.01	3.64 ± 0.00	8.07 ± 0.01
WB AX + 500 g kg^{-1} Sorbitol	27.56 ± 0.00	5.54 ± 0.01	6.55 ± 0.01	28.65 ± 0.00	7.32 ± 0.01	8.24 ± 0.01	27.95 ± 0.00	3.54 ± 0.01	7.36 ± 0.01
WB AX + 100 g kg^{-1} Glycerol	28.27 ± 0.01	5.90 ± 0.01	7.49 ± 0.01	29.46 ± 0.01	7.79 ± 0.01	9.31 ± 0.01	28.72 ± 0.00	3.77 ± 0.01	8.43 ± 0.01
WB AX + 250 g kg^{-1} Glycerol	27.99 ± 0.01	5.72 ± 0.01	7.20 ± 0.00	29.14 ± 0.00	7.59 ± 0.01	8.96 ± 0.01	28.42 ± 0.01	3.65 ± 0.01	8.09 ± 0.01
WB AX + 500 g kg^{-1} Glycerol	28.16 ± 0.00	5.95 ± 0.02	7.72 ± 0.03	29.37 ± 0.00	7.87 ± 0.02	9.57 ± 0.02	28.63 ± 0.00	3.81 ± 0.02	8.69 ± 0.06
MB AX + 100 g kg^{-1} Sorbitol	41.07 ± 0.00	0.66 ± 0.01	3.19 ± 0.01	41.40 ± 0.00	2.14 ± 0.01	3.40 ± 0.01	41.09 ± 0.00	0.30 ± 0.01	3.45 ± 0.00
MB AX + 250 g kg^{-1} Sorbitol	41.00 ± 0.00	0.67 ± 0.02	3.49 ± 0.00	41.34 ± 0.00	2.21 ± 0.02	3.70 ± 0.00	41.03 ± 0.00	0.29 ± 0.01	3.79 ± 0.01
MB AX + 500 g kg^{-1} Sorbitol	41.11 ± 0.00	0.65 ± 0.01	3.43 ± 0.02	41.45 ± 0.00	2.17 ± 0.01	3.63 ± 0.02	41.14 ± 0.00	0.29 ± 0.01	3.71 ± 0.02

Table 4.7. CIELab lightness, redness, and yellowness values of arabinoxylan films using D65 light, A light, and CWF-2 light (continued).

	D65			A			CWF-2		
Film composition	L	a	b	L	a	b	L	a	b
MB AX + 100 g kg^{-1} Glycerol	40.82 ± 0.00	0.68 ± 0.00	3.50 ± 0.01	41.16 ± 0.00	2.22 ± 0.00	3.72 ± 0.01	40.85 ± 0.00	0.30 ± 0.00	3.81 ± 0.01
MB AX + 250 g kg^{-1} Glycerol	41.63 ± 0.00	0.58 ± 0.01	2.45 ± 0.01	41.90 ± 0.00	1.84 ± 0.01	2.61 ± 0.00	41.62 ± 0.00	0.26 ± 0.01	2.62 ± 0.00
MB AX + 500 g kg^{-1} Glycerol	39.39 ± 0.02	0.76 ± 0.01	4.73 ± 0.02	39.82 ± 0.02	2.58 ± 0.01	5.00 ± 0.02	39.48 ± 0.02	0.35 ± 0.01	5.20 ± 0.02
DDG AX + 100 g kg^{-1} Sorbitol	37.98 ± 0.00	1.37 ± 0.01	7.83 ± 0.01	38.67 ± 0.00	3.57 ± 0.01	8.45 ± 0.01	38.28 ± 0.00	0.68 ± 0.00	8.72 ± 0.01
DDG AX + 250 g kg^{-1} Sorbitol	38.48 ± 0.00	1.19 ± 0.02	7.35 ± 0.02	39.13 ± 0.00	3.33 ± 0.02	7.90 ± 0.02	38.75 ± 0.00	0.57 ± 0.02	8.18 ± 0.02
DDG AX + 500 g kg^{-1} Sorbitol	38.77 ± 0.00	1.12 ± 0.01	7.15 ± 0.01	39.40 ± 0.00	3.23 ± 0.01	7.68 ± 0.01	39.03 ± 0.00	0.52 ± 0.01	7.94 ± 0.01
DDG AX + 100 g kg^{-1} Glycerol	37.72 ± 0.00	1.35 ± 0.01	8.00 ± 0.01	38.42 ± 0.00	3.60 ± 0.01	8.61 ± 0.01	38.02 ± 0.01	0.66 ± 0.01	8.92 ± 0.01
DDG AX + 250 g kg^{-1} Glycerol	38.20 ± 0.00	1.19 ± 0.01	7.64 ± 0.02	38.86 ± 0.00	3.39 ± 0.01	8.19 ± 0.02	38.48 ± 0.00	0.57 ± 0.01	8.50 ± 0.02
DDG AX + 500 g kg^{-1} Glycerol	37.10 ± 0.01	1.34 ± 0.00	7.99 ± 0.01	37.79 ± 0.01	3.57 ± 0.00	8.60 ± 0.00	37.41 ± 0.01	0.66 ± 0.01	8.92 ± 0.01

[a] Wheat bran arabinoxylan
[b] Maize bran arabinoxylan
[c] Dried distillers grain arabinoxylan

Table 4.8. Hunter Lab lightness, redness, and yellowness analysis of arabinoxylan films using D65 light, A light, and CWF-2 light.

	D65			A			CWF-2		
Film composition	L	a	b	L	a	b	L	a	b
				Smooth side of films					
WB AX[a] + 100 g kg^{-1} Sorbitol	24.24 ± 0.00	3.58 ± 0.01	4.03 ± 0.01	25.11 ± 0.00	4.90 ± 0.00	4.98 ± 0.01	24.56 ± 0.00	2.26 ± 0.00	4.51 ± 0.01
WB AX + 250 g kg^{-1} Sorbitol	24.02 ± 0.00	3.55 ± 0.01	3.85 ± 0.01	24.88 ± 0.00	4.84 ± 0.01	4.79 ± 0.01	24.33 ± 0.00	2.25 ± 0.01	4.30 ± 0.01
WB AX + 500 g kg^{-1} Sorbitol	23.37 ± 0.01	3.79 ± 0.02	3.85 ± 0.01	24.26 ± 0.00	5.08 ± 0.01	4.84 ± 0.01	23.69 ± 0.00	2.41 ± 0.01	4.30 ± 0.00
WB AX + 100 g kg^{-1} Glycerol	24.94 ± 0.01	3.69 ± 0.02	4.44 ± 0.02	25.87 ± 0.01	5.10 ± 0.01	5.43 ± 0.01	25.30 ± 0.01	2.33 ± 0.01	4.98 ± 0.01
WB AX + 250 g kg^{-1} Glycerol	23.77 ± 0.00	3.79 ± 0.01	4.02 ± 0.01	24.68 ± 0.00	5.11 ± 0.01	5.02 ± 0.01	24.10 ± 0.01	2.41 ± 0.01	4.49 ± 0.01
WB AX + 500 g kg^{-1} Glycerol	23.71± 0.00	4.13 ± 0.01	4.46 ± 0.00	24.70 ± 0.00	5.54 ± 0.01	5.53 ± 0.00	24.08 ± 0.00	2.62 ± 0.01	4.98 ± 0.00
MB AX[b] + 100 g kg^{-1} Sorbitol	34.52 ± 0.00	0.49 ± 0.01	2.36 ± 0.00	34.82 ± 0.00	1.62 ± 0.01	2.51 ± 0.00	34.54 ± 0.00	0.22 ± 0.01	2.55 ± 0.01
MB AX + 250 g kg^{-1} Sorbitol	34.39 ± 0.01	0.49 ± 0.00	2.50 ± 0.01	34.71 ± 0.01	1.65 ± 0.00	2.66 ± 0.01	34.42 ± 0.01	0.21 ± 0.00	2.72 ± 0.01
MB AX + 500 g kg^{-1} Sorbitol	34.50 ± 0.00	0.49 ± 0.01	2.49 ± 0.00	34.81 ± 0.00	1.64 ± 0.00	2.64 ± 0.00	34.53 ± 0.00	0.21 ± 0.01	2.70 ± 0.01
MB AX + 100 g kg^{-1} Glycerol	34.33 ± 0.00	0.51 ± 0.01	2.53 ± 0.00	34.65 ± 0.00	1.67 ± 0.00	2.69 ± 0.00	34.37 ± 0.00	0.23 ± 0.01	2.75 ± 0.00
MB AX + 250 g kg^{-1} Glycerol	34.84 ± 0.00	0.45 ± 0.00	1.67 ± 0.00	35.08 ± 0.00	1.40 ± 0.00	1.79 ± 0.01	34.82 ± 0.00	0.20 ± 0.00	1.77 ± 0.01
MB AX + 500 g kg^{-1} Glycerol	32.79 ± 0.01	0.50 ± 0.01	3.10 ± 0.01	33.15 ± 0.01	1.76 ± 0.00	3.27 ± 0.01	32.87 ± 0.01	0.22 ± 0.00	3.40 ± 0.01

Table 4.8. Hunter Lab lightness, redness, and yellowness analysis of arabinoxylan films using D65 light, A light, and CWF-2 light (continued).

	D65			A			CWF-2		
Film composition	L	a	b	L	a	b	L	a	b
DDG AX[c] + 100 g kg^{-1} Sorbitol	32.16 ± 0.00	0.86 ± 0.00	4.80 ± 0.00	32.73 ± 0.00	2.43 ± 0.00	5.17 ± 0.00	32.39 ± 0.00	0.41 ± 0.00	5.31 ± 0.01
DDG AX + 250 g kg^{-1} Sorbitol	31.72 ± 0.00	0.96 ± 0.01	5.19 ± 0.01	32.33 ± 0.00	2.61 ± 0.01	5.59 ± 0.01	31.99 ± 0.00	0.46 ± 0.01	5.75 ± 0.01
DDG AX + 500 g kg^{-1} Sorbitol	32.54 ± 0.00	0.80 ± 0.00	4.72 ± 0.01	33.09 ± 0.00	2.35 ± 0.01	5.07 ± 0.01	32.76 ± 0.01	0.37 ± 0.01	5.21 ± 0.01
DDG AX + 100 g kg^{-1} Glycerol	31.57 ± 0.01	0.94 ± 0.00	5.25 ± 0.01	32.19 ± 0.01	2.61 ± 0.00	5.64 ± 0.01	31.84 ± 0.01	0.46 ± 0.00	5.81 ± 0.01
DDG AX + 250 g kg^{-1} Glycerol	31.68 ± 0.01	0.90 ± 0.01	5.18 ± 0.01	32.28 ± 0.01	2.56 ± 0.01	5.57 ± 0.01	31.93 ± 0.01	0.43 ± 0.00	5.73 ± 0.00
DDG AX + 500 g kg^{-1} Glycerol	31.36 ± 0.01	0.89 ± 0.00	5.15 ± 0.00	31.96 ± 0.01	2.52 ± 0.00	5.53 ± 0.01	31.63 ± 0.01	0.43 ± 0.00	5.71 ± 0.01
				<u>Rough side of films</u>					
WB AX + 100 g kg^{-1} Sorbitol	23.84 ± 0.00	3.78 ± 0.00	4.41 ± 0.00	24.77 ± 0.00	5.18 ± 0.00	5.40 ± 0.00	24.19 ± 0.00	2.40 ± 0.00	4.94 ± 0.01
WB AX + 250 g kg^{-1} Sorbitol	23.44 ± 0.01	3.80 ± 0.00	4.22 ± 0.00	24.36 ± 0.01	5.15 ± 0.00	5.21 ± 0.00	23.78 ± 0.01	2.41 ± 0.00	4.72 ± 0.00
WB AX + 500 g kg^{-1} Sorbitol	23.01 ± 0.00	3.67 ± 0.01	3.86 ± 0.01	23.88 ± 0.00	4.95 ± 0.00	4.81 ± 0.00	23.32 ± 0.00	2.33 ± 0.01	4.31 ± 0.01
WB AX + 100 g kg^{-1} Glycerol	23.57 ± 0.00	3.94 ± 0.01	4.40 ± 0.01	24.53 ± 0.00	5.33 ± 0.01	5.42 ± 0.01	23.9 ± 0.00	2.50 ± 0.01	4.92 ± 0.00
WB AX + 250 g kg^{-1} Glycerol	23.35 ± 0.00	3.81 ± 0.01	4.23 ± 0.00	24.28 ± 0.00	5.17 ± 0.01	5.22 ± 0.00	23.69 ± 0.01	2.42 ± 0.01	4.72 ± 0.00
WB AX + 500 g kg^{-1} Glycerol	23.49 ± 0.00	3.98 ± 0.02	4.51 ± 0.01	24.46 ± 0.00	5.38 ± 0.01	5.55 ± 0.01	23.87 ± 0.00	2.53 ± 0.02	5.05 ± 0.03
MB AX + 100 g kg^{-1} Sorbitol	34.51 ± 0.00	0.49 ± 0.01	2.28 ± 0.01	34.81 ± 0.00	1.59 ± 0.01	2.42 ± 0.01	34.53 ± 0.00	0.22 ± 0.00	2.46 ± 0.00
MB AX + 250 g kg^{-1} Sorbitol	34.44 ± 0.00	0.49 ± 0.01	2.48 ± 0.00	34.75 ± 0.00	1.65 ± 0.01	2.63 ± 0.00	34.47 ± 0.00	0.22 ± 0.01	2.68 ± 0.00
MB AX + 500 g kg^{-1} Sorbitol	34.55 ± 0.00	0.48 ± 0.01	2.44 ± 0.01	34.85 ± 0.00	1.62 ± 0.01	2.59 ± 0.01	34.57 ± 0.00	0.21 ± 0.00	2.63 ± 0.01
MB AX + 100 g kg^{-1} Glycerol	34.28 ± 0.00	0.50 ± 0.00	2.48 ± 0.01	34.59 ± 0.00	1.65 ± 0.00	2.64 ± 0.01	34.31 ± 0.00	0.22 ± 0.00	2.69 ± 0.01
MB AX + 250 g kg^{-1} Glycerol	35.02 ± 0.00	0.43 ± 0.01	1.77 ± 0.00	35.26 ± 0.00	1.38 ± 0.01	1.89 ± 0.00	35.01 ± 0.00	0.19 ± 0.01	1.89 ± 0.00
MB AX + 500 g kg^{-1} Glycerol	32.99 ± 0.02	0.56 ± 0.01	3.26 ± 0.01	33.38 ± 0.02	1.90 ± 0.01	3.46 ± 0.01	33.08 ± 0.02	0.25 ± 0.01	3.57 ± 0.01
DDG AX + 100 g kg^{-1} Sorbitol	31.74 ± 0.00	0.99 ± 0.00	5.15 ± 0.01	32.35 ± 0.00	2.61 ± 0.00	5.56 ± 0.01	32.01 ± 0.00	0.49 ± 0.00	5.70 ± 0.01
DDG AX + 250 g kg^{-1} Sorbitol	32.19 ± 0.00	0.86 ± 0.01	4.88 ± 0.01	32.76 ± 0.00	2.45 ± 0.01	5.26 ± 0.01	32.43 ± 0.00	0.41 ± 0.01	5.40 ± 0.01
DDG AX + 500 g kg^{-1} Sorbitol	32.45 ± 0.00	0.81 ± 0.01	4.78 ± 0.00	33.00 ± 0.00	2.38 ± 0.01	5.13 ± 0.00	32.68 ± 0.00	0.38 ± 0.01	5.28 ± 0.01
DDG AX + 100 g kg^{-1} Glycerol	31.51 ± 0.00	0.97 ± 0.01	5.24 ± 0.01	32.13 ± 0.00	2.63 ± 0.01	5.64 ± 0.01	31.78 ± 0.00	0.47 ± 0.01	5.80 ± 0.01
DDG AX + 250 g kg^{-1} Glycerol	31.93 ± 0.00	0.86 ± 0.01	5.05 ± 0.01	32.52 ± 0.00	2.49 ± 0.01	5.42 ± 0.01	32.18 ± 0.00	0.41 ± 0.01	5.58 ± 0.01
DDG AX + 500 g kg^{-1} Glycerol	23.84 ± 0.00	3.78 ± 0.00	4.41 ± 0.00	24.77 ± 0.00	5.18 ± 0.00	5.40 ± 0.00	24.19 ± 0.00	2.40 ± 0.00	4.94 ± 0.01

[a] Wheat bran arabinoxylan

[b] Maize bran arabinoxylan

[c] Dried distillers grain arabinoxylan

The colors of the smooth sides of all AX films analyzed with CIELab were averaged across the type of AX used, type of plasticizer used, and the level of the plasticizer used to make the film. The separation of all the means for these values is given in Table 4.9. Firstly, the color of each film (and side of each film) was very similar across all three types of light used. Compared to D65 light, the films appeared lighter, redder, and yellower under A light. In addition, when compared to D65 light, the films appeared lighter, greener, and yellower under CWF-2 light. Secondly, the smooth side of the WB AX films were significantly ($P≤0.01$) darker, and redder than the MB AX films and DDG AX films. In addition, the MB AX films were significantly ($P≤0.01$) lighter, less red, and less yellow than the other films. The MB AX films had values very close to those for a film that is clear (L,a,b = 50,0,0). The DDG AX films were significantly ($P≤0.01$) lighter and less red than the WB AX films, but yellower than both the WB AX and MB AX films. Thirdly, when averaged across the type of plasticizer used, the glycerol films appeared significantly ($P≤0.01$) darker, yellower, and redder than the films made with sorbitol. Fourthly, as the level of plasticizer increased, the films became significantly ($P≤0.01$) lighter. In addition, the films made with 500 g kg^{-1} plasticizer were the reddest and films made with 250 g kg^{-1} plasticizer were significantly ($P≤0.01$) bluer than the other films. When analyzed using Hunter Lab, the results were very similar as seen in Table 4.10.

When the colors of the rough sides of the AX films were analyzed with CIELab, very similar trends were seen. Firstly, the trends in color variation amongst the three types of light were the same as those seen in the smooth sides of the films. Secondly, the same trends were seen in the colors of the rough sides of the films when averaged across the type of AX used. However, the rough sides of the films were lighter, redder, and yellower than the smooth sides of the films. Thirdly, the same trends were also seen in the color of the rough sides of the films when averaged across the type of plasticizer used as those seen in the smooth sides. The rough side of the films was darker, redder, and yellower than the smooth side. Fourthly, as the plasticizer level increased, the rough side of the AX film became lighter and yellower. The rough side of the films with 100 g kg^{-1} plasticizer were the reddest. When the separation of the means for the color of all AX films was performed on the data obtained using Hunter Lab, the results were very similar to the results of the analysis with CIELab.

Table 4.9. Mean separation of CIELab lightness, redness, and yellowness analysis of arabinoxylan films using D65 light, A light, and CWF-2 light.

			D65			A			CWF-2		
Arabinoxylan type	Plasticizer type	Plasticizer level (g kg^{-1})	L	a	b	L	a	b	L	a	b
							Smooth side of films				
WB[a]			28.72	5.65	7.02	29.88	7.47	8.72	29.17	3.59	7.85
MB[b]			40.76	0.66	3.45	41.10	2.19	3.66	40.79	0.29	3.75
DDG[c]			38.09	1.24	7.66	38.75	3.43	8.22	38.37	0.59	8.53
LSD ($P \le 0.05$)[d]			0.00	0.01	0.01	0.04	0.04	0.08	0.04	0.02	0.08
LSD ($P \le 0.01$)			0.01	0.01	0.01	0.05	0.05	0.11	0.06	0.03	0.11
	Glycerol		35.73	2.56	6.21	36.46	4.44	7.07	35.99	1.52	6.92
	Sorbitol		35.98	2.47	5.88	36.69	4.29	6.67	36.23	1.46	6.51
	LSD ($P \le 0.05$)		0.00	0.01	0.01	0.03	0.03	0.07	0.03	0.02	0.07
	LSD ($P \le 0.01$)		0.01	0.01	0.01	0.04	0.04	0.09	0.05	0.02	0.09
		100	36.12	2.49	6.16	36.87	4.35	6.96	36.40	1.46	6.82
		250	35.93	2.47	5.80	36.62	4.29	6.62	36.16	1.46	6.45
		500	35.52	2.59	6.16	36.25	4.46	7.03	35.77	1.55	6.86
		LSD ($P \le 0.05$)	0.00	0.01	0.01	0.04	0.04	0.08	0.04	0.02	0.08
		LSD ($P \le 0.01$)	0.01	0.01	0.01	0.05	0.05	0.11	0.06	0.03	0.11
							Rough side of films				
WB			28.11	5.74	7.27	29.27	7.61	9.04	28.55	3.67	8.18
MB			40.84	0.67	3.46	41.18	2.19	3.68	40.87	0.30	3.76
DDG			38.04	1.26	7.66	38.71	3.45	8.24	38.33	0.61	8.53
LSD ($P \le 0.05$)			0.00	0.01	0.01	0.04	0.01	0.01	0.01	0.01	0.01
LSD ($P \le 0.01$)			0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02
	Glycerol		35.47	2.61	6.30	36.21	4.50	7.17	35.74	1.56	7.02
	Sorbitol		35.85	2.50	5.96	36.56	4.34	6.80	36.09	1.49	6.63
	LSD ($P \le 0.05$)		0.00	0.01	0.01	0.00	0.01	0.01	0.00	0.01	0.01
	LSD ($P \le 0.01$)		0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
		100	35.74	2.60	6.25	36.48	4.48	7.12	36.00	1.55	6.96
		250	35.90	2.51	5.88	36.60	4.32	6.72	36.14	1.50	6.54
		500	35.35	2.56	6.26	36.08	4.46	7.12	35.61	1.53	6.97
		LSD ($P \le 0.05$)	0.00	0.01	0.01	0.00	0.01	0.01	0.01	0.01	0.01
		LSD ($P \le 0.01$)	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02

[a] Wheat bran

[b] Maize bran

[c] Dried distillers grain

[d] Least significant difference

Table 4.10. Mean separation of Hunter Lab lightness, redness, and yellowness analysis of arabinoxylan films using D65 light, A light, and CWF-2 light.

			D65			A			CWF-2		
Arabinoxylan type	Plasticizer type	Plasticizer level (g kg^{-1})	L	a	b	L	a	b	L	a	b
						Smooth side of films					
WB[a]			24.01	3.76	4.11	24.92	5.10	5.10	24.34	2.38	4.59
MB[b]			34.23	0.49	2.44	34.53	1.62	2.59	34.25	0.22	2.65
DDG[c]			31.84	0.89	5.05	32.43	2.51	5.43	32.09	0.43	5.59
LSD (*P*≤0.05)[d]			0.00	0.01	0.01	0.00	0.01	0.01	0.00	0.01	0.01
LSD (*P*≤0.01)			0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
	Glycerol		29.89	1.76	3.98	30.51	3.14	4.50	30.10	1.03	4.40
	Sorbitol		30.16	1.67	3.75	30.75	3.01	4.25	30.36	0.98	4.15
	LSD (*P*≤0.05)		0.00	0.01	0.01	0.00	0.00	0.01	0.00	0.00	0.01
	LSD (*P*≤0.01)		0.00	0.01	0.01	0.00	0.01	0.01	0.00	0.01	0.01
		100	30.29	1.68	3.90	30.89	3.06	4.40	30.50	0.98	4.32
		250	30.07	1.69	3.74	30.66	3.03	4.24	30.26	0.99	4.13
		500	29.71	1.77	3.96	30.33	3.15	4.48	29.93	1.04	4.38
		LSD (*P*≤0.05)	0.00	0.01	0.01	0.00	0.01	0.01	0.00	0.01	0.01
		LSD (*P*≤0.01)	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
						Rough side of films					
WB			23.45	3.83	4.27	24.38	5.19	5.27	23.80	2.43	4.77
MB			34.30	0.49	2.45	34.61	1.63	2.60	34.33	0.22	2.65
DDG			31.80	0.91	5.05	32.39	2.52	5.43	32.05	0.44	5.59
LSD (*P*≤0.05)			0.00	0.01	0.01	0.00	0.01	0.01	0.00	0.01	0.01
LSD (*P*≤0.01)			0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
	Glycerol		29.68	1.78	4.01	30.30	3.17	4.54	29.90	1.05	4.44
	Sorbitol		30.02	1.71	3.83	30.62	3.06	4.34	30.22	1.01	4.23
	LSD (*P*≤0.05)		0.00	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.01
	LSD (*P*≤0.01)		0.00	0.01	0.01	0.00	0.01	0.01	0.00	0.01	0.01
		100	29.91	1.78	3.99	30.53	3.17	4.51	30.13	1.05	4.42
		250	30.06	1.71	3.77	30.66	3.05	4.27	30.26	1.01	4.17
		500	29.58	1.74	4.01	30.19	3.14	4.52	29.79	1.03	4.43
		LSD (*P*≤0.05)	0.00	0.01	0.01	0.00	0.01	0.01	0.00	0.01	0.01
		LSD (*P*≤0.01)	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01

[a] Wheat bran
[b] Maize bran
[c] Dried distillers grain
[d] Least significant difference

The color of the AX films depended on many things including the interactions of the materials they are made from. The three factors that impacted the color of the AX films were the type of AX used, the type of plasticizer used, and the amount of the plasticizer used. The result of this is that there were numerous significant (*P*≤0.05) two-way interactions between these three factors as shown in Table 4.11. To begin, the lightness of the films will be discussed. Firstly, for both the smooth and rough sides of the AX films, the effect of the two-way interaction between the type of AX used and type of plasticizer used resulted in multiple responses from the factors. As the type of plasticizer was switched from glycerol to sorbitol, the WB AX had a converging response with both the MB AX and DDG AX. Secondly, a similar trend was observed in the two-way interaction between the type of AX used and the level of plasticizer for both the smooth and rough sides of the AX films. As the level of plasticizer increased, the WB and MB films became darker, but the DDG AX films became lighter. The result of this was a converging response between these factors. Thirdly, in the interaction between plasticizer type over increasing levels of plasticizer (both the smooth and rough sides), as plasticizer level increased the films became darker.

The second color value, redness to greenness (a), was also affected by significant (*P*≤0.05) two-way interactions. Firstly, for both the smooth and rough sides of the films, as the type of plasticizer was changed from glycerol to sorbitol, the films became greener regardless of the type of AX used. This showed a lack of true interaction for this two-way interaction. Secondly, for the smooth side of the films, the responses of the interaction between AX type and plasticizer level was a diverging response for the WB AX films and both the DDG AX films and MB AX films as plasticizer level increased. However, for the rough side of the films, there was a diverging response for the MB AX films and both the WB AX films and DDG AX films as the level of plasticizer increased. Thirdly, for both the smooth side and rough side of the AX films, there was a diverging response for the films made with glycerol and those made with sorbitol as the level of plasticizer increased.

Once again, all trends seen in the data for the color of AX films produced using Hunter Lab was very like that produced by CIELab as seen in Table 4.12. The similarities in the data were due to the calculation of the L,a,b values from the same x,y,z values.

Table 4.11. Mean separation of two-way interactions for lightness, redness, and yellowness analysis of arabinoxylan films obtained by CIELab using D65 light, A light, and CWF-2 light.

				D65			A			CWF-2	
Arabinoxylan type	Plasticizer type	Plasticizer level (g kg^{-1})	L	a	b	L	a	b	L	a	b
							Smooth side of films				
WB[a]	Glycerol		28.97	5.75	7.25	30.13	7.64	9.03	29.40	3.67	8.15
WB	Sorbitol		28.48	5.54	6.79	29.64	7.32	8.40	28.93	3.51	7.55
MB[b]	Glycerol		40.49	0.67	3.46	40.83	2.18	3.67	40.52	0.30	3.76
MB	Sorbitol		41.03	0.66	3.45	41.37	2.20	3.66	41.05	0.29	3.75
DDG[c]	Glycerol		37.75	1.27	7.93	38.43	3.51	8.51	38.04	0.61	8.83
DDG	Sorbitol		38.43	1.21	7.39	39.08	3.36	7.94	38.70	0.58	8.22
LSD (*P*≤0.05)[d]			0.01	0.01	0.01	0.05	0.06	0.11	0.06	0.03	0.11
LSD (*P*≤0.01)			0.01	0.02	0.02	0.07	0.08	0.15	0.08	0.04	0.15
WB		100	29.28	5.54	7.45	30.50	7.39	9.02	29.80	3.48	8.25
WB		250	28.67	5.48	6.59	29.76	7.27	8.27	29.06	3.49	7.40
WB		500	28.22	5.92	7.04	29.39	7.77	8.86	28.65	3.79	7.91
MB		100	40.98	0.68	3.44	41.32	2.21	3.66	41.01	0.30	3.74
MB		250	41.19	0.64	2.92	41.49	2.05	3.11	41.19	0.28	3.15
MB		500	40.11	0.67	3.99	40.48	2.30	4.22	40.17	0.30	4.37
DDG		100	38.12	1.25	7.61	38.79	3.44	8.19	38.40	0.60	8.48
DDG		250	37.93	1.29	7.90	38.62	3.53	8.49	38.23	0.62	8.80
DDG		500	38.21	1.17	7.46	38.86	3.33	8.00	38.49	0.55	8.30
LSD (*P*≤0.05)			0.01	0.02	0.02	0.07	0.07	0.14	0.07	0.04	0.14
LSD (*P*≤0.01)			0.01	0.02	0.02	0.09	0.09	0.18	0.10	0.05	0.18
	Glycerol	100	36.21	2.48	6.33	36.94	4.39	7.17	36.47	1.46	7.05
	Glycerol	250	35.95	2.51	5.66	36.64	4.29	6.49	36.17	1.49	6.28
	Glycerol	500	35.04	2.69	6.65	35.82	4.64	7.56	35.33	1.61	7.42
	Sorbitol	100	36.04	2.50	6.01	36.80	4.30	6.75	36.34	1.46	6.59
	Sorbitol	250	35.91	2.43	5.95	36.60	4.28	6.76	36.14	1.44	6.62
	Sorbitol	500	35.98	2.48	5.68	36.67	4.28	6.50	36.21	1.48	6.31
	LSD (*P*≤0.05)		0.01	0.01	0.01	0.05	0.06	0.11	0.06	0.03	0.11
	LSD (*P*≤0.01)		0.01	0.02	0.02	0.07	0.08	0.15	0.08	0.04	0.15
							Rough side of films				
WB	Glycerol		28.14	5.85	7.47	29.32	7.75	9.28	28.59	3.74	8.40
WB	Sorbitol		28.08	5.63	7.07	29.22	7.48	8.80	28.51	3.59	7.95
MB	Glycerol		40.61	0.67	3.56	40.96	2.22	3.78	40.65	0.30	3.88
MB	Sorbitol		41.06	0.66	3.37	41.40	2.17	3.58	41.09	0.29	3.65
DDG	Glycerol		37.67	1.30	7.88	38.36	3.52	8.47	37.97	0.63	8.78
DDG	Sorbitol		38.41	1.23	7.44	39.06	3.38	8.01	38.69	0.59	8.28
LSD (*P*≤0.05)			0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02
LSD (*P*≤0.01)			0.01	0.01	0.02	0.01	0.01	0.02	0.01	0.01	0.02
WB		100	28.43	5.77	7.49	29.61	7.68	9.27	28.88	3.68	8.43
WB		250	28.04	5.71	7.19	29.19	7.57	8.95	28.47	3.65	8.08

Table 4.11. Mean separation of two-way interactions for lightness, redness, and yellowness analysis of arabinoxylan films obtained by CIELab using D65 light, A light, and CWF-2 light (continued).

			D65			A			CWF-2		
Arabinoxylan type	Plasticizer type	Plasticizer level (g kg^{-1})	L	a	b	L	a	b	L	a	b
WB		500	27.86	5.74	7.14	29.01	7.59	8.90	28.29	3.67	8.03
MB		100	40.95	0.67	3.35	41.28	2.18	3.56	40.97	0.30	3.63
MB		250	41.32	0.62	2.97	41.62	2.03	3.16	41.32	0.28	3.20
MB		500	40.25	0.71	4.08	40.64	2.38	4.32	40.31	0.32	4.46
DDG		100	37.85	1.36	7.91	38.54	3.59	8.53	38.15	0.67	8.82
DDG		250	38.34	1.19	7.49	38.99	3.36	8.05	38.62	0.57	8.34
DDG		500	37.94	1.23	7.57	38.60	3.40	8.14	38.22	0.59	8.43
LSD (*P*≤0.05)			0.01	0.01	0.02	0.01	0.01	0.02	0.01	0.01	0.02
LSD (*P*≤0.01)			0.01	0.02	0.02	0.01	0.02	0.02	0.01	0.02	0.03
	Glycerol	100	35.60	2.64	6.33	36.35	4.54	7.21	35.86	1.58	7.05
	Glycerol	250	35.94	2.50	5.76	36.63	4.27	6.59	36.17	1.49	6.40
	Glycerol	500	34.88	2.69	6.81	35.66	4.67	7.72	35.17	1.61	7.60
	Sorbitol	100	35.88	2.56	6.17	36.61	4.42	7.02	36.14	1.52	6.87
	Sorbitol	250	35.86	2.52	6.00	36.57	4.37	6.84	36.10	1.50	6.68
	Sorbitol	500	35.81	2.44	5.71	36.50	4.24	6.52	36.04	1.45	6.34
	LSD (*P*≤0.05)		0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02
	LSD (*P*≤0.01)		0.01	0.01	0.02	0.01	0.01	0.02	0.01	0.01	0.02

[a] Wheat bran
[b] Maize bran
[c] Dried distillers grain
[d] Least significant difference

Table 4.12. Mean separation of two-way interactions for lightness, redness, and yellowness analysis of arabinoxylan films obtained by Hunter Lab using D65 light, A light, and CWF-2 light.

			D65			A			CWF-2		
Arabinoxylan type	Plasticizer type	Plasticizer level (g kg^{-1})	L	a	b	L	a	b	L	a	b
							Smooth side of films				
WB[a]	Glycerol		24.14	3.87	4.31	25.08	5.25	5.33	24.49	2.45	4.82
WB	Sorbitol		23.88	3.64	3.91	24.75	4.94	4.87	24.19	2.31	4.37
MB[b]	Glycerol		33.99	0.49	2.43	34.29	1.61	2.59	34.02	0.22	2.64
MB	Sorbitol		34.47	0.49	2.45	34.78	1.64	2.60	34.49	0.22	2.65
DDG[c]	Glycerol		31.54	0.91	5.19	32.14	2.56	5.58	31.80	0.44	5.75
DDG	Sorbitol		32.14	0.87	4.90	32.72	2.46	5.28	32.38	0.42	5.42
LSD (*P*≤0.05)[d]			0.01	0.01	0.01	0.00	0.01	0.01	0.01	0.01	0.01
LSD (*P*≤0.01)			0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
WB		100	24.59	3.64	4.24	25.49	5.00	5.20	24.93	2.29	4.75
WB		250	23.90	3.67	3.93	24.78	4.97	4.90	24.21	2.33	4.40
WB		500	23.54	3.96	4.15	24.48	5.31	5.18	23.88	2.52	4.64
MB		100	34.42	0.50	2.44	34.73	1.64	2.60	34.45	0.22	2.65
MB		250	34.62	0.47	2.09	34.89	1.53	2.22	34.62	0.21	2.24

Table 4.12. Mean separation of two-way interactions for lightness, redness, and yellowness analysis of arabinoxylan films obtained by Hunter Lab using D65 light, A light, and CWF-2 light (continued).

			D65			A			CWF-2		
Arabinoxylan type	Plasticizer type	Plasticizer level (g kg^{-1})	L	a	b	L	a	b	L	a	b
MB		500	33.64	0.49	2.79	33.98	1.70	2.96	33.70	0.22	3.05
DDG		100	31.87	0.90	5.03	32.46	2.52	5.41	32.12	0.43	5.56
DDG		250	31.70	0.93	5.19	32.31	2.58	5.58	31.96	0.45	5.74
DDG		500	31.95	0.84	4.93	32.52	2.43	5.30	32.19	0.40	5.46
LSD (*P*≤0.05)			0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
LSD (*P*≤0.01)			0.01	0.02	0.01	0.01	0.01	0.01	0.01	0.01	0.01
	Glycerol	100	30.28	1.71	4.08	30.90	3.13	4.59	30.50	1.00	4.51
	Glycerol	250	30.10	1.71	3.63	30.68	3.03	4.13	30.28	1.01	4.00
	Glycerol	500	29.28	1.84	4.24	29.94	3.27	4.78	29.52	1.09	4.69
	Sorbitol	100	30.30	1.65	3.73	30.89	2.98	4.22	30.50	0.97	4.12
	Sorbitol	250	30.04	1.67	3.85	30.64	3.03	4.34	30.24	0.98	4.26
	Sorbitol	500	30.14	1.69	3.68	30.72	3.02	4.18	30.33	1.00	4.07
	LSD (*P*≤0.05)		0.01	0.01	0.01	0.00	0.01	0.01	0.01	0.01	0.01
	LSD (*P*≤0.01)		0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
							<u>Rough side of films</u>				
WB	Glycerol		23.47	3.91	4.38	24.42	5.29	5.40	23.83	2.48	4.89
WB	Sorbitol		23.43	3.75	4.16	24.34	5.09	5.14	23.77	2.38	4.65
MB	Glycerol		34.10	0.50	2.50	34.41	1.64	2.66	34.13	0.22	2.72
MB	Sorbitol		34.50	0.49	2.40	34.80	1.62	2.55	34.52	0.22	2.59
DDG	Glycerol		31.47	0.93	5.16	32.08	2.57	5.55	31.74	0.45	5.71
DDG	Sorbitol		32.13	0.89	4.94	32.71	2.48	5.32	32.37	0.43	5.46
LSD (*P*≤0.05)			0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
LSD (*P*≤0.01)			0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
WB		100	23.71	3.86	4.40	24.65	5.25	5.41	24.07	2.45	4.93
WB		250	23.39	3.80	4.22	24.32	5.16	5.22	23.74	2.42	4.72
WB		500	23.25	3.82	4.18	24.17	5.17	5.18	23.59	2.43	4.68
MB		100	34.40	0.50	2.38	34.70	1.62	2.53	34.42	0.22	2.57
MB		250	34.73	0.46	2.12	35.01	1.51	2.26	34.74	0.20	2.29
MB		500	33.77	0.52	2.85	34.12	1.76	3.02	33.82	0.23	3.10
DDG		100	31.63	0.98	5.19	32.24	2.62	5.60	31.90	0.48	5.75
DDG		250	32.06	0.86	4.97	32.64	2.47	5.34	32.31	0.41	5.49
DDG		500	31.71	0.89	4.99	32.29	2.48	5.37	31.96	0.43	5.52
LSD (*P*≤0.05)			0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
LSD (*P*≤0.01)			0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02
	Glycerol	100	29.79	1.80	4.04	30.42	3.20	4.57	30.01	1.07	4.47
	Glycerol	250	30.10	1.70	3.68	30.69	3.01	4.18	30.30	1.01	4.06
	Glycerol	500	29.15	1.83	4.32	29.81	3.29	4.87	29.39	1.08	4.79
	Sorbitol	100	30.03	1.75	3.95	30.64	3.13	4.46	30.24	1.04	4.36
	Sorbitol	250	30.02	1.72	3.86	30.63	3.08	4.37	30.23	1.01	4.27
	Sorbitol	500	30.00	1.65	3.69	30.58	2.98	4.18	30.19	0.97	4.07
	LSD (*P*≤0.05)		0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
	LSD (*P*≤0.01)		0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01

[a] Wheat bran

[b] Maize bran

[c] Dried distillers grain

[d] Least significant difference

There were also numerous significant (*P*≤0.05) three-way interactions listed in Table 4.13 for the color of films analyzed by CIELab. In these interactions, the effect of two of the factors over the entire range of the third factor influenced the color of the films. For films made with glycerol, there were trends for each color parameter (L,a,b). Firstly, for both the smooth and rough sides of the films, there did not appear to be a true interaction between the AX type and plasticizer level for the lightness of the films. This was because for all three types of AX, as plasticizer level increased, the films (both the smooth and rough sides) became darker. Secondly, for the redness of the smooth sides of the films, as the level of plasticizer increased for films made with glycerol, there was a diverging response between WB AX films and both the MB AX films and DDG AX films. However, for the rough side of these films as the level of plasticizer increased (for films made with glycerol), there was a diverging response between MB AX films and WB AX films, but there was a converging response between MB AX films and DDG AX films. Thirdly, for both the smooth and rough sides of films made with glycerol, as the level of plasticizer increased, there was a converging response between DDG AX films and both WB AX films and MB AX films.

For films made with sorbitol, the lightness, redness, and yellowness of the films were affected by the three-way interaction between the three factors of the AX film composition. Firstly, for the smooth side of the films, as the plasticizer level increased, there was a diverging response between WB AX and DDG AX and a converging response between DDG AX and MB AX. For the rough side of these same films, as plasticizer level increased there was a diverging response between DDG AX and both WB AX and MB AX. Secondly, for the rough side of the films, there did not appear to be a true three-way interaction as the films all become bluer as the plasticizer level was increased for all types of AX. However, there was a diverging response between WB AX and both MB AX and DDG AX as plasticizer level increased for the yellowness of the smooth side of all films made with sorbitol. Thirdly, for both the rough and smooth sides of the films, there was the same trend. As plasticizer level increased, there was a crossover response between the WB AX and DDG AX. In addition, there was a converging response between MB AX and WB AX as the plasticizer level increased.

Table 4.13. Mean separation of three-way interactions for lightness, redness, and yellowness analysis of arabinoxylan films obtained by CIELab using D65 Light, A light, and CWF-2 light.

Arabinoxylan type	Plasticizer type	Plasticizer level (g kg^{-1})	D65			A			CWF-2		
			L	a	b	L	a	b	L	a	b
						Smooth side of films					
WB[a]	Glycerol	100	29.96	5.43	7.40	31.10	7.36	9.10	30.40	3.45	8.35
WB	Glycerol	250	28.51	5.66	6.77	29.64	7.48	8.51	28.92	3.62	7.59
WB	Glycerol	500	28.43	6.15	7.59	29.66	8.07	9.48	28.90	3.93	8.53
MB[b]	Glycerol	100	40.87	0.70	3.56	41.23	2.25	3.79	40.91	0.31	3.89
MB	Glycerol	250	41.44	0.61	2.32	41.69	1.88	2.48	41.41	0.27	2.46
MB	Glycerol	500	39.15	0.69	4.48	39.56	2.40	4.74	39.24	0.31	4.94
DDG[c]	Glycerol	100	37.79	1.31	8.02	38.48	3.58	8.61	38.09	0.63	8.93
DDG	Glycerol	250	37.90	1.25	7.89	38.59	3.50	8.47	38.20	0.59	8.78
DDG	Glycerol	500	37.55	1.24	7.87	38.22	3.46	8.45	37.85	0.59	8.78
WB	Sorbitol	100	28.59	5.64	7.49	29.90	7.42	8.95	29.19	3.52	8.15
WB	Sorbitol	250	28.82	5.29	6.41	29.88	7.07	8.04	29.20	3.37	7.21
WB	Sorbitol	500	28.02	5.69	6.49	29.13	7.46	8.23	28.41	3.64	7.29
MB	Sorbitol	100	41.08	0.67	3.32	41.41	2.17	3.53	41.10	0.30	3.59
MB	Sorbitol	250	40.94	0.66	3.53	41.29	2.22	3.74	40.97	0.29	3.84
MB	Sorbitol	500	41.06	0.66	3.50	41.40	2.20	3.71	41.09	0.29	3.80
DDG	Sorbitol	100	38.45	1.20	7.21	39.09	3.31	7.76	38.71	0.57	8.03
DDG	Sorbitol	250	37.95	1.33	7.90	38.65	3.57	8.51	38.25	0.65	8.81
DDG	Sorbitol	500	38.88	1.10	7.04	39.50	3.19	7.56	39.13	0.51	7.83
LSD (*P*≤0.05)[d]			0.01	0.02	0.02	0.09	0.10	0.19	0.10	0.05	0.19
LSD (*P*≤0.01)			0.01	0.03	0.03	0.13	0.13	0.26	0.14	0.07	0.26
						Rough side of films					
WB	Glycerol	100	28.27	5.90	7.49	29.46	7.79	9.31	28.72	3.77	8.43
WB	Glycerol	250	27.99	5.72	7.20	29.14	7.59	8.96	28.42	3.65	8.09
WB	Glycerol	500	28.16	5.95	7.72	29.37	7.87	9.57	28.63	3.81	8.69
MB	Glycerol	100	40.82	0.68	3.50	41.16	2.22	3.72	40.85	0.30	3.81
MB	Glycerol	250	41.63	0.58	2.45	41.90	1.84	2.61	41.62	0.26	2.62
MB	Glycerol	500	39.39	0.76	4.73	39.82	2.58	5.00	39.48	0.35	5.20
DDG	Glycerol	100	37.72	1.35	8.00	38.42	3.60	8.61	38.02	0.66	8.92
DDG	Glycerol	250	38.20	1.19	7.64	38.86	3.39	8.19	38.48	0.57	8.50
DDG	Glycerol	500	37.10	1.34	7.99	37.79	3.57	8.60	37.41	0.66	8.92
WB	Sorbitol	100	28.59	5.64	7.49	29.76	7.56	9.23	29.04	3.60	8.43
WB	Sorbitol	250	28.10	5.70	7.18	29.25	7.56	8.93	28.53	3.64	8.07
WB	Sorbitol	500	27.56	5.54	6.55	28.65	7.32	8.24	27.95	3.54	7.36
MB	Sorbitol	100	41.07	0.66	3.19	41.40	2.14	3.40	41.09	0.30	3.45
MB	Sorbitol	250	41.00	0.67	3.49	41.34	2.21	3.70	41.03	0.29	3.79
MB	Sorbitol	500	41.11	0.65	3.43	41.45	2.17	3.63	41.14	0.29	3.71
DDG	Sorbitol	100	37.98	1.37	7.83	38.67	3.57	8.45	38.28	0.68	8.72
DDG	Sorbitol	250	38.48	1.19	7.35	39.13	3.33	7.90	38.75	0.57	8.18
DDG	Sorbitol	500	38.77	1.12	7.15	39.40	3.23	7.68	39.03	0.52	7.94
LSD (*P*≤0.05)			0.01	0.02	0.02	0.01	0.02	0.02	0.01	0.02	0.03
LSD (*P*≤0.01)			0.01	0.03	0.03	0.01	0.02	0.03	0.01	0.02	0.04

[a] Wheat bran

[b] Maize bran

[c] Dried distillers grain

[d] Least significant difference

Mean separation for three-way interactions for the color of films analyzed by Hunter Lab is given in Table 4.14. The same trends for the effects of the three-way interactions on the color of both the smooth and rough sides of the AX films were seen in the color data produced using Hunter Lab as when CIELab was used. This is because the interactions are due to the chemical composition of the AX films. The similarities between the calculations performed using CIELab and Hunter Lab result in the same trends and numbers that vary slightly.

In Table 4.15, the relationship between numerous chemical characteristics of the AX films made with sorbitol and the colors of the films (assessed using CIELab) are given. Firstly, for both the smooth and rough sides of the films and the three types of light, there was a relationship between the PI of the film solution after 24 hours of stirring and the color of the film. As the PI increased, the films became darker and redder. Secondly, for almost all six combinations (three types of light and both sides of the films), there was a significant ($P \leq 0.01$ or $P \leq 0.05$) correlation between an increase in Mw of the solution after stirring for 24 hours and an increase in the blueness of the films. Thirdly, as the PI of the heated film solutions increased, the films became bluer. Fourthly, there was a significant ($P \leq 0.001$) correlation between an increase in the Mw of the heated solutions and an increase in the darkness and redness of the films for all lights and sides of the films. Fifthly, there was a significant ($P \leq 0.05$) correlation between an increase in the PI of the solutions after the addition of the plasticizer and an increase in the blueness of both the smooth and rough sides of the films when illuminated with D65 or CWF-2 light. Sixthly, there was a significant ($P \leq 0.01$ or $P \leq 0.05$) correlation between an increase in the Mw of the film solutions after the addition of the plasticizer and an increase in the darkness, redness, and yellowness of the films for both the smooth and rough sides. Seventhly, there was a significant ($P \leq 0.05$) correlation between an increase in the moisture content of the films and an increase in the darkness and redness of both the smooth and rough sides of the films when illuminated by all three types of light.

Table 4.14. Mean separation of three-way interactions for lightness, redness, and yellowness analysis of arabinoxylan films obtained by Hunter Lab using D65 Light, A light, and CWF-2 light.

			D65			A			CWF-2		
Arabinoxylan type	Plasticizer type	Plasticizer level (g kg^{-1})	L	a	b	L	a	b	L	a	b
Smooth side of films											
WB[a]	Glycerol	100	24.94	3.69	4.45	25.87	5.10	5.43	25.30	2.33	4.98
WB	Glycerol	250	23.77	3.79	4.02	24.68	5.11	5.02	24.10	2.41	4.49
WB	Glycerol	500	23.71	4.13	4.46	24.70	5.54	5.53	24.08	2.62	4.98
MB[b]	Glycerol	100	34.33	0.51	2.53	34.65	1.67	2.69	34.37	0.23	2.75
MB	Glycerol	250	34.84	0.45	1.67	35.08	1.40	1.79	34.82	0.20	1.77
MB	Glycerol	500	32.79	0.50	3.10	33.15	1.76	3.27	32.87	0.22	3.40
DDG[c]	Glycerol	100	31.57	0.94	5.25	32.19	2.61	5.64	31.84	0.46	5.81
DDG	Glycerol	250	31.68	0.90	5.18	32.28	2.56	5.57	31.93	0.43	5.73
DDG	Glycerol	500	31.36	0.89	5.15	31.96	2.52	5.53	31.63	0.43	5.71
WB	Sorbitol	100	24.24	3.58	4.03	25.11	4.90	4.98	24.56	2.26	4.51
WB	Sorbitol	250	24.02	3.55	3.85	24.88	4.84	4.79	24.33	2.25	4.30
WB	Sorbitol	500	23.37	3.79	3.85	24.26	5.08	4.84	23.69	2.41	4.30
MB	Sorbitol	100	34.52	0.49	2.36	34.82	1.62	2.51	34.54	0.22	2.55
MB	Sorbitol	250	34.39	0.49	2.50	34.71	1.65	2.66	34.42	0.21	2.72
MB	Sorbitol	500	34.50	0.49	2.49	34.81	1.64	2.64	34.53	0.21	2.70
DDG	Sorbitol	100	32.16	0.86	4.80	32.73	2.43	5.17	32.39	0.41	5.31
DDG	Sorbitol	250	31.72	0.96	5.19	32.33	2.61	5.59	31.99	0.46	5.75
DDG	Sorbitol	500	32.54	0.80	4.72	33.09	2.35	5.07	32.76	0.37	5.21
LSD ($P \leq 0.05$)[d]			0.01	0.02	0.01	0.01	0.01	0.01	0.01	0.01	0.01
LSD ($P \leq 0.01$)			0.01	0.02	0.02	0.01	0.02	0.02	0.01	0.02	0.02
Rough side of films											
WB	Glycerol	100	23.57	3.94	4.40	24.53	5.33	5.42	23.94	2.50	4.92
WB	Glycerol	250	23.35	3.81	4.23	24.28	5.17	5.22	23.69	2.42	4.72
WB	Glycerol	500	23.49	3.98	4.51	24.46	5.38	5.55	23.87	2.53	5.05
MB	Glycerol	100	34.28	0.50	2.48	34.59	1.65	2.64	34.31	0.22	2.69
MB	Glycerol	250	35.02	0.43	1.77	35.26	1.38	1.89	35.01	0.19	1.89
MB	Glycerol	500	32.99	0.56	3.26	33.38	1.90	3.46	33.08	0.25	3.57
DDG	Glycerol	100	31.51	0.97	5.24	32.13	2.63	5.64	31.78	0.47	5.80
DDG	Glycerol	250	31.93	0.86	5.05	32.52	2.49	5.42	32.18	0.41	5.58
DDG	Glycerol	500	30.97	0.96	5.20	31.58	2.59	5.60	31.24	0.47	5.76
WB	Sorbitol	100	23.84	3.78	4.41	24.77	5.18	5.40	24.19	2.40	4.94
WB	Sorbitol	250	23.44	3.80	4.22	24.36	5.15	5.21	23.78	2.41	4.72
WB	Sorbitol	500	23.01	3.67	3.86	23.88	4.95	4.81	23.32	2.33	4.31
MB	Sorbitol	100	34.51	0.49	2.28	34.81	1.59	2.42	34.53	0.22	2.46
MB	Sorbitol	250	34.44	0.49	2.48	34.75	1.65	2.63	34.47	0.22	2.68
MB	Sorbitol	500	34.55	0.48	2.44	34.85	1.62	2.59	34.57	0.21	2.63
DDG	Sorbitol	100	31.74	0.99	5.15	32.35	2.61	5.56	32.01	0.49	5.70
DDG	Sorbitol	250	32.19	0.86	4.88	32.76	2.45	5.26	32.43	0.41	5.40
DDG	Sorbitol	500	32.45	0.81	4.78	33.00	2.38	5.13	32.68	0.38	5.28
LSD ($P \leq 0.05$)			0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02
LSD ($P \leq 0.01$)			0.01	0.02	0.02	0.01	0.02	0.02	0.01	0.02	0.02

[a] Wheat bran

[b] Maize bran

[c] Dried distillers grain

[d] Least significant difference

The same correlations between the chemical characteristics of the films and their color were present for films made with glycerol (analyzed by CIELab). However, there were two distinct differences. Firstly, there was no significant ($P \leq 0.05$) correlation between the color of the films and their moisture content. Secondly, there was a significant ($P \leq 0.01$) correlation between an increase in the Mw of the films and an increase in the darkness and redness of the films when both all three types of light illuminate the smooth and dark sides of the films. These correlations are given in Table 4.16.

The correlations between linkages present in the AX and the color of the films were also analyzed. There was only one significant ($P \leq 0.05$ or $P \leq 0.01$) correlation present between linkages and the color of the films. The correlation was that as the xylose backbone was more substituted, the films became yellower. This is indicated by the positive correlation between R1-R6 and the b-values for each type of light. The negative correlation between the abundance of R7 and the b-value of the color for the films also supports this.

The correlations between the chemical characteristics and the color of the AX films analyzed using Hunter Lab are given in Table 4.17 for films made with sorbitol and 4.18 for films made with glycerol. The same correlations were present when the films were analyzed using Hunter Lab as when the colors of the AX films were analyzed using CIELab. Once again, this is due to the similarities in which CIELab and Hunter Lab determine the L,a,b values for each color.

Table 4.15. Correlations between lightness, redness, and yellowness of arabinoxylan films made with sorbitol assessed with CIELab (using D65 light, A light, and CWF-2 light) and the chemical characteristics of the films.

	D65			A			CWF-2		
	L	a	b	L	a	b	L	a	b
				Smooth side of films					
PI[a] for 24 hours of stirring	-0.809 **	0.861 **	-0.045	-0.816 **	0.796 *	0.179	-0.816 **	0.872 **	-0.045
Mw[b] for 24 hours of stirring	-0.002	0.097	-0.818 **	-0.014	-0.019	-0.680 *	-0.015	0.119	-0.822 **
PI for heated solutions	-0.279	0.187	0.936 ***	-0.268	0.299	0.855 **	-0.267	0.165	0.941 ***
Mw for heated solutions	-0.982 ***	0.995 ***	0.378	-0.984 ***	0.977 ***	0.578	-0.984 ***	0.997 ***	0.380
PI for solutions with plasticizer	0.055	-0.139	0.703 *	0.066	-0.036	0.571	0.067	-0.158	0.706 *
Mw for solutions with plasticizer	-0.764 *	0.708 *	0.822 **	-0.759 *	0.773 *	0.895 **	-0.759 *	0.697 *	0.832 **
PI for films	0.581	-0.551	-0.587	0.575	-0.588	-0.636	0.574	-0.540	-0.582
Mw for films	-0.630	0.655	0.019	-0.637	0.619	0.195	-0.637	0.666	0.031
R1	0.157	-0.250	0.724 *	0.169	-0.137	0.561	0.170	-0.272	0.727 *
R2	0.338	-0.426	0.588	0.349	-0.318	0.399	0.350	-0.446	0.727 *
R3	0.522	-0.601	0.417	0.532	-0.504	0.207	0.533	-0.618	0.419
R4	0.333	-0.421	0.592	0.344	-0.313	0.404	0.345	-0.441	0.595
R5	0.525	-0.603	0.414	0.535	-0.507	0.204	0.536	-0.621	0.416
R6	0.367	-0.454	0.563	0.378	-0.348	0.371	0.379	-0.474	0.566
R7	-0.304	0.394	-0.616	-0.316	0.284	-0.431	-0.317	0.414	-0.619
Film moisture content (g kg^{-1})	-0.737 *	0.741 *	0.282	-0.740 *	0.730 *	0.440	-0.740 *	0.745 *	0.290
				Rough side of films					
PI for 24 hours of stirring	-0.810 **	0.860 **	0.011	-0.818 **	0.796 *	0.231	-0.818 **	0.872 **	0.030
Mw for 24 hours of stirring	-0.005	0.094	-0.792 *	-0.018	-0.020	-0.643	-0.018	0.117	-0.781 *
PI for heated solutions	-0.277	0.190	0.926 ***	-0.264	0.300	0.829 **	-0.263	0.168	0.920 ***
Mw for heated solutions	-0.982 ***	0.995 ***	0.433	-0.984 ***	0.978 ***	0.621	-0.984 ***	0.997 ***	0.450 ^{NS}
PI for solutions with plasticizer	0.054	-0.140	0.688 *	0.066	-0.036	0.544	0.067	-0.160	0.677 *
Mw for solutions with plasticizer	-0.762 *	0.701 *	0.832 **	-0.757 *	0.760 *	0.882 **	-0.756 *	0.688 *	0.838 **
PI for films	0.577	-0.584	-0.636	0.570	-0.624	-0.685 *	0.571	-0.576	-0.642
Mw for films	-0.634	0.637	0.045	-0.641	0.596	0.203	-0.640	0.644	0.058
R1	0.160	-0.248	0.691 *	0.173	-0.136	0.518	0.174	-0.270	0.677 *
R2	0.341	-0.424	0.547	0.353	-0.318	0.352	0.354	-0.444	0.531
R3	0.524	-0.599	0.369	0.535	-0.504	0.156	0.536	-0.617	0.351
R4	0.336	-0.419	0.552	0.348	-0.313	0.357	0.349	-0.440	0.536
R5	0.527	-0.602	0.366	0.538	-0.507	0.153	0.539	-0.619	0.348
R6	0.370	-0.452	0.521	0.382	-0.348	0.323	0.383	-0.472	0.505
R7	-0.307	0.391	-0.576	-0.319	0.284	-0.385	-0.320	0.412	-0.561
Film moisture content (g kg^{-1})	-0.748 *	0.737 *	0.334	-0.751 *	0.725 *	0.472	-0.750 *	0.738 *	0.347

[a] Polydispersity index

[b] Molecular weight

* Significant at *P*≤0.05

** Significant at *P*≤0.01

*** Significant at *P*≤0.001

R1 = Arabinose C-(O)-3 Linked to Anomeric Xylose, R2 and R3 = Anomeric Proton of Arabinose C-(O)-3 to Disubstituted Xylose, R4 and R5 = R1 = Arabinose C-(O)-3 Linked to Anomeric Xylose, R2 = Anomeric Proton of Arabinose C-(O)-3 to Disubstituted Xylose, R3 = Anomeric Proton of Arabinose C-(O)-2 to Disubstituted Xylose with Adjoining Disubstituted Xylose, R4 = Anomeric Proton of Arabinose C-(O)-2 to Disubstituted Xylose, R5 = Anomeric Proton of Disubstituted Xylose, R6 = Anomeric Proton of Monosubstituted Xylose, R7 = Anomeric Proton of Xylopyranosyl Unit

Table 4.16. Correlations between colors of arabinoxylan films made with glycerol assessed with CIELab (using D65 light, A light, and CWF-2 light) and the chemical characteristics of the films.

	D65			A			CWF-2		
	L	a	b	L	a	b	L	a	b
					<u>Smooth side of films</u>				
PI[a] for 24 hours of stirring	-0.782 *	0.856 **	-0.045	-0.791 *	0.782 *	0.176	-0.792 *	0.869 **	-0.028
Mw[b] for 24 hours of stirring	0.031	0.090	-0.804 **	0.016	-0.038	-0.669 *	0.015	0.115	-0.795 *
PI for heated solutions	-0.308	0.193	0.921 ***	-0.294	0.316	0.841 **	-0.293	0.168	0.915 ***
Mw for heated solutions	-0.967 ***	0.992 ***	0.371	-0.969 ***	0.971 ***	0.568	-0.970 ***	0.995 ***	0.386
PI for solutions with plasticizer	-0.440	0.413	0.592	-0.432	0.467	0.614	-0.433	0.400	0.592
Mw for solutions with plasticizer	-0.818 **	0.752 *	0.874 **	-0.810 **	0.824 **	0.941 ***	-0.809 **	0.737 *	0.879 **
PI for films	-0.556	0.538	0.289	-0.557	0.538	0.383	-0.557	0.539	0.296
Mw for films	-0.855 **	0.867 **	0.278	-0.859 **	0.840 **	0.457	-0.859 **	0.872 **	0.291
R1	0.124	-0.244	0.712 *	0.138	-0.118	0.552	0.140	-0.268	0.700 *
R2	0.305	-0.420	0.579	0.319	-0.300	0.393	0.320	-0.442	0.565
R3	0.490	-0.595	0.410	0.502	-0.486	0.204	0.503	-0.614	0.395
R4	0.300	-0.415	0.583	0.314	-0.295	0.398	0.315	-0.437	0.569
R5	0.493	-0.597	0.407	0.505	-0.489	0.201	0.506	-0.617	0.392
R6	0.334	-0.448	0.554	0.348	-0.329	0.365	0.349	-0.470	0.541
R7	-0.271	0.387	-0.606	-0.285	0.266	-0.424	-0.286	0.410	-0.593
					<u>Rough of films</u>				
PI for 24 hours of stirring	-0.787 *	0.857 **	0.008	-0.794 *	0.789 *	0.222	-0.796 *	0.869 **	0.024
Mw for 24 hours of stirring	0.028	0.089	-0.773 *	0.016	-0.028	-0.634	0.014	0.112	-0.763 *
PI for heated solutions	-0.307	0.195	0.902 ***	-0.295	0.308	0.816 **	-0.293	0.172	0.897 **
Mw for heated solutions	-0.971 ***	0.995 ***	0.418	-0.973 ***	0.974 ***	0.606	-0.974 ***	0.997 ***	0.433
PI for solutions with plasticizer	-0.421	0.384	0.581	-0.415	0.428	0.592	-0.414	0.373	0.583
Mw for solutions with plasticizer	-0.819 **	0.751 *	0.894 **	-0.812 **	0.816 **	0.948 ***	-0.811 **	0.736 *	0.900 **
PI for films	-0.526	0.508	0.327	-0.527	0.513	0.405	-0.526	0.507	0.334
Mw for films	-0.834 **	0.847 **	0.333	-0.837 **	0.826 **	0.496	-0.837 **	0.851 **	0.346
R1	0.127	-0.243	0.674 *	0.139	-0.127	0.512	0.141	-0.266	0.663
R2	0.308	-0.419	0.535	0.320	-0.309	0.349	0.322	-0.440	0.522
R3	0.494	-0.595	0.361	0.505	-0.495	0.158	0.506	-0.614	0.347
R4	0.303	-0.414	0.539	0.315	-0.304	0.354	0.317	-0.436	0.526
R5	0.496	-0.597	0.359	0.507	-0.498	0.154	0.509	-0.616	0.344
R6	0.337	-0.447	0.510	0.349	-0.339	0.320	0.351	-0.468	0.496
R7	-0.274	0.387	-0.563	-0.286	0.275	-0.381	-0.288	0.408	-0.550

[a] Polydispersity index

[b] Molecular weight

* Significant at *P*≤0.05

** Significant at *P*≤0.01

*** Significant at *P*≤0.001

R1 = Arabinose C-(O)-3 Linked to Anomeric Xylose, R2 = Anomeric Proton of Arabinose C-(O)-3 to Disubstituted Xylose, R3 = Anomeric Proton of Arabinose C-(O)-2 to Disubstituted Xylose with Adjoining Disubstituted Xylose, R4 = Anomeric Proton of Arabinose C-(O)-2 to Disubstituted Xylose, R5 = Anomeric Proton of Disubstituted Xylose, R6 = Anomeric Proton of Monosubstituted Xylose, R7 = Anomeric Proton of Xylopyranosyl Unit

Table 4.17. Correlations between lightness, redness, and yellowness of arabinoxylan films made with sorbitol assessed with Hunter Lab (using D65 light, A light, and CWF-2 light) and the chemical characteristics of the films.

	D65			A			CWF-2		
	L	a	b	L	a	b	L	a	b
Smooth side of films									
PI[a] for 24 hours of stirring	-0.800 **	0.856 **	-0.314	-0.809 **	0.781 *	-0.048	-0.809 **	0.868 **	-0.286
Mw[b] for 24 hours of stirring	0.012	0.087	-0.945 ***	-0.003	-0.044	-0.830 **	-0.004	0.112	-0.936 ***
PI for heated solutions	-0.292	0.196	0.991 ***	-0.278	0.323	0.950 ***	-0.277	0.172	0.990 ***
Mw for heated solutions	-0.978 ***	0.994 ***	0.119	-0.981 ***	0.972 ***	0.381	-0.981 ***	0.996 ***	0.148
PI for solutions with plasticizer	0.043	-0.131	0.828 **	0.057	-0.014	0.713 *	0.057	-0.153	0.819 **
Mw for solutions with plasticizer	-0.774 *	0.719 *	0.723 *	-0.766 *	0.789 *	0.852 **	-0.766 *	0.704 *	0.739 *
PI for films	0.580	-0.548	-0.457	0.576	-0.589	-0.564	0.576	-0.540	-0.472
Mw for films	-0.631	0.663	-0.135	-0.637	0.619	0.060	-0.637	0.670 *	-0.116
R1	0.144	-0.241	0.887 **	0.158	-0.112	0.735 *	0.159	-0.265	0.874 **
R2	0.325	-0.417	0.789 *	0.339	-0.295	0.598	0.340	-0.440	0.771 *
R3	0.510	-0.593	0.650 ^{NS}	0.523	-0.482	0.425	0.523	-0.613	0.628
R4	0.320	-0.412	0.792 *	0.334	-0.289	0.603	0.335	-0.435	0.774 *
R5	0.513	-0.596	0.648 ^{NS}	0.526	-0.485	0.422	0.526	-0.615	0.626
R6	0.355	-0.445	0.770 *	0.368	-0.324	0.573	0.369	-0.467	0.751 *
R7	-0.292	0.385	-0.810 **	-0.306	0.260	-0.626	-0.306	0.407	-0.793 *
Film moisture content ($g\ kg^{-1}$)	-0.739 *	0.748 *	0.116	-0.741 *	0.733 *	0.311	-0.741 *	0.749 *	0.136
Rough side of films									
PI for 24 hours of stirring	-0.804 **	0.856 **	-0.203	-0.812 **	0.783 *	0.038	-0.813 **	0.868 **	-0.178
Mw for 24 hours of stirring	0.006	0.086	-0.901 ***	-0.008	-0.041	-0.778 *	-0.009	0.110	-0.891 **
PI for heated solutions	-0.287	0.197	0.979 ***	-0.273	0.320	0.919 ***	-0.273	0.174	0.975 ***
Mw for heated solutions	-0.980 ***	0.994 ***	0.230	-0.983 ***	0.973 ***	0.457	-0.983 ***	0.997 ***	0.255
PI for solutions with plasticizer	0.044	-0.133	0.796 *	0.057	-0.017	0.673 *	0.058	-0.154	0.785 *
Mw for solutions with plasticizer	-0.767 *	0.704 *	0.749 *	-0.761 *	0.768 *	0.845 **	-0.760 *	0.690 *	0.761 *
PI for films	0.581	-0.591	-0.554	0.574	-0.636	-0.640	0.575	-0.582	-0.564
Mw for films	-0.629	0.631	-0.103	-0.636	0.583	0.071	-0.635	0.639	-0.085
R1	0.149	-0.240	0.827 **	0.164	-0.115	0.673 *	0.164	-0.263	0.813 **
R2	0.331	-0.417	0.712 *	0.344	-0.298	0.526	0.345	-0.438	0.694 *
R3	0.515	-0.593	0.559	0.528	-0.485	0.345	0.528	-0.612	0.537
R4	0.326	-0.412	0.716 *	0.339	-0.292	0.530	0.340	-0.433	0.698 *
R5	0.518	-0.595	0.556	0.530	-0.488	0.342	0.531	-0.614	0.535
R6	0.360	-0.445	0.691 *	0.373	-0.327	0.499	0.374	-0.466	0.672 *
R7	-0.297	0.384	-0.736 *	-0.311	0.263	-0.556	-0.311	0.406	-0.719 *
Film moisture content ($g\ kg^{-1}$)	-0.747 *	0.735 *	0.186	-0.749 *	0.720 *	0.354	-0.749 *	0.736 *	0.204

[a] Polydispersity index

[b] Molecular weight

* Significant at *P*≤0.05

** Significant at *P*≤0.01

*** Significant at *P*≤0.001

R1 = Arabinose C-(O)-3 Linked to Anomeric Xylose, R2 = Anomeric Proton of Arabinose C-(O)-3 to Disubstituted Xylose, R3 = Anomeric Proton of Arabinose C-(O)-2 to Disubstituted Xylose with Adjoining Disubstituted Xylose, R4 = Anomeric Proton of Arabinose C-(O)-2 to Disubstituted Xylose, R5 = Anomeric Proton of Disubstituted Xylose, R6 = Anomeric Proton of Monosubstituted Xylose, R7 = Anomeric Proton of Xylopyranosyl Unit

Table 4.18. Correlations between lightness, redness, and yellowness of arabinoxylan films made with glycerol assessed with Hunter Lab (using D65 light, A light, and CWF-2 light) and the chemical characteristics of the films.

	D65			A			CWF-2		
	L	a	b	L	a	b	L	a	b
					Smooth side of films				
PI[a] for 24 hours of stirring	-0.775 *	0.853 **	-0.213	-0.785 *	0.771 *	0.016	-0.786 *	0.866 **	-0.191
Mw[b] for 24 hours of stirring	0.042	0.084	-0.878 **	0.026	-0.055	-0.770 *	0.025	0.110	-0.868 **
PI for heated solutions	-0.318	0.199	0.948 ***	-0.303	0.333	0.902 ***	-0.302	0.174	0.944 ***
Mw for heated solutions	-0.964 ***	0.992 ***	0.206	-0.967 ***	0.966 ***	0.427	-0.968 ***	0.994 ***	0.227
PI for solutions with plasticizer	-0.442	0.415	0.547	-0.434	0.473	0.594	-0.435	0.401	0.549
Mw for solutions with plasticizer	-0.823 **	0.756 *	0.789 *	-0.814 **	0.832 **	0.894 **	-0.814 **	0.740 *	0.799 **
PI for films	-0.555	0.535	0.198	-0.557	0.533	0.306	-0.556	0.536	0.208
Mw for films	-0.851 **	0.864 **	0.126	-0.856 **	0.833 **	0.322	-0.856 **	0.869 **	0.144
R1	0.113	-0.238	0.809 **	0.129	-0.100	0.670 *	0.130	-0.263	0.796 *
R2	0.294	-0.414	0.700 *	0.309	-0.283	0.529	0.310	-0.437	0.684 *
R3	0.480	-0.590	0.553	0.494	-0.471	0.355	0.495	-0.610	0.535
R4	0.289	-0.410	0.703 *	0.304	-0.278	0.534	0.305	-0.433	0.688 *
R5	0.483	-0.593	0.551	0.496	-0.474	0.352	0.497	-0.613	0.533
R6	0.323	-0.443	0.679 *	0.338	-0.312	0.504	0.339	-0.465	0.663
R7	-0.260	0.382	-0.723 *	-0.276	0.249	-0.558	-0.276	0.405	-0.708 *
					Rough side of films				
PI for 24 hours of stirring	-0.779 *	0.854 **	-0.186	-0.788 *	0.777 *	0.044	-0.788 *	0.866 **	-0.164
Mw for 24 hours of stirring	0.040	0.082	-0.864 **	0.026	-0.047	-0.751 *	0.025	0.106	-0.855 **
PI for heated solutions	-0.318	0.202	0.941 ***	-0.304	0.325	0.889 **	-0.303	0.178	0.938 ***
Mw for heated solutions	-0.968 ***	0.994 ***	0.231	-0.971 ***	0.969 ***	0.451	-0.971 ***	0.996 ***	0.253
PI for solutions with plasticizer	-0.425	0.388	0.540	-0.419	0.435	0.578	-0.418	0.376	0.544
Mw for solutions with plasticizer	-0.825 **	0.755 *	0.803 **	-0.817 **	0.825 **	0.902 ***	-0.817 **	0.740 *	0.814 **
PI for films	-0.527	0.508	0.236	-0.528	0.512	0.334	-0.527	0.507	0.246
Mw for films	-0.831 **	0.846 **	0.169	-0.835 **	0.821 **	0.357	-0.835 **	0.850 **	0.187
R1	0.115	-0.236	0.791 *	0.129	-0.108	0.648	0.130	-0.259	0.779 *
R2	0.297	-0.413	0.679 *	0.310	-0.291	0.504	0.311	-0.435	0.664
R3	0.483	-0.589	0.529	0.495	-0.478	0.328	0.496	-0.608	0.511
R4	0.291	-0.408	0.683 *	0.305	-0.286	0.509	0.306	-0.430	0.667 *
R5	0.486	-0.592	0.527	0.498	-0.481	0.325	0.499	-0.611	0.509
R6	0.326	-0.441	0.658	0.339	-0.321	0.479	0.341	-0.463	0.642
R7	-0.263	0.380	-0.702 *	-0.276	0.257	-0.533	-0.277	0.402	-0.688 *

[a] Polydispersity index
[b] Molecular weight
* Significant at $P \le 0.05$
** Significant at $P \le 0.01$
*** Significant at $P \le 0.001$
R1 = Arabinose C-(O)-3 Linked to Anomeric Xylose, R2 = Anomeric Proton of Arabinose C-(O)-3 to Disubstituted Xylose, R3 = Anomeric Proton of Arabinose C-(O)-2 to Disubstituted Xylose with Adjoining Disubstituted Xylose, R4 = Anomeric Proton of Arabinose C-(O)-2 to Disubstituted Xylose, R5 = Anomeric Proton of Disubstituted Xylose, R6 = Anomeric Proton of Monosubstituted Xylose, R7 = Anomeric Proton of Xylopyranosyl Unit

4.4.6. Atomic Force Microscopy

Atomic force microscopy was used to quantify the surface features and characteristics of the AX films. This is accomplished by tapping a cantilever onto the surface of the film while moving the cantilever in a predictable pattern (Alvarez and Siqueiros 2014). When this was done, parameters of the surface of the film including the height, phase, and amplitude were measured. Firstly, the height profile of the AX film obtained using AFM provided a visual representation of the topographical features of the film. For example, Figure 4.4 provides the height profile obtained when the smooth side of a WB AX film made with 100 g kg^{-1} sorbitol was scanned. For comparison, Figure 4.5 provides the height profile obtained when the rough side of the same film was scanned. Graphically, it is clear that the smooth side of the film has fewer changes in height than the rough side. Secondly, AFM analysis of the phase of the surface of the film provides information about the phase shifts that take place in the film. Some of the common phase shifts are due to differences in density, viscoelasticity, and adhesion. Figure 4.6 provides the phase diagram for the smooth side of the WB AX film made with 100 g kg^{-1} sorbitol, and Figure 4.7 provides the phase diagram for the rough side of the same film. From these images, it appears that there were many phase changes in both sides of the films, but the changes were more drastic on the rough side of the film. Thirdly, amplitude is a measure of the deflection that occurred when the image of the surface of the AX films was taken. These images and the numerical data that goes along with them represent the interaction of the cantilever tip and the topographical features of the AX films. Figure 4.8 shows the amplitude image produced when the smooth side of the WB AX film with 100 g kg^{-1} sorbitol is scanned, and Figure 4.9 shows the amplitude image produced after scanning the rough side of the same film. It is clear that there is more feedback from the interaction of the AX film surface when the cantilever tip taps the rough side of the film than when the smooth side is tapped. Table 4.19 provides all surface characteristics for the AX films obtained using AFM.

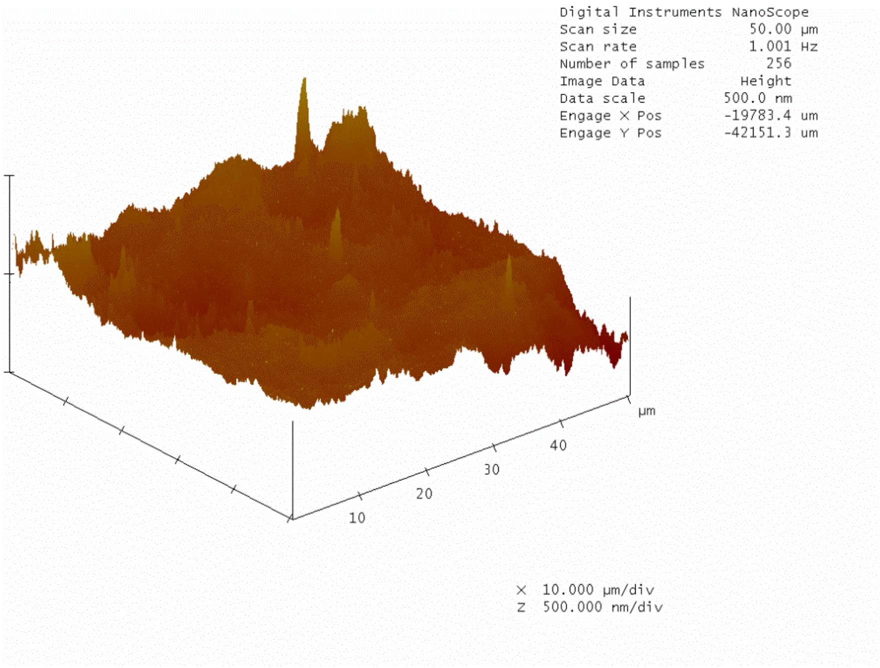

Figure 4.4. Atomic force microscopy for height analysis of the smooth side of a wheat bran arabinoxylan film plasticized with 100 g kg^{-1} sorbitol.

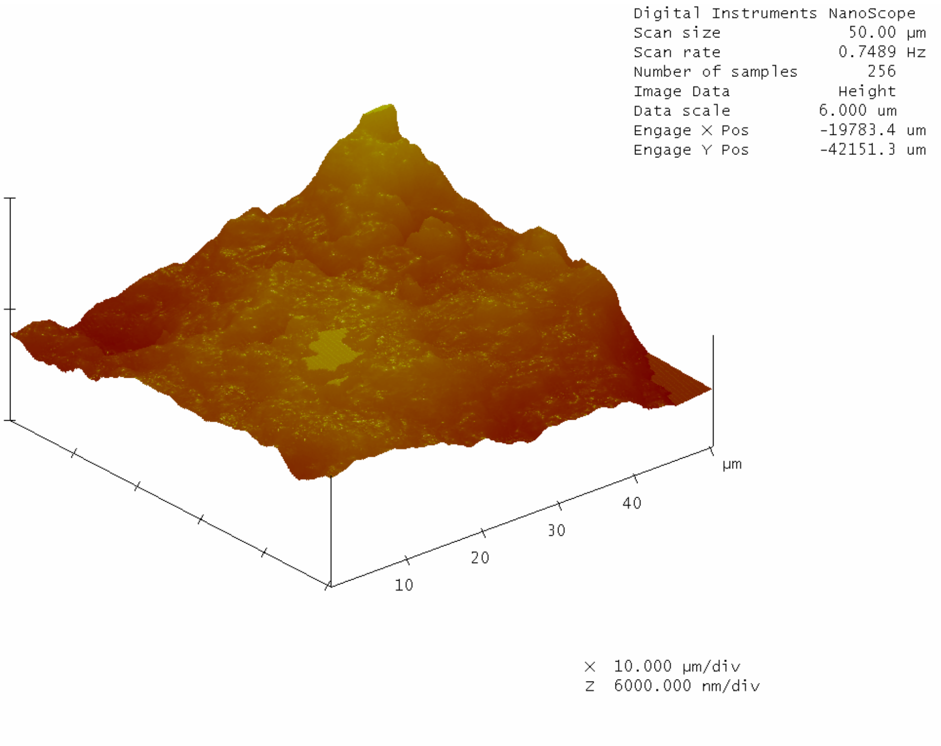

Figure 4.5. Atomic force microscopy for height analysis of the rough side of a wheat bran arabinoxylan film plasticized with 100 g kg^{-1} sorbitol.

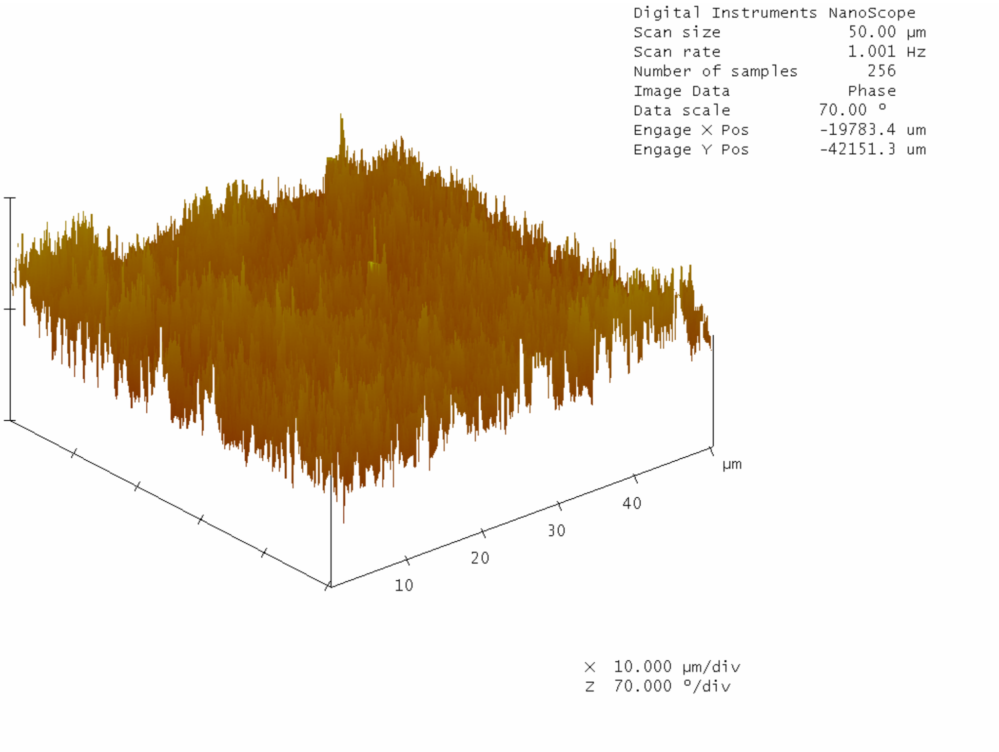

Figure 4.6. Atomic force microscopy for phase analysis of the smooth side of a wheat bran arabinoxylan film plasticized with 100 g kg^{-1} sorbitol.

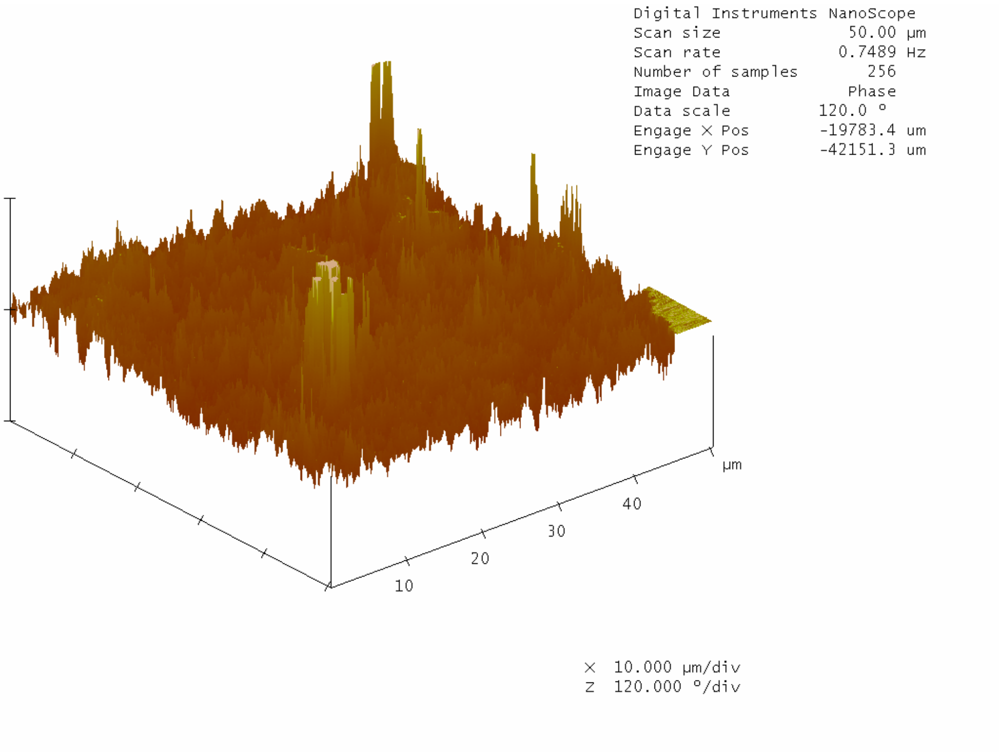

Figure 4.7. Atomic force microscopy for phase analysis of the rough side of a wheat bran arabinoxylan film plasticized with 100 g kg^{-1} sorbitol.

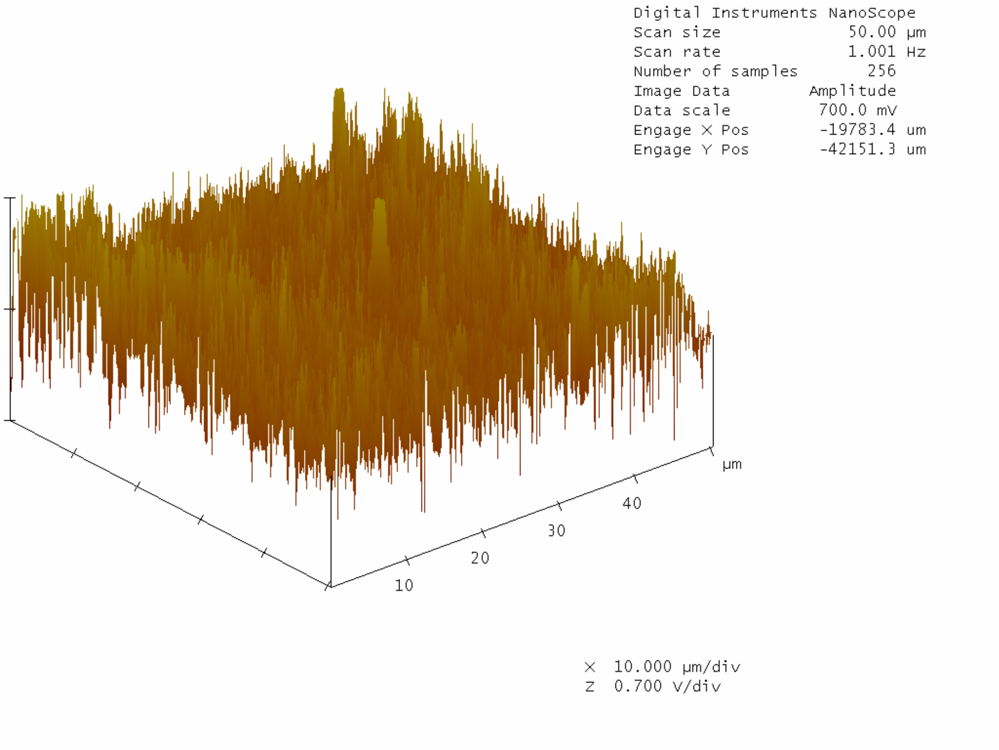

Figure 4.8. Atomic force microscopy for amplitude analysis of the smooth side of wheat bran arabinoxylan film plasticized with 100 g kg^{-1} sorbitol.

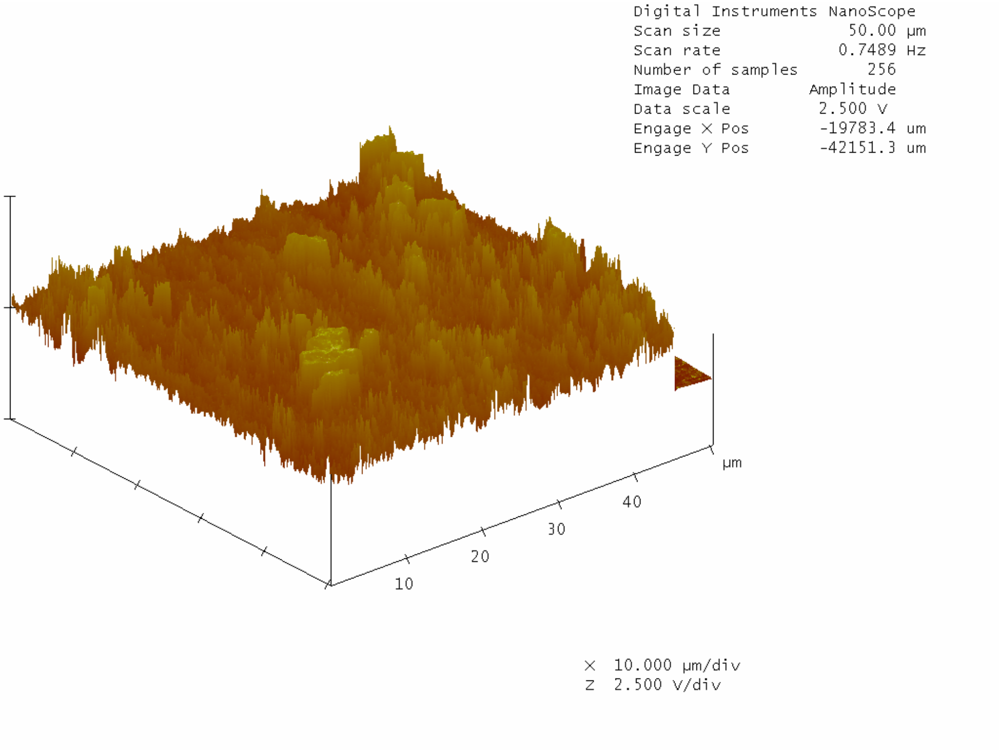

Figure 4.9. Atomic force microscopy for amplitude analysis of the rough side of wheat bran arabinoxylan film plasticized with 100 g kg^{-1} sorbitol.

Table 4.19. Atomic force microscopy characteristics for wheat bran arabinoxylan films, maize bran arabinoxylan films, and dried distillers grain arabinoxylan films made with varying levels of either glycerol or sorbitol.

	Height				Phase			Amplitude		
Film Composition	Z Range (nm)	RMS[d] (nm)	Ra[e] (nm)	Image SA[f] (μm^2)	Z Range (deg)	RMS (deg)	Ra (deg)	Z Range (V)	RMS (V)	Ra (V)
				Smooth side of films						
WB AX[a] + 100 g kg^{-1} Sorbitol	810.30 ± 269.66	93.77 ± 51.87	73.98 ± 42.31	2517.50 ± 8.50	175.16 ± 45.86	9.83 ± 3.41	7.62 ± 3.18	1.25 ± 0.04	0.15 ± 0.06	0.11 ± 0.05
WB AX + 250 g kg^{-1} Sorbitol	878.10 ± 72.00	119.08 ± 18.20	95.93 ± 13.74	2537.77 ± 16.42	99.38 ± 13.95	10.97 ± 1.25	8.53 ± 1.22	1.59 ± 0.10	0.16 ± 0.03	0.40 ± 0.48
WB AX + 500 g kg^{-1} Sorbitol	1701.33 ± 122.86	243.97 ± 38.26	191.16 ± 32.88	2540.77 ± 2.84	101.65 ± 3.66	15.95 ± 0.94	13.19 ± 1.09	1.61 ± 0.07	0.17 ± 0.01	0.13 ± 0.01
WB AX + 100 g kg^{-1} Glycerol	642.35 ± 73.44	84.70 ± 6.81	66.93 ± 6.91	2514.97 ± 0.50	71.94 ± 9.61	8.19 ± 0.68	6.39 ± 0.68	1.99 ± 0.75	0.22 ± 0.02	0.18 ± 0.03
WB AX + 250 g kg^{-1} Glycerol	1253.67 ± 48.81	167.74 ± 40.84	133.82 ± 38.15	2586.53 ± 50.12	62.58 ± 7.86	7.86 ± 1.34	6.07 ± 1.36	3.88 ± 0.58	0.40 ± 0.11	0.30 ± 0.09
WB AX + 500 g kg^{-1} Glycerol	4356.00 ± 309.05	647.73 ± 79.77	516.61 ± 70.39	2670.50 ± 29.75	99.46 ± 57.46	10.36 ± 1.26	7.58 ± 0.31	4.60 ± 0.94	0.33 ± 0.14	0.20 ± 0.04
MB AX[b] + 100 g kg^{-1} Sorbitol	285.90 ± 36.03	29.25 ± 3.20	21.47 ± 1.06	2521.53 ± 3.84	73.30 ± 9.70	13.96 ± 2.38	11.56 ± 2.21	0.89 ± 0.16	0.11 ± 0.01	0.07 ± 0.01
MB AX + 250 g kg^{-1} Sorbitol	671.43 ± 81.29	76.66 ± 18.93	59.69 ± 15.48	2618.90 ± 22.37	100.42 ± 3.91	14.05 ± 1.29	11.15 ± 0.95	1.02 ± 0.02	0.24 ± 0.01	0.20 ± 0.01
MB AX + 500 g kg^{-1} Sorbitol	573.77 ± 207.31	38.41 ± 10.57	29.08 ± 8.02	2521.53 ± 4.86	114.66 ± 30.35	13.70 ± 1.11	11.28 ± 1.14	1.03 ± 0.03	0.14 ± 0.02	0.11 ± 0.01
MB AX + 100 g kg^{-1} Glycerol	221.09 ± 39.55	20.41 ± 2.60	15.81 ± 1.74	2516.97 ± 4.24	86.71 ± 2.64	13.42 ± 2.11	10.92 ± 1.84	0.79 ± 0.11	0.13 ± 0.01	0.11 ± 0.01
MB AX + 250 g kg^{-1} Glycerol	461.25 ± 140.93	34.44 ± 8.90	25.42 ± 5.43	2526.80 ± 9.97	102.47 ± 34.56	14.73 ± 1.89	12.21 ± 1.78	1.07 ± 0.04	0.15 ± 0.02	0.12 ± .01
MB AX + 500 g kg^{-1} Glycerol	205.18 ± 160.89	26.30 ± 20.53	19.67 ± 15.31	103.44 ± 100.79	56.08 ± 56.07	5.49 ± 5.80	4.32 ± 4.63	0.76 ± 0.58	0.10 ± 0.15	0.06 ± 0.10
DDG AX[c] + 100 g kg^{-1} Sorbitol	349.17 ± 51.87	34.45 ± 3.46	26.15 ± 1.02	2552.13 ± 9.46	85.70 ± 6.93	16.83 ± 4.15	14.51 ± 4.08	0.99 ± 0.07	0.18 ± 0.02	0.15 ± 0.03
DDG AX + 250 g kg^{-1} Sorbitol	548.35 ± 262.44	35.60 ± 5.23	26.74 ± 2.50	2530.07 ± 8.05	115.92 ± 70.43	13.09 ± 6.35	10.42 ± 5.56	278.80 ± 481.09	0.14 ± 0.02	0.12 ± 0.02
DDG AX + 500 g kg^{-1} Sorbitol	906.47 ± 190.89	108.35 ± 5.06	85.19 ± 4.30	2555.80 ± 7.14	135.49 ± 58.92	16.03 ± 0.49	14.08 ± 0.59	1.06 ± 0.01	0.18 ± 0.00	0.16 ± 0.00
DDG AX + 100 g kg^{-1} Glycerol	296.33 ± 30.62	35.89 ± 1.68	27.96 ± 1.61	2539.93 ± 6.06	96.64 ± 4.27	17.16 ± 0.20	14.45 ± 0.28	1.01 ± 0.02	0.18 ± 0.01	0.14 ± 0.01
DDG AX + 250 g kg^{-1} Glycerol	321.00 ± 112.63	23.68 ± 3.67	18.29 ± 2.76	2523.93 ± 8.87	95.48 ± 6.01	17.74 ± 1.76	14.79 ± 1.97	0.95 ± 0.10	0.13 ± 0.02	0.11 ± 0.02
DDG AX + 500 g kg^{-1} Glycerol	146.47 ± 44.09	14.38 ± 0.56	10.53 ± 0.79	100.47 ± 0.46	30.78 ± 13.00	2.65 ± 0.85	1.98 ± 0.68	0.63 ± 0.42	0.02 ± 0.01	0.01 ± 0.00

Table 4.19. Atomic force microscopy characteristics for wheat bran arabinoxylan films, maize bran arabinoxylan films, and dried distillers grain arabinoxylan films made with varying levels of either glycerol or sorbitol (continued).

	Height			Phase				Amplitude		
Film Composition	Z Range (nm)	RMS[d] (nm)	Ra[e] (nm)	Image SA[f] (μm^2)	Z Range (deg)	RMS (deg)	Ra (deg)	Z Range (V)	RMS (V)	Ra (V)
					Rough side of films					
WB AX + 100 g kg^{-1} Sorbitol	5316.67 ± 1130.91	946.73 ± 166.51	756.07 ± 125.60	2681.70 ± 41.05	228.26 ± 74.75	16.05 ± 4.53	12.26 ± 4.60	3.14 ± 2.09	0.48 ± 0.36	0.34 ± 0.23
WB AX + 250 g kg^{-1} Sorbitol	5119.67 ± 1146.50	989.28 ± 199.39	796.04 ± 129.18	2704.60 ± 73.46	252.62 ± 35.85	13.42 ± 2.84	10.44 ± 1.56	1.82 ± 0.08	0.21 ± 0.04	0.15 ± 0.02
WB AX + 500 g kg^{-1} Sorbitol	5201.00 ± 930.78	931.43 ± 171.49	743.72 ± 140.76	2724.23 ± 25.94	231.66 ± 114.38	22.37 ± 7.11	15.62 ± 2.69	3.00 ± 0.32	0.37 ± 0.09	0.26 ± 0.05
WB AX + 100 g kg^{-1} Glycerol	5353.67 ± 502.79	873.81 ± 144.43	689.83 ± 112.02	2766.70 ± 94.71	254.99 ± 67.10	32.66 ± 16.70	22.66 ± 14.53	4.40 ± 0.46	0.63 ± 0.12	0.46 ± 0.08
WB AX + 250 g kg^{-1} Glycerol	4794.33 ± 594.16	748.26 ± 172.98	591.99 ± 143.87	2621.43 ± 3.14	79.62 ± 4.63	11.10 ± 0.65	8.81 ± 0.54	2.72 ± 0.38	0.18 ± 0.01	0.13 ± 0.01
WB AX + 500 g kg^{-1} Glycerol	3973.67 ± 2827.52	806.76 ± 621.57	666.46 ± 539.87	1063.69 ± 1656.63	167.42 ± 83.74	25.78 ± 20.96	19.59 ± 15.56	0.82 ± 0.78	0.23 ± 0.18	0.09 ± 0.10
MB AX + 100 g kg^{-1} Sorbitol	697.13 ± 327.51	109.39 ± 38.17	81.53 ± 22.33	1893.35 ± 1094.61	86.94 ± 5.56	14.59 ± 2.82	11.68 ± 3.21	0.91 ± 0.18	0.23 ± 0.20	0.17 ± 0.14
MB AX + 250 g kg^{-1} Sorbitol	616.61 ± 73.53	67.42 ± 10.28	48.66 ± 2.47	2543.40 ± 14.73	96.88 ± 8.79	13.77 ± 4.85	11.16 ± 4.10	0.96 ± 0.12	0.18 ± 0.02	0.15 ± 0.02
MB AX + 500 g kg^{-1} Sorbitol	1465.27 ± 1200.24	191.55 ± 232.50	131.65 ± 156.85	2576.37 ± 28.58	194.72 ± 113.94	17.50 ± 3.30	13.98 ± 2.32	1.17 ± 0.27	0.19 ± 0.05	0.14 ± 0.04
MB AX + 100 g kg^{-1} Glycerol	696.08 ± 244.10	86.10 ± 36.27	65.20 ± 26.41	2536.77 ± 4.91	89.91 ± 7.71	14.16 ± 1.98	11.91 ± 1.59	1.01 ± 0.01	0.15 ± 0.01	0.13 ± 0.01
MB AX + 250 g kg^{-1} Glycerol	445.24 ± 133.17	48.51 ± 8.51	37.98 ± 6.41	2552.13 ± 9.61	82.25 ± 0.72	12.70 ± 1.15	10.15 ± 1.16	1.03 ± 0.06	0.20 ± 0.02	0.17 ± 0.01
MB AX + 500 g kg^{-1} Glycerol	252.26 ± 98.65	22.43 ± 8.28	16.03 ± 3.84	102.08 ± 2.52	32.93 ± 16.45	3.13 ± 2.61	2.25 ± 1.78	0.91 ± 0.22	0.04 ± 0.03	0.01 ± 0.01
DDG AX + 100 g kg^{-1} Sorbitol	2426.00 ± 881.52	432.74 ± 147.66	346.78 ± 111.17	2583.60 ± 61.61	161.56 ± 117.77	14.22 ± 6.61	10.52 ± 4.54	1.08 ± 0.07	0.17 ± 0.08	0.13 ± 0.07
DDG AX + 250 g kg^{-1} Sorbitol	2167.00 ± 856.60	317.73 ± 128.87	254.43 ± 102.89	2617.47 ± 19.73	101.56 ± 10.62	15.74 ± 0.89	13.51 ± 0.52	1.10 ± 0.05	0.26 ± 0.02	0.22 ± 0.02
DDG AX + 500 g kg^{-1} Sorbitol	1067.45 ± 170.86	170.18 ± 26.74	129.43 ± 11.56	2545.57 ± 22.25	98.87 ± 2.78	17.20 ± 0.53	14.55 ± 0.48	1.10 ± 0.07	0.15 ± 0.04	0.12 ± 0.04
DDG AX + 100 g kg^{-1} Glycerol	1643.67 ± 321.34	261.52 ± 105.36	211.90 ± 88.84	2546.30 ± 8.87	156.40 ± 110.42	19.08 ± 5.66	14.41 ± 1.91	1.23 ± 0.29	0.18 ± 0.02	0.15 ± 0.01
DDG AX + 250 g kg^{-1} Glycerol	1783.67 ± 474.14	239.83 ± 46.28	185.27 ± 39.43	2579.53 ± 18.63	251.38 ± 132.83	21.89 ± 6.31	17.32 ± 5.18	1.06 ± 0.12	0.22 ± 0.06	0.18 ± 0.06
DDG AX + 500 g kg^{-1} Glycerol	222.65 ± 67.97	17.76 ± 3.57	11.87 ± 1.48	101.14 ± 1.39	44.20 ± 20.35	3.74 ± 3.24	2.69 ± 2.32	0.63 ± 0.31	0.03 ± 0.03	0.01 ± 0.01

[a] Wheat bran arabinoxylan
[b] Maize bran arabinoxylan
[c] Dried distillers grain arabinoxylan
[d] Root mean square
[e] Roughness average
[f] Surface area

To determine if there were significant (*P*≤0.01 or (*P*≤0.05) differences between the surface characteristics of the AX films, separation of the means was performed as seen in Table 4.20. The Z-range is the entire range of data points, the RMS is a squared mean of all values, and the Ra is the absolute mean of all values. The Ra is more sensitive to the presence of peaks and valleys than the RMS. To begin, the means were separated based on the type of AX used to make the film. When this was done, it was determined that the WB AX films were significantly (*P*≤0.01) more rough (on both the smooth and rough sides) than the surfaces of the films made from MB AX or DDG AX. However, for the smooth side of the films, the DDG AX films had significantly (*P*≤0.01) higher values for the amplitude than the films made with WB AX or MB AX. This indicates that the cantilever tip experienced more feedback when tapping the DDG AX films than when tapping the other two types of films. For the rough side of the AX films, the WB AX films had a significantly (*P*≤0.01) higher amplitude than the other two types of films.

Next, the surface features of the films were analyzed by averaging the height, phase, and amplitude values across the type of plasticizer used in the films. For the smooth side of the films, the height profiles suggest that the films made with glycerol were significantly (*P*≤0.01) rougher. The phase profiles suggest that the smooth side of the glycerol films had fewer phase changes than the glycerol films. Lastly, the amplitude profiles suggest that the smooth side of the films made with glycerol provided a smoother tapping profile than those made with glycerol. Overall, these three profiles suggest that the smooth side of the glycerol films had more peaks and valleys, but the composition was more even, which resulted in fewer phase changes. For the rough side of the films, the films made with sorbitol were significantly (*P*≤0.05) rougher than the films made with glycerol based on their height profiles.

Lastly, the level of the plasticizer in the films was used as the basis for comparison between the types of films. For the smooth side of the films, the roughness significantly (*P*≤0.01) increased as the level of plasticizer increased. However, there were fewer changes in phase as the level of plasticizer increased. For the rough side of the films, as the level of plasticizer increases from 100 g kg^{-1} to 500 g kg^{-1}, the films became significantly (*P*≤0.05) less rough. In addition, the films had fewer phase shifts as the level of plasticizer increased, which was also observed on the smooth side of the film. As the level of plasticizer increased, the rough side of the film had a significantly (*P*≤0.01) lower amplitude range. This indicates that the cantilever tip traveled more smoothly over the films with a higher level of plasticization.

Table 4.20. Mean separation of surface characteristics of films made from wheat bran arabinoxylan, maize bran arabinoxylan, or dried distillers grain arabinoxylan. These characteristics were determined using atomic force microscopy.

			Height				Phase			Amplitude		
AX[a] Type	Plasticizer Type	Plasticizer level (g kg^{-1})	Z range (nm)	RMS[f] (nm)	Ra[g] (nm)	Image SA[h] (μm^2)	Z range (deg)	RMS (deg)	Ra (deg)	Z range (V)	RMS (V)	Ra (V)
Smooth side of films												
WB[b]			1607	226	180	2561	101.70	10.53	8.23	2.49	0.24	0.22
MB[b]			403	38	29	2135	88.94	12.56	10.24	0.93	0.14	0.11
DDG[d]			428	42	32	2134	93.34	13.92	11.71	47.24	0.14	0.11
LSD (*P*≤0.05)[e]			103	19	16	19	22.45	1.82	1.61	76.66	0.04	0.08
LSD (*P*≤0.01)			138	25	22	26	30.10	2.43	2.17	102.79	0.05	0.11
	Glycerol		878	117	93	2009	78.02	10.84	8.75	1.74	0.19	0.14
	Sorbitol		747	87	68	2544	111.30	13.82	11.37	32.03	0.16	0.16
	LSD (*P*≤0.05)		84	15	13	16	18.33	1.48	1.32	62.59	0.03	0.07
	LSD (*P*≤0.01)		113	20	18	21	24.58	1.99	1.77	83.93	0.04	0.09
		100	434	50	39	2527	98.24	13.23	10.91	1.15	0.16	0.13
		250	689	76	60	2554	96.04	13.07	10.53	47.88	0.20	0.21
		500	1315	180	142	1749	89.69	10.70	8.74	1.62	0.16	0.11
		LSD (*P*≤0.05)	103	19	16	19	22.45	1.82	1.61	76.66	0.04	0.08
		LSD (*P*≤0.01)	138	25	22	26	30.10	2.43	2.17	102.79	0.05	0.11
Rough side of films												
WB			4960	883	707	2427	202.43	20.23	14.89	2.65	0.35	0.24
MB			695	88	64	2034	97.27	12.64	10.19	1.00	0.16	0.13
DDG			1552	240	190	2162	135.66	15.31	12.17	1.03	0.17	0.14
LSD (*P*≤0.05)			628	128	105	317	47.58	4.99	3.80	0.38	0.08	0.05
LSD (*P*≤0.01)			843	171	141	425	63.80	6.69	5.10	0.51	0.10	0.07
	Glycerol		2129	345	275	1874	128.79	16.03	12.20	1.53	0.21	0.15
	Sorbitol		2675	462	365	2541	161.45	16.10	12.63	1.59	0.25	0.19
	LSD (*P*≤0.05)		513	104	86	259	38.45	4.07	3.10	0.31	0.06	0.04
	LSD (*P*≤0.01)		688	140	115	347	52.09	5.46	4.20	0.42	0.09	0.06
		100	2689	452	359	2501	163.01	18.46	13.90	1.96	0.31	0.23
		250	2488	402	319	2603	144.05	14.77	11.90	1.45	0.21	0.17
		500	2030	357	283	1519	128.30	14.95	11.45	1.27	0.17	0.11
		LSD (*P*≤0.05)	629	128	105	317	47.58	4.99	3.80	0.38	0.08	0.05
		LSD (*P*≤0.01)	843	171	141	425	63.80	6.69	5.10	0.51	0.10	0.07

[a] Arabinoxylan

[b] Wheat bran

[c] Maize bran

[d] Dried distillers grain

[e] Root mean square

[f] Least significant difference

[g] Roughness average

[h] Surface area

There were numerous significant ($P \leq 0.05$) two- and three-way interactions that affected the overall surface features of the smooth and rough sides of the AX films. The significant ($P \leq 0.05$) two-way interactions are shown in Table 4.21. For the smooth side of the films, the two-way interactions that affected the surface characteristics of the films included the AX type by plasticizer type interaction, AX type by plasticizer level interaction, and plasticizer type by plasticizer level interaction. However, when it came to the rough side of the films, only the AX type by plasticizer level and plasticizer type by plasticizer level interactions were significant ($P \leq 0.05$). For the smooth side of the films, these two-way interactions mainly affected the height profile of the films. But for the rough side of the films, the significant ($P \leq 0.05$) interactions mainly affected the phase and amplitude of the films. This indicates that the effects of these two-way interactions were limited to these aspects of the surface characteristics of the films.

Like the two-way interactions, the effects of the significant ($P \leq 0.05$) three-way interactions varied between the smooth and rough sides of the AX films as seen in Table 4.22. The three-way interaction between AX type, plasticizer type, and plasticizer level affected the height, phase, and amplitude of the smooth side of the AX films. The result of this was that the cumulative effect of combining one level from each of the three main factors affected all three of the surface parameters measured. However, for the rough side of the film, this three-way interaction significantly ($P \leq 0.05$) affected only the z-ranges for phase and amplitude. This means that this three-way interaction affected the number of phase shifts and feedback measured by the cantilever tip, but the height profile was not.

The correlations between the chemical properties of the AX films and their surface properties are given in Table 4.23 for films made with sorbitol. For the smooth side of the films, there were three notable correlations. Firstly, there was a significant ($P \leq 0.05$) correlation between an increase in the Mw of the film solutions after being heated and an increase in the roughness of the film as measured by the range in height of the films. Secondly, there was a significant ($P \leq 0.05$) correlation between an increase in the Mw of the film solutions after the addition of sorbitol and an increase in the height variance of the films. Thirdly, there was a significant ($P \leq 0.01$) correlation between an increase in the Mw of the films and an increase in the topographical variations on the surface of the film. These three correlations demonstrate that as the Mw of the film solutions and films increased, so did the roughness of the surface of the films.

For the rough side of the films made with sorbitol, there were more notable correlations. Firstly, as the PI of the film solutions after 24 hours of stirring increased, the roughness of the films increased significantly ($P \leq 0.05$). Correlations between not only the PI and the height profile of the rough side of the film but also the phase and amplitude profiles demonstrate this. Secondly, there was a significant ($P \leq 0.001$) correlation between an increase in the Mw of the film solutions after heating and an increase in the roughness of the surface of the films. Once again, all three surface profiles indicate this. Thirdly, the significant ($P \leq 0.05$) positive correlation between the Mw of the film solutions after heating and the height profile of the rough side of the films indicates that as the Mw increased, the surface became rougher. Fourthly, there was a significant ($P \leq 0.05$) correlation between an increase in the moisture content of the film and an increase in the roughness of the rough side of the films made with sorbitol.

The correlations between the chemical properties of the AX films and their surface properties are given in Table 4.23 for films made with sorbitol. For the smooth side of the films, there were three notable correlations. Firstly, there was a significant ($P \leq 0.05$) correlation between an increase in the Mw of the film solutions after being heated and an increase in the roughness of the film as measured by the range in height of the films. Secondly, there was a significant ($P \leq 0.05$) correlation between an increase in the Mw of the film solutions after the addition of sorbitol and an increase in the height variance of the films. Thirdly, there was a significant ($P \leq 0.01$) correlation between an increase in the Mw of the films and an increase in the topographical variations on the surface of the film. These three correlations demonstrate that as the Mw of the film solutions and films increased, so did the roughness of the surface of the films.

For the rough side of the films made with sorbitol, there were more notable correlations. Firstly, as the PI of the film solutions after 24 hours of stirring increased, the roughness of the films increased significantly ($P \leq 0.05$). Correlations between not only the PI and the height profile of the rough side of the film but also the phase and amplitude profiles demonstrate this. Secondly, there was a significant ($P \leq 0.001$) correlation between an increase in the Mw of the film solutions after heating and an increase in the roughness of the surface of the films. Once again, all three surface profiles indicate this. Thirdly, the significant ($P \leq 0.05$) positive correlation between the Mw of the film solutions after heating and the height profile of the rough side of the films indicates that as the Mw increased, the surface became rougher. Fourthly, there was a significant ($P \leq 0.05$) correlation between an increase in the moisture content of the film and an increase in the roughness of the rough side of the films made with sorbitol.

Table 4.21. Two-way interactions influencing surface features of wheat bran arabinoxylan, maize bran arabinoxylan, and dried distillers grain arabinoxylan films between combinations of type of arabinoxylan, plasticizer type, and level of plasticizer used. The surface feature characteristics were determined using atomic force microscopy.

			Height				Phase			Amplitude		
AX[a] type	Plasticizer type	Plasticizer level (g kg^{-1})	Z Range (nm)	RMS[i] (nm)	Ra[j] (nm)	Image SA[k] (µm^2)	Z Range (deg)	RMS (deg)	Ra (deg)	Z Range (V)	RMS (V)	Ra (V)
							Smooth side of films					
WB[b]	Gly[f]		2084.00	300.06	239.12	NS	NS	NS	NS	NS	0.32	NS
WB	Sor[g]		1129.91	152.27	120.36	NS	NS	NS	NS	NS	0.16	NS
MB[c]	Gly		295.84	27.05	20.30	NS	NS	NS	NS	NS	0.13	NS
MB	Sor		510.36	48.11	36.75	NS	NS	NS	NS	NS	0.16	NS
DDG[d]	Gly		254.60	24.65	18.93	NS	NS	NS	NS	NS	0.11	NS
DDG	Sor		601.33	59.46	46.02	NS	NS	NS	NS	NS	0.17	NS
LSD ($P≤0.05$)[e]			145.70	26.31	22.68	NS	NS	NS	NS	NS	0.06	NS
LSD ($P≤0.01$)			195.37	35.28	30.41	NS	NS	NS	NS	NS	0.08	NS
WB		100	726.32	89.24	70.45	NS	NS	9.01	7.01	NS	0.18	NS
WB		250	1065.88	143.41	114.87	NS	NS	9.41	7.30	NS	0.28	NS
WB		500	3028.67	445.85	353.89	NS	NS	13.15	10.38	NS	0.25	NS
MB		100	253.50	24.83	18.64	NS	NS	13.69	11.24	NS	0.12	NS
MB		250	566.34	55.55	42.55	NS	NS	14.39	11.68	NS	0.20	NS
MB		500	389.48	32.35	24.38	NS	NS	9.59	7.80	NS	0.12	NS
DDG		100	322.75	35.17	27.06	NS	NS	17.00	14.48	NS	0.18	NS
DDG		250	434.68	29.64	22.52	NS	NS	15.41	12.61	NS	0.14	NS
DDG		500	526.47	61.36	47.86	NS	NS	9.34	8.03	NS	0.10	NS
LSD ($P≤0.05$)			178.44	32.22	27.77	NS	NS	3.14	2.80	NS	0.07	NS
LSD ($P≤0.01$)			239.28	43.21	37.24	NS	NS	4.22	3.75	NS	0.09	NS
	Gly	100	386.59	47.00	36.90	NS	NS	12.92	10.58	NS	NS	NS
	Gly	250	678.64	75.29	59.18	NS	NS	13.44	11.03	NS	NS	NS
	Gly	500	1569.22	229.47	182.27	NS	NS	6.17	4.63	NS	NS	NS
	Sor	100	481.79	52.49	40.53	NS	NS	13.54	11.23	NS	NS	NS
	Sor	250	699.29	77.11	60.79	NS	NS	12.70	10.03	NS	NS	NS
	Sor	500	1060.52	130.24	101.81	NS	NS	15.23	12.85	NS	NS	NS
	LSD ($P≤0.05$)		145.70	26.31	22.68	NS	NS	2.57	2.28	NS	NS	NS
	LSD ($P≤0.01$)		195.37	35.28	30.41	NS	NS	3.44	3.06	NS	NS	NS

Table 4.21. Two-way interactions influencing surface features of wheat bran arabinoxylan, maize bran arabinoxylan, and dried distillers grain arabinoxylan films between combinations of type of arabinoxylan, plasticizer type, and level of plasticizer used. The surface feature characteristics were determined using atomic force microscopy (continued).

			Height				Phase			Amplitude		
AX[a] type	Plasticizer type	Plasticizer level	Z Range	RMS[i]	Ra[j]	Image SA[k]	Z Range	RMS	Ra	Z Range	RMS	Ra
		(g kg^{-1})	(nm)	(nm)	(nm)	(µm^2)	(deg)	(deg)	(deg)	(V)	(V)	(V)
							Rough Side of Films					
WB	Gly		NS[h]	NS	NS	NS	NS	NS	NS	NS	NS	NS
WB	Sor		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
MB	Gly		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
MB	Sor		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
DDG	Gly		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
DDG	Sor		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
LSD ($P≤0.05$)			NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
LSD ($P≤0.01$)			NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
WB		100	NS	NS	NS	NS	NS	24.35	17.46	3.77	0.56	0.40
WB		250	NS	NS	NS	NS	NS	12.26	9.63	2.27	0.19	0.14
WB		500	NS	NS	NS	NS	NS	24.08	17.60	1.91	0.30	0.18
MB		100	NS	NS	NS	NS	NS	14.37	11.79	0.96	0.19	0.15
MB		250	NS	NS	NS	NS	NS	13.23	10.65	1.00	0.19	0.16
MB		500	NS	NS	NS	NS	NS	10.32	8.12	1.04	0.11	0.08
DDG		100	NS	NS	NS	NS	NS	16.65	12.46	1.16	0.18	0.14
DDG		250	NS	NS	NS	NS	NS	18.81	15.42	1.08	0.24	0.20
DDG		500	NS	NS	NS	NS	NS	10.47	8.62	0.87	0.09	0.07
LSD ($P≤0.05$)			NS	NS	NS	NS	NS	8.64	6.65	0.66	0.14	0.09
LSD ($P≤0.01$)			NS	NS	NS	NS	NS	11.58	8.92	0.89	0.18	0.12
	Gly	100	NS	NS	NS	2616.59	NS	21.97	16.32	2.21	NS	0.25
	Gly	250	NS	NS	NS	2584.37	NS	15.23	12.10	1.60	NS	0.16
	Gly	500	NS	NS	NS	422.31	NS	10.88	8.17	0.79	NS	0.04
	Sor	100	NS	NS	NS	2386.22	NS	14.95	11.48	1.71	NS	0.21
	Sor	250	NS	NS	NS	2621.82	NS	14.31	11.70	1.29	NS	0.18
	Sor	500	NS	NS	NS	2615.39	NS	19.02	14.72	1.76	NS	0.18
	LSD ($P≤0.05$)		NS	NS	NS	448.74	NS	7.05	5.43	0.54	NS	0.07
	LSD ($P≤0.01$)		NS	NS	NS	601.71	NS	9.46	7.28	0.73	NS	0.10

[a] Arabinoxylan

[b] Wheat bran

[c] Maize bran

[d] Dried distillers grain

[e] Least significant difference

[f] Glycerol

[g] Sorbitol

[h] Not significant ($P≤0.05$)

[l] Root mean square

[j] Roughness average

[k] Surface area

Table 4.22. Three-way interactions influencing surface features of wheat bran arabinoxylan, maize bran arabinoxylan, and dried distillers grain arabinoxylan films between the type of arabinoxylan, plasticizer type, and level of plasticizer used.

			Height				Phase			Amplitude		
AX[a] type	Plasticizer type	Plasticizer level (g kg^{-1})	Z Range (nm)	RMS[i] (nm)	Ra[j] (nm)	Image SA[k] (µm^2)	Z Range (deg)	RMS (deg)	Ra (deg)	Z Range (V)	RMS (V)	Ra (V)
Smooth side of films												
WB[b]	Gly[f]	100	642.35	84.70	66.93	2514.97	71.94	8.19	6.39	NS	0.22	NS
WB	Gly	250	1253.67	167.74	133.82	2586.53	62.58	7.86	6.07	NS	0.40	NS
WB	Gly	500	4356.00	647.73	516.61	2670.50	99.46	10.36	7.58	NS	0.33	NS
MB[c]	Gly	100	221.09	20.41	15.81	2516.97	86.71	13.42	10.92	NS	0.13	NS
MB	Gly	250	461.25	34.44	25.42	2526.80	102.47	14.73	12.21	NS	0.15	NS
MB	Gly	500	205.18	26.30	19.67	103.44	56.08	5.49	4.32	NS	0.10	NS
DDG[d]	Gly	100	296.33	35.89	27.96	2539.93	96.64	17.16	14.45	NS	0.18	NS
DDG	Gly	250	321.00	23.68	18.29	2523.93	95.48	17.74	14.79	NS	0.13	NS
DDG	Gly	500	146.47	14.38	10.53	100.47	30.78	2.65	1.98	NS	0.02	NS
WB	Sor[g]	100	810.30	93.77	73.98	2517.50	175.16	9.83	7.62	NS	0.15	NS
WB	Sor	250	878.10	119.08	95.93	2537.77	99.38	10.97	8.53	NS	0.16	NS
WB	Sor	500	1701.33	243.97	191.16	2540.77	101.65	15.95	13.19	NS	0.17	NS
MB	Sor	100	285.90	29.25	21.47	2521.53	73.30	13.96	11.56	NS	0.11	NS
MB	Sor	250	671.43	76.66	59.69	2618.90	100.42	14.05	11.15	NS	0.24	NS
MB	Sor	500	573.77	38.41	29.08	2521.53	114.66	13.70	11.28	NS	0.14	NS
DDG	Sor	100	349.17	34.45	26.15	2552.13	85.70	16.83	14.51	NS	0.18	NS
DDG	Sor	250	548.35	35.60	26.74	2530.07	115.92	13.09	10.42	NS	0.14	NS
DDG	Sor	500	906.47	108.35	85.19	2555.80	135.49	16.03	14.08	NS	0.18	NS
LSD ($P \leq 0.05$)[e]			252.36	45.57	39.28	47.62	54.99	4.45	3.96	NS	0.10	NS
LSD ($P \leq 0.01$)			338.39	61.11	52.67	63.85	73.73	5.96	5.30	NS	0.13	NS

Table 4.22. Three-way interactions influencing surface features of wheat bran arabinoxylan, maize bran arabinoxylan, and dried distillers grain arabinoxylan films between the type of arabinoxylan, plasticizer type, and level of plasticizer used (continued).

			Height				Phase			Amplitude		
AX[a] type	Plasticizer type	Plasticizer level (g kg^{-1})	Z Range (nm)	RMS[i] (nm)	Ra[j] (nm)	Image SA[k] (µm^2)	Z Range (deg)	RMS (deg)	Ra (deg)	Z Range (V)	RMS (V)	Ra (V)
							Rough side of films					
WB	Gly	100	NS[h]	NS	NS	NS	254.99	NS	NS	4.40	NS	NS
WB	Gly	250	NS	NS	NS	NS	79.62	NS	NS	2.72	NS	NS
WB	Gly	500	NS	NS	NS	NS	167.42	NS	NS	0.82	NS	NS
MB	Gly	100	NS	NS	NS	NS	89.91	NS	NS	1.01	NS	NS
MB	Gly	250	NS	NS	NS	NS	82.25	NS	NS	1.03	NS	NS
MB	Gly	500	NS	NS	NS	NS	32.93	NS	NS	0.91	NS	NS
DDG	Gly	100	NS	NS	NS	NS	156.40	NS	NS	1.23	NS	NS
DDG	Gly	250	NS	NS	NS	NS	251.38	NS	NS	1.06	NS	NS
DDG	Gly	500	NS	NS	NS	NS	44.20	NS	NS	0.63	NS	NS
WB	Sor	100	NS	NS	NS	NS	228.26	NS	NS	3.14	NS	NS
WB	Sor	250	NS	NS	NS	NS	252.62	NS	NS	1.82	NS	NS
WB	Sor	500	NS	NS	NS	NS	231.66	NS	NS	3.00	NS	NS
MB	Sor	100	NS	NS	NS	NS	86.94	NS	NS	0.91	NS	NS
MB	Sor	250	NS	NS	NS	NS	96.88	NS	NS	0.96	NS	NS
MB	Sor	500	NS	NS	NS	NS	194.72	NS	NS	1.17	NS	NS
DDG	Sor	100	NS	NS	NS	NS	161.56	NS	NS	1.08	NS	NS
DDG	Sor	250	NS	NS	NS	NS	101.56	NS	NS	1.10	NS	NS
DDG	Sor	500	NS	NS	NS	NS	98.87	NS	NS	1.10	NS	NS
LSD ($P \leq 0.05$)			NS	NS	NS	NS	116.54	NS	NS	0.94	NS	NS
LSD ($P \leq 0.01$)			NS	NS	NS	NS	156.27	NS	NS	1.26	NS	NS

[a] Arabinoxylan
[b] Wheat bran
[c] Maize bran
[d] Dried distillers grain
[e] Least significant difference
[f] Glycerol
[g] Sorbitol
[h] Not significant ($P \leq 0.05$)
[l] Root mean square
[j] Roughness average
[k] Surface area

Table 4.23. Correlations between chemical properties of wheat bran arabinoxylan films, maize bran arabinoxylan films, and dried distillers grain arabinoxylan films and all corresponding film solutions throughout the film solution making process. These films were made with sorbitol and their surface features measured with atomic force microscopy.

	Height			Phase			Amplitude		
	Z Range (nm)	RMS[c] (nm)	Ra[d] (nm)	Z Range (deg)	RMS (deg)	Ra (deg)	Z Range (V)	RMS (V)	Ra (V)
				Smooth Side of Films					
PI[a] for 24 hours of stirring	0.584	0.625	0.632	0.224	-0.572	-0.594	-0.406	-0.122	0.333
Mw[b] for 24 hours of stirring	0.043	0.072	0.071	-0.161	-0.358	-0.406	-0.474	-0.096	0.028
PI for heated solutions	0.153	0.134	0.137	0.265	0.218	0.266	0.411	0.070	0.083
Mw for heated solutions	0.686 *	0.721 *	0.730 *	0.357	-0.505	-0.507	-0.242	-0.097	0.389
PI for solutions with plasticizer	-0.077	-0.132	-0.135	0.114	0.443	0.481	0.390	0.034	-0.172
Mw for solutions with plasticizer	0.682 *	0.699 *	0.704 *	0.328	-0.008	0.019	0.120	0.019	0.247
PI for Films	0.008	-0.036	-0.052	-0.368	0.663	0.632	-0.149	0.001	-0.598
Mw for Films	0.896 **	0.864 **	0.866 **	0.243	-0.067	-0.075	-0.355	0.012	0.203
Film moisture content (g kg^{-1})	0.650	0.652	-0.528	-0.091	-0.064	-0.300	-0.445	0.087	0.689 *
				Rough side of films					
PI for 24 hours of stirring	0.780 *	0.786 *	0.780 *	0.788 *	0.264	-0.007	0.809 **	0.654	0.587
Mw for 24 hours of stirring	-0.016	-0.013	-0.022	0.203	-0.003	-0.124	0.141	0.156	0.106
PI for heated solutions	0.290	0.288	0.298	0.038	0.095	0.141	0.117	0.046	0.081
Mw for heated solutions	0.956 ***	0.962 ***	0.960 ***	0.849 **	0.322	0.057	0.908 ***	0.712 *	0.657
PI for solutions with plasticizer	0.038	0.016	0.020	-0.026	0.134	0.178	-0.105	-0.142	-0.106
Mw for solutions with plasticizer	0.696 *	0.704 *	0.710 *	0.401	0.418	0.319	0.613	0.424	0.417
PI for Films	-0.568	-0.596	-0.606	-0.344	0.475	0.503	-0.322	-0.304	-0.305
Mw for Films	0.567	0.557	0.547	0.651	0.750 *	0.605	0.669 *	0.362	0.310
Film moisture content (g kg^{-1})	0.689 *	0.714 *	0.714 *	0.556	0.452	0.209	0.671 *	0.510	0.416

[a] Polydispersity index
[b] Molecular weight
[c] Root mean square
[d] Roughness average
* Significant at $P \leq 0.05$
** Significant at $P \leq 0.01$
*** Significant at $P \leq 0.001$
R1 = Arabinose C-(O)-3 Linked to Anomeric Xylose, R2 and R3 = Anomeric Proton of Arabinose C-(O)-3 to Disubstituted Xylose, R4 and R5 = Anomeric Proton of Arabinose C-(O)-2 to Disubstituted Xylose with Adjoining Disubstituted Xylose, R6 = Anomeric Proton of Arabinose C-(O)-2 to Disubstituted Xylose, R7 = Anomeric Proton of Disubstituted Xylose, R8 = Anomeric Proton of Monosubstituted Xylose, R9 and R10 = Anomeric Proton of Xylopyranosyl Unit

Table 4.24 provides the correlations between the chemical properties of AX films made with glycerol and their surface features. For the smooth side of the films made with glycerol, there were four notable correlations. Firstly, there was a significant ($P \leq 0.01$) correlation between an increase in the PI of the film solutions after stirring for 24 hours and an increase in the amplitude values. This indicates that as the number of Mw increased, there was more feedback felt by the cantilever tip when measuring the surface features of the AX films. Secondly, there was a significant ($P \leq 0.05$ for height and $P \leq 0.01$ for amplitude) correlation between an increase in the Mw of the film solutions after heating and an increase in surface roughness. This indicates that the larger polymers create a rougher surface of the AX film. Thirdly, there was a significant ($P \leq 0.05$) correlation between the increasing PI of the films and an increase in the surface roughness of the film. Fourthly, as the Mw of the film increased, the films became rougher. The significant ($P \leq 0.01$ for height and $P \leq 0.001$ for amplitude) correlation between the Mw of the films and the height profile and the amplitude profile support this conclusion. The surface profile of the rough side of the films made with glycerol has the same number of correlations with the chemical characteristics of the film as the smooth side did. Firstly, there was a significant ($P \leq 0.05$) correlation between an increase in the PI of the film solutions after 24 hours of stirring and an increase in the surface roughness of the films. Secondly, there was a significant ($P \leq 0.001$) correlation between an increase in the Mw of the heated film solutions and an increase in the surface roughness of the films. Thirdly, the Mw of the solutions after the addition of glycerol was significantly ($P \leq 0.05$) correlated to the roughness of the films. As the Mw increased, so did the roughness of the surface of the films. Fourthly, there was a significant ($P \leq 0.05$) correlation between an increase in the Mw of the films and an increase in the roughness of the surface of the films.

Table 4.24. Correlations between chemical properties of wheat bran arabinoxylan films, maize bran arabinoxylan films, and dried distillers grain arabinoxylan films and all corresponding film solutions throughout the film solution making process. These films were made with glycerol and their surface features measured with atomic force microscopy.

	Height			Phase			Amplitude		
	Z Range (nm)	RMS[c] (nm)	Ra[d] (nm)	Z Range (nm)	RMS (nm)	Ra (nm)	Z Range (V)	RMS (V)	Ra (V)
				Smooth side of films					
Plasticizer level	0.392	0.404	0.403	-0.442	-0.604	-0.616	0.197	-0.132	-0.330
PI[a] for 24 hours of stirring	0.616	0.611	0.611	0.054	-0.310	-0.360	0.806 **	0.795 *	0.752 *
Mw[b] for 24 hours of stirring	0.146	0.137	0.136	0.129	-0.163	-0.185	0.178	0.226	0.226
PI for heated solutions	0.045	0.053	0.054	-0.132	0.082	0.090	0.073	0.013	-0.001
Mw for heated solutions	0.671 *	0.669 *	0.670 *	-0.002	-0.290	-0.339	0.885 **	0.846 **	0.794 *
PI for solutions with plasticizer	0.532	0.522	0.523	0.006	-0.043	-0.073	0.441	0.195	0.090
Mw for solutions with plasticizer	0.512	0.519	0.520	-0.196	-0.253	-0.280	0.621	0.478	0.403
PI for films	0.783 *	0.790 *	0.790 *	-0.233	-0.547	-0.590	0.637	0.295	0.083
Mw for films	0.854 **	0.856 **	0.856 **	-0.094	-0.466	-0.520	0.904 ***	0.705 *	0.552
				Rough side of films					
Plasticizer level	-0.238	-0.149	-0.133	-0.451	-0.475	-0.492	-0.501	-0.536	-0.680 *
PI for 24 hours of stirring	0.795 *	0.818 **	0.819 **	0.137	0.407	0.379	0.616	0.528	0.389
Mw for 24 hours of stirring	0.031	0.049	0.050	-0.350	-0.104	-0.101	0.138	0.082	0.061
PI for heated solutions	0.241	0.228	0.227	0.456	0.262	0.250	0.054	0.088	0.064
Mw for heated solutions	0.949 ***	0.968 ***	0.969 ***	0.352	0.549	0.514	0.675 *	0.599	0.440
PI for solutions with plasticizer	0.291	0.342	0.351	0.341	0.288	0.308	-0.122	0.062	-0.052
Mw for solutions with plasticizer	0.699 *	0.719 *	0.721 *	0.438	0.439	0.407	0.361	0.344	0.206
PI for films	0.261	0.372	0.390	-0.110	-0.004	-0.025	-0.170	-0.114	-0.335
Mw for films	0.675 *	0.752 *	0.764 *	0.063	0.259	0.231	0.261	0.228	0.011

[a] Polydispersity index
[b] Molecular weight
[c] Root mean square
[d] Roughness average
* Significant at *P*≤0.05
** Significant at *P*≤0.01
*** Significant at *P*≤0.001
R1 = Arabinose C-(O)-3 Linked to Anomeric Xylose, R2 and R3 = Anomeric Proton of Arabinose C-(O)-3 to Disubstituted Xylose, R4 and R5 = Anomeric Proton of Arabinose C-(O)-2 to Disubstituted Xylose with Adjoining Disubstituted Xylose, R6 = Anomeric Proton of Arabinose C-(O)-2 to Disubstituted Xylose, R7 = Anomeric Proton of Disubstituted Xylose, R8 = Anomeric Proton of Monosubstituted Xylose, R9 and R10 = Anomeric Proton of Xylopyranosyl Unit

4.5. Summary and Conclusions

Commercial samples of WB, MB, and DDG that were milled either on a commercial mill or Perten hammer mill with varying compositions were obtained. The MB had the highest total starch and AX content, while the DDG was the highest in moisture, ash, and N contents. In the starting materials, both the WB and DDG had A:X of about 0.7, while the MB had a much lower A:X at about 0.5. The AX was extracted and purified from all materials providing a basis for developing biodegradable films. The purity of the AX extracted was the purest in the MB, followed by the WB and lastly the DDG. The A:X for all three extracted AX materials was the same (0.51). The main trends demonstrated in the Mw throughout the film making process are a decrease upon heating due to hydrolysis and initial addition of the plasticizer but an increase after the films had dried. In addition, increasing plasticizer level and utilizing glycerol instead of sorbitol resulted in an increased film Mw. This shows an increase in the intermolecular interactions between the plasticizers and AX polymers as the films dry. Linkage analysis using ^{1}H NMR demonstrated variations in the relative abundances of the linkages present in each type of AX extracted. This most likely plays a role in the Mw differences in both the extracted AX as well as the film solutions and dried films.

All films created had an overall percentage of biodegradable material of 490 to 650 g kg^{-1}. There were correlations between the biodegradability of the films and the PI of the films. In addition, there were correlations between both the PI and the Mw of the film solution as it was heated and the biodegradability of the film after curing. The color of the AX films showed variation depending upon the type of AX, type of plasticizer, and level of plasticizer used. The WB AX films were the darkest, and the MB AX films were the lightes. The DDG AX films were light, but the yellowest. In addition, films made with WB AX had much rougher surface topography than those made from either MB AX or DDG AX. Numerous types of biodegradable films with varying physical properties were created by modifying the starting material, type of plasticizer, and amount of plasticizer. The result of this is numerous possibilities for these biodegradable materials in the future.

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CHPATER 5. CONCLUSION AND FUTURE RESEARCH

5.1. Overall Conclusion

Throughout this research, three main objectives were met. Firstly, arabinoxylan (AX) from wheat bran (WB), maize bran (MB), and dried distillers grains (DDG) was extracted with a purity higher than that of previously published research for alkaline extracted AX. This method was developed for commercial upscale, and is highly effective for AX extraction and purification. Secondly, this AX was used as the basis of films that had a wide range of properties. These films were mechanically sound, and show promise for the food packaging industry. Thirdly, the mechanical, physical, and biodegradability characteristics were determined for all films, which provides a comprehensive materials profile for each type of film. These materials profiles can be used to match a food with proper packaging.

Development of a novel AX extraction method from the combination of methods for AX extraction previously published produced an AX extract that is more pure than that previously obtained using alkaline extraction (Xiang et al. 2014; Swennen et al. 2006). The MB AX was the purest, at 850 g kg^{-1} AX, followed by the WB AX at 730 g kg^{-1} AX, and lastly the DDG AX had a purity of 580 g kg^{-1}. In addition, the residual ash, N, and starch in the extracted AX is, on average, lower than the amounts of these materials remaining in AX using previously published works. The purity of the AX extracted in this research demonstrated the increased effectiveness of the method developed. This method was developed with commercial upscale in mind, and as such involved methods that balance the time, money, and resources required for extraction with the purity of the AX extracted. This extraction method can easily be modified for the needs of the user, which is highly desirable for both commercial and research use. In addition, this method can be used to extract and purify AX from a variety of materials and when coupled with plasticizers, a variety of films can easily be made by simply modifying one or two steps of the process. This allows for a wide variety of applications for the methods developed in this research.

After extraction and purification of the AX, the weight average molecular weight (Mw), polydispersity index (PI), and linkages for each type of AX were analyzed. These values were also determined for the film solutions throughout the film making process. The three types of AX (WB, MB, and DDG) had a wide range of Mw, PI, and linkages, which resulted in varying materials properties in the films they were used to make. The result of this is that a wide variety of materials can be made by simply changing the type of material that the AX is extracted from. When the chemical properties of the film solutions were analyzed, there was a demonstrated increase in intermolecular interactions as the films were cured. This most likely it took place when the plasticizer interacted with the AX polymers.

These chemical characteristics of the AX and intermolecular interactions that took place during the curing process directly influenced the mechanical strengths of the AX films. In general, as the plasticizers interacted with the AX polymers and the intermolecular interactions increased, the films lost mechanical strength but gained flexibility. This was demonstrated by increased puncture resistance but decreased tensile strength and tear resistance at increased levels of plasticization. In addition, increased disorder in the films resulted in increased biodegradability, which is highly favorable in some applications. The interactions of the films with water were influenced more by the hydrophilicity of the plasticizers and AX polymers than the PI or Mw of the film. With this in mind, it is possible to choose an AX material that will be appropriate for numerous applications that would involve varying levels of interaction with water.

Many different materials were made by using three types of AX (WB, MB, and DDG), two types of plasticizers (glycerol and sorbitol), and three levels of each of the plasticizers (100 g kg^{-1}, 250 g kg^{-1}, and 500 g kg^{-1}). By utilizing a three factor factorial arrangement, 18 different AX materials were created. All 18 materials have different characteristics that could be used for packaging food. This is greatly beneficial because it allows for numerous options for packaging food by changing the formula of the material only slightly. This creates opportunity in the milling, ethanol, and food packaging industries by recycling the byproducts of the milling and ethanol industries for use in food packaging. An increased level of sustainability is created by connecting these industries through this value-added food packaging.

This research presented characteristics of AX films that had previously been left unexplored including the biodegradability and surface topography of all films. In addition, this research analyzed the mechanical properties of DDG AX films that had previously not been published. The mechanical properties of the MB AX films and WB AX films were researched and used as a basis for comparison between the mechanical properties analyzed in this research and previously published research. The color of all films was also analyzed and quantified, which has not been published for films of this composition. Overall, this research furthered the knowledge basis for films made out of AX from WB, MB, and DDG.

5.2. Future Research

There are numerous additional analyses that could be performed on the AX films developed throughout this research. The first additional analysis that could be performed is determination of the oxygen permeability of the films. This would provide information about the feasibility of using these films to package foods requiring a barrier from oxygen. Secondly, analysis of the interaction of the films with light via Fourier Transform Infrared Spectroscopy (FTIR) would be beneficial because this would provide more information about the physicochemical composition of the films. This analysis could be done using photoacoustic, reflectance, and/or transmission FTIR. Thirdly, analyses of the alignment of AX polymers and their interaction with sorbitol or glycerol would be necessary to demonstrate usability as a food packaging material. This could be done using gel rheology and glass transition temperature analysis. Finally, sensory evaluation of the materials would be beneficial if the AX films were to be marketed as edible food packaging material.

5.2.1. Oxygen Permeability

The standard method for determining the oxygen permeability of AX films is the American Society of Testing and Materials (ASTM) Method D3985-05(2010)e1 can be used to determine the oxygen permeability of AX films (ASTM International 2010). When extracted, AX has the ability to form a network that is very dense and has a low level of molecular mobility (Zhang et al. 2011). The result of this is that AX films have good barrier characteristics including low oxygen permeability. Films with an oxygen permeability less than 20 cm^3 µm m^{-2} d^{-1} kPa^{-1} are considered good barriers (Heikkinen et al. 2013). WB films have an oxygen permeability of 7.6 20 cm^3 µm m^{-2} d^{-1} kPa^{-1} when a relative humidity difference of 50 % to 75 % is used. When 35 g kg^{-1} (w w^{-1}) sorbitol is used in WB films, the films have an oxygen permeability of 0.21 cm^3 µm m^{-2} d^{-1} kPa^{-1} (Hansen and Plackett 2008).

Oxygen permeability is also very important to food packaging because oxygen can react with food systems resulting in oxidation reactions. When this occurs, the food will spoil much more quickly than when oxygen is not present. For this reason, the oxygen permeability of food packaging material must be properly measured. In addition, a material suitable for packaging a specific type of food must be chosen.

5.2.2. Fourier Transform Infrared Spectroscopy

When AX films are analyzed using FTIR, typically the range of 4000 to 600 Hz is measured (Peroval et al. 2004; Peroval et al. 2003). In addition, four or more scans are necessary of this range of wavelengths to obtain a clean spectrum. Due to the impurities present in AX films, there can be a large amount of noise in the FTIR spectra obtained. Using FTIR, the common vibration bands include CH_2 stretching at 2918 and 2850 Hz, C=O stretching at 1736 Hz, CH_3 bending at 1736 Hz, CH_2 bending at 1407 Hz, and C-O stretching at 1257 Hz.

The most common methods for analyzing films include photoacoustic and reflectance FTIR. The method for analyzing an AX film with FTIR is often chosen based upon what method is physically possible due to the limitations of working with films. Photoacoustic FTIR is used because it eliminates the need to reduce the particle size of the film and subsequently dilute the AX film with KBr (Rehman and Bonfield 1997). Doing these steps for traditional absorbance FTIR are typically not possible with AX films due to their materials properties. In reflectance FTIR, the film is placed in to the FTIR without modifying it in any way except for possibly cutting the film so it fits properly. In this method, the surface of the film is exposed to specified wavelengths of light and the spectrum is obtained (Flach et al. 1994). Using one or both of these methods for FTIR analysis of the AX films would provide more information about the chemical species present in the films.

5.2.3. Crystallinity

5.2.3.1. Gel Rheology

Gel rheology analysis determines the gelling characteristics of a material, which will provide information about the degree of cross-linking present in the films (Ayala-Soto et al. 2014). The cross-linkages that typically occur in AX films are due to formation of dehydrodimers (5-5', 8-O-4', 8-5', and 8-8') and/or dehydrotrimers (8-O-4/8-O-4, 8-8’/8-O-4) dimerization reactions. These interactions can form covalent bonds between AX and AX polymers or AX and ferulic acid. This is measured by determining the intrinsic viscosity and viscosimetric molecular weight (Berlanga-Reyes et al. 2011). Water unextractable AX films with an extraction time of 30 to 120 minutes can have an intrinsic viscosity of 206 to 180 mL/g and a viscosimetric molecular weight of 74 to 66 kDa.

Determination of if the cross-linkages present in AX films is important because it provides information about how the material will react under mechanical stress. When cross-linking is present, the material can be stronger. However, if cross-linking is inconsistent throughout the material, it will result in the material being unsuitable for commercial use. When materials are inconsistent mechanically, they can have failure such as food package breaking where and when it should not during transportation.

5.2.3.2. Glass Transition Temperature

The glass transition temperature of a material can be determined using differential scanning calorimetry (Souza et al. 2012). This is the temperature range at which the amorphous regions of the AX film turn from a glass to a viscous material. This arrangement change is due to the polymer chains gaining energy, which results in an increase in chain motion. The temperature range provides information about the crystalline structure of the AX film because as a material becomes more crystalline. As such, this is one way to determine the level of crystallinity in an AX film. This can be important in food packaging because some foods require a very flexible package that will not be heated, while some foods require a package that is not flexible and needs to be heated.

5.2.4. Sensory

Sensory analysis of the AX films is one final examination that could be performed. This would be necessary if the material would be marketed as edible food packaging and the consumer was expected to eat the packaging. AX by itself is edible, and it is a very good source of dietary fiber, thus if all materials used during manufacturing are generally regarded as safe, the food packaging would be edible. However, edible food packaging has not yet been accepted in all markets. Examining the market’s approval of edible AX food packaging through a set of sensory panels would be highly beneficial to selling this type of food packaging.

5.3. References

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APPENDIX A. ANOVA TABLES

Table A1. Analysis of variance for proximate compositions of wheat bran, maize bran, and dried distillers grain.

	Source	DF	Mean Square	*F* Value	*Pr>F*
Moisture	Material	2	0.36	330000000000	-
	Residual	0	0.00	-	-
Ash	Material	2	24.37	1302370	<.0001*
	Residual	3	0.00	-	-
N	Material	2	283.37	5296	<.0001*
	Residual	3	0.05	-	-
Total starch	Material	2	102.25	2349	<.0001*
	Residual	9	0.04	-	-
Total lipid	Material	2	20.12	211	0.0006*
	Residual	3	0.10	-	-
Arabinoxylan	Material	2	1275.90	6521	<.0001*
	Residual	3	0.20	-	-
Arabinose	Material	2	161.09	3918	<.0001*
	Residual	3	0.04	-	-
Xylose	Material	2	791.40	8807	<.0001*
	Residual	3	0.09	-	-
Galactose	Material	2	0.49	29575	<.0001*
	Residual	3	0.00	-	-
Glucose	Material	2	105.03	10627	<.0001*
	Residual	3	0.01	-	-
Arabinose to xylose ratio	Material	2	0.03	1489	<.0001*
	Residual	3	0.00	-	-

Table A2. Analysis of variance for proximate compositions of defatted wheat bran, maize bran, and dried distillers grain.

	Source	DF	Mean Square	*F* Value	*Pr>F*
Moisture	Material	2	1.21	9.64	0.0940^{NS}
	Residual	2	0.13	-	-
Ash	Material	2	26.46	877.31	<.0001*
	Residual	3	0.03	-	-
N	Material	2	480.99	10943.40	<.0001*
	Residual	6	0.04	-	-
Total starch	Material	2	37.11	279.27	<.0001*
	Residual	9	0.13	-	-
Total lipid	Material	2	0.21	5.34	0.1028^{NS}
	Residual	3	0.04	-	-
Arabinoxylan	Material	2	629.30	1775.84	<.0001*
	Residual	3	0.35	-	-
Arabinose	Material	2	81.11	1098.19	<.0001*
	Residual	3	0.07	-	-
Xylose	Material	2	386.36	2352.14	<.0001*
	Residual	3	0.16	-	-
Galactose	Material	2	0.01	300.50	0.0004*
	Residual	3	0.00	-	-
Glucose	Material	2	5.75	166.23	0.0008*
	Residual	3	0.04	-	-
Arabinose to xylose ratio	Material	2	0.02	967.00	<.0001*
	Residual	3	0.00	-	-

Table A3. Analysis of variance for proximate compositions of arabinoxylan extracted from wheat bran, maize bran, and dried distillers grain.

	Source	DF	Mean Square	*F* Value	*Pr>F*
Moisture	Material	2	15.67	81.86	0.0024*
	Residual	3	0.19	-	-
Ash	Material	2	32.89	95597.70	<.0001*
	Residual	3	0.00	-	-
N	Material	2	72.94	433.78	0.0002*
	Residual	3	0.17	-	-
Total starch	Material	2	1.87	428.57	<.0001*
	Residual	3	0.00	-	-
Arabinoxylan	Material	2	357.00	410.47	0.0002*
	Residual	3	0.87	-	-
Arabinose	Material	2	51.90	501.07	0.0002*
	Residual	3	0.10	-	-
Xylose	Material	2	203.54	365.93	0.0003*
	Residual	3	0.56	-	-
Galactose	Material	2	0.03	759.50	<.0001*
	Residual	3	0.00	-	-
Glucose	Material	2	0.36	122.08	0.0013*
	Residual	3	0.00	-	-
Arabinose to xylose ratio	Material	2	0.00	0.00	1.0000^{NS}
	Residual	3	0.00	-	-

Table A4. Analysis of variance for molecular weight and polydispersity index of arabinoxylan.

	Source	DF	Mean Square	*F* Value	*Pr>F*
Molecular weight	Material	2	217846020000000	162034	<.0001*
	Treatment	13	147354920000000	109603	<.0001*
	Material*treatment	26	8637852100000	6425	<.0001*
	Residual	84	1344444445	-	-
Polydispersity index	Material	2	0.666	32.30	<.0001*
	Treatment	13	0.477	23.12	<.0001*
	Material*treatment	26	0.168	8.12	<.0001*
	Residual	84	0.021	-	-

Table A5. Analysis of variance for tear properties of arabinoxylan films.

	Source	DF	Mean Square	*F* Value	*Pr>F*
Load	Material	2	1.40	15.44	<.0001*
	Plasticizer	1	0.17	1.93	0.169^{NS}
	Plasticizer level	2	0.61	6.71	0.0021*
	Material*plasticizer	2	0.19	2.13	0.1268^{NS}
	Material*plasticizer level	4	0.98	10.83	<.0001*
	Plasticizer* plasticizer level	2	5.31	58.67	<.0001*
	Material*plasticizer* plasticizer level	4	0.24	2.67	0.0387*
	Residual	72	0.09	-	-
Extension	Material	2	456.53	77.90	<.0001*
	Plasticizer	1	1806.35	308.22	<.0001*
	Plasticizer level	2	1763.97	300.98	<.0001*
	Material*plasticizer	2	436.26	74.44	<.0001*
	Material*plasticizer level	4	402.77	68.72	<.0001*
	Plasticizer* plasticizer level	2	1513.32	258.22	<.0001*
	Material*plasticizer* plasticizer level	4	385.55	65.79	<.0001*
	Residual	72	5.86	-	-

Table A6. Analysis of variance for tensile properties of arabinoxylan films.

	Source	DF	Mean Square	*F* Value	*Pr>F*
Maximum tensile strength	Material	2	1017.70	246.78	<.0001*
	Plasticizer	1	66.83	16.21	0.0001*
	Plasticizer level	2	1243.60	301.56	<.0001*
	Material*plasticizer	2	24.24	5.88	0.0043*
	Material*plasticizer level	4	180.02	43.65	<.0001*
	Plasticizer* plasticizer level	2	481.80	116.83	<.0001*
	Material*plasticizer* plasticizer level	4	37.57	9.11	<.0001*
	Residual	72	4.12	-	-
Modulus	Material	2	19.3105	354.74	<.0001*
	Plasticizer	1	1.17993	21.68	<.0001*
	Plasticizer level	2	28.899	530.88	<.0001*
	Material*plasticizer	2	1.61435	29.66	<.0001*
	Material*plasticizer level	4	0.73825	13.56	<.0001*
	Plasticizer* plasticizer level	2	20.4035	374.82	<.0001*
	Material*plasticizer* plasticizer level	4	2.44148	44.85	<.0001*
	Residual	72	0.05444	-	-
Breaking factor	Material	2	4302186.00	417.41	<.0001*
	Plasticizer	1	162470.00	15.76	0.0002*
	Plasticizer level	2	1829382.00	177.49	<.0001*
	Material*plasticizer	2	11292.00	1.10	0.3399
	Material*plasticizer level	4	485393.00	47.09	<.0001*
	Plasticizer* plasticizer level	2	1361524.00	132.10	<.0001*
	Material*plasticizer* plasticizer level	4	102555.00	9.95	<.0001*
	Residual	72	10307.00	-	-
Elongation	Material	2	11155.00	517.05	<.0001*
	Plasticizer	1	15443.00	715.79	<.0001*
	Plasticizer level	2	38353.00	1777.73	<.0001*
	Material*plasticizer	2	9262.13	429.31	<.0001*
	Material*plasticizer level	4	10364.00	480.38	<.0001*
	Plasticizer* plasticizer level	2	14451.00	669.81	<.0001*
	Material*plasticizer* plasticizer level	4	7936.36	367.86	<.0001*
	Residual	72	21.57	-	-

Table A7. Analysis of variance for puncture properties of arabinoxylan films.

	Source	DF	Mean Square	*F* Value	*Pr>F*
Resistance	Material	2	143.01	175.28	<.0001*
	Plasticizer	1	0.06	0.08	0.7836^{NS}
	Plasticizer level	2	12.87	15.77	<.0001*
	Material*plasticizer	2	6.01	7.37	0.0015*
	Material*plasticizer level	4	8.66	10.61	<.0001*
	Plasticizer* plasticizer level	2	44.09	54.04	<.0001*
	Material*plasticizer* plasticizer level	4	11.31	13.86	<.0001*
	Residual	54	0.82	-	-
Extensibility	Material	2	1.30	0.80	0.4534^{NS}
	Plasticizer	1	100.61	61.88	<.0001*
	Plasticizer level	2	67.79	41.70	<.0001*
	Material*plasticizer	2	0.73	0.45	0.64^{NS}
	Material*plasticizer level	4	15.96	9.81	<.0001*
	Plasticizer* plasticizer level	2	7.70	4.74	0.0127^{NS}
	Material*plasticizer* plasticizer level	4	1.86	1.15	0.3453^{NS}
	Residual	54	1.63	-	-
Total energy	Material	2	0.00	13.00	<.0001*
	Plasticizer	1	0.00	12.61	0.0008*
	Plasticizer level	2	0.00	14.11	<.0001*
	Material*plasticizer	2	0.00	4.53	0.0152*
	Material*plasticizer level	4	0.00	4.76	0.0023*
	Plasticizer* plasticizer level	2	0.00	12.83	<.0001*
	Material*plasticizer* plasticizer level	4	0.00	0.88	0.4802^{NS}
	Residual	54	0.00	-	-
Energy to peak	Material	2	0.00	7.64	0.0012*
	Plasticizer	1	0.00	16.47	0.0002*
	Plasticizer level	2	0.00	15.86	<.0001*
	Material*plasticizer	2	0.00	4.66	0.0135*
	Material*plasticizer level	4	0.00	6.68	0.0002*
	Plasticizer* plasticizer level	2	0.00	12.48	<.0001*
	Material*plasticizer* plasticizer level	4	0.00	1.66	0.1718^{NS}
	Residual	54	0.00	-	-

Table A8. Analysis of variance for water solubility of arabinoxylan films.

Source	DF	Mean Square	*F* Value	*Pr>F*
Material	2	7344.36	496.28	<.0001*
Plasticizer	1	654.85	44.25	<.0001*
Plasticizer level	2	547.78	37.02	<.0001*
Material*plasticizer	2	29.17	1.97	0.1683^{NS}
Material*plasticizer level	4	154.98	10.47	0.0001*
Plasticizer* plasticizer level	2	17.49	1.18	0.3295^{NS}
Material*plasticizer* plasticizer level	4	95.26	6.44	0.0021*
Residual	18	14.80	-	-

Table A9. Analysis of variance for water vapor transmission properties of arabinoxylan films.

	Source	DF	Mean Square	*F* Value	*Pr>F*
Water vapor transmission rate	Material	2	472.20	51.41	<.0001*
	Plasticizer	1	4392.15	478.19	<.0001*
	Plasticizer level	2	954.99	103.97	<.0001*
	Material*plasticizer	2	153.99	16.77	<.0001*
	Material*plasticizer level	4	40.84	4.45	0.0051*
	Plasticizer* plasticizer level	2	476.82	51.91	<.0001*
	Material*plasticizer* plasticizer level	4	20.52	2.23	0.0846^{NS}
	Residual	36	9.19	-	-
Permeance	Material	2	2.49	50.42	<.0001*
	Plasticizer	1	23.70	479.53	<.0001*
	Plasticizer level	2	5.14	103.94	<.0001*
	Material*plasticizer	2	0.81	16.44	<.0001*
	Material*plasticizer level	4	0.22	4.44	0.0051*
	Plasticizer* plasticizer level	2	2.57	51.92	<.0001*
	Material*plasticizer* plasticizer level	4	0.11	2.22	0.0857^{NS}
	Residual	36	0.05	-	-

Table A10. Analysis of variance for moisture content of arabinoxylan films.

Source	DF	Mean Square	*F* Value	*Pr>F*
Material	2	18.95	29.37	<.0001*
Plasticizer	1	926.62	1435.94	<.0001*
Plasticizer level	2	372.46	577.19	<.0001*
Material*plasticizer	2	16.30	25.25	<.0001*
Material*plasticizer level	4	51.10	79.19	<.0001*
Plasticizer* plasticizer level	2	362.76	562.16	<.0001*
Material*plasticizer* plasticizer level	4	50.60	78.41	<.0001*
Residual	36	0.65	-	-

Table A11. Analysis of variance for color of arabinoxylan films (smooth side) analyzed using CIE Lab.

Light	L, a, b	Source	DF	Mean Square	*F* Value	*Pr>F*
D65	L	Material	2	718.77	19260000	<.0001*
		Plasticizer	1	0.79	21305	<.0001*
		Plasticizer level	2	1.75	46809	<.0001*
		Material*plasticizer	2	1.85	49446	<.0001*
		Material*plasticizer level	4	1.02	27251	<.0001*
		Plasticizer* plasticizer level	2	1.66	44518	<.0001*
		Material*plasticizer* plasticizer level	4	1.15	30909	<.0001*
		Residual	36	0.00	-	-
D65	a	Material	2	133.75	663584	<.0001*
		Plasticizer	1	0.11	527	<.0001*
		Plasticizer level	2	0.07	371	<.0001*
		Material*plasticizer	2	0.05	247	<.0001*
		Material*plasticizer level	4	0.15	750	<.0001*
		Plasticizer* plasticizer level	2	0.06	311	<.0001*
		Material*plasticizer* plasticizer level	4	0.08	403	<.0001*
		Residual	36	0.00	-	-
D65	b	Material	2	92.51	473452	<.0001*
		Plasticizer	1	1.52	7766	<.0001*
		Plasticizer level	2	0.79	4023	<.0001*
		Material*plasticizer	2	0.37	1901	<.0001*
		Material*plasticizer level	4	1.17	5969	<.0001*
		Plasticizer* plasticizer level	2	1.79	9151	<.0001*
		Material*plasticizer* plasticizer level	4	0.48	2470	<.0001*
		Residual	36	0.00	-	-
A	L	Material	2	629.72	191691	<.0001*
		Plasticizer	1	0.71	215	<.0001*
		Plasticizer level	2	1.76	537	<.0001*
		Material*plasticizer	2	1.79	544	<.0001*
		Material*plasticizer level	4	0.98	300	<.0001*
		Plasticizer* plasticizer level	2	1.35	411	<.0001*
		Material*plasticizer* plasticizer level	4	1.01	308	<.0001*
		Residual	36	0.00	-	-
A	a	Material	2	137.64	39344	<.0001*
		Plasticizer	1	0.31	89	<.0001*
		Plasticizer level	2	0.15	42	<.0001*
		Material*plasticizer	2	0.13	37	<.0001*

Table A11. Analysis of variance for color of arabinoxylan films (smooth side) analyzed using CIE Lab (continued).

Light	L, a, b	Source	DF	Mean Square	*F* Value	*Pr>F*
		Material*plasticizer level	4	0.21	60	<.0001*
		Plasticizer* plasticizer level	2	0.16	45	<.0001*
		Material*plasticizer* plasticizer level	4	0.10	29	<.0001*
		Residual	36	0.00	-	-
A	b	Material	2	139.77	10232	<.0001*
		Plasticizer	1	2.17	159	<.0001*
		Plasticizer level	2	0.84	61	<.0001*
		Material*plasticizer	2	0.52	38	<.0001*
		Material*plasticizer level	4	1.16	85	<.0001*
		Plasticizer* plasticizer level	2	2.00	146	<.0001*
		Material*plasticizer* plasticizer level	4	0.46	34	<.0001*
		Residual	36	0.01	-	-
CFW-2	L	Material	2	676.49	178944	<.0001*
		Plasticizer	1	0.77	205	<.0001*
		Plasticizer level	2	1.83	485	<.0001*
		Material*plasticizer	2	1.72	454	<.0001*
		Material*plasticizer level	4	1.04	275	<.0001*
		Plasticizer* plasticizer level	2	1.40	371	<.0001*
		Material*plasticizer* plasticizer level	4	1.04	276	<.0001*
		Residual	36	0.00	-	-
CFW-2	a	Material	2	59.80	58820	<.0001*
		Plasticizer	1	0.05	52	<.0001*
		Plasticizer level	2	0.04	40	<.0001*
		Material*plasticizer	2	0.03	28	<.0001*
		Material*plasticizer level	4	0.07	72	<.0001*
		Plasticizer* plasticizer level	2	0.02	20	<.0001*
		Material*plasticizer* plasticizer level	4	0.02	24	<.0001*
		Residual	36	0.00	-	-
CFW-2	b	Material	2	120.13	8734	<.0001*
		Plasticizer	1	2.26	165	<.0001*
		Plasticizer level	2	0.93	68	<.0001*
		Material*plasticizer	2	0.53	38	<.0001*
		Material*plasticizer level	4	1.39	101	<.0001*
		Plasticizer* plasticizer level	2	2.39	173	<.0001*
		Material*plasticizer* plasticizer level	4	0.50	37	<.0001*
		Residual	36	0.01	-	-

Table A12. Analysis of variance for color of arabinoxylan films (smooth side) analyzed using Hunter Lab.

Light	L, a, b	Source	DF	Mean Square	*F* Value	*Pr>F*
D65	L	Material	2	514.34	18390000	<.0001*
		Plasticizer	1	1.02	36334	<.0001*
		Plasticizer level	2	1.55	55555	<.0001*
		Material*plasticizer	2	0.99	35447	<.0001*
		Material*plasticizer level	4	0.93	33144	<.0001*
		Plasticizer* plasticizer level	2	1.14	40682	<.0001*
		Material*plasticizer* plasticizer level	4	0.77	27651	<.0001*
		Residual	36	0.00	-	-
D65	a	Material	2	57.11	572218	<.0001*
		Plasticizer	1	0.11	1055	<.0001*
		Plasticizer level	2	0.04	396	<.0001*
		Material*plasticizer	2	0.07	685	<.0001*
		Material*plasticizer level	4	0.08	828	<.0001*
		Plasticizer* plasticizer level	2	0.01	124	<.0001*
		Material*plasticizer* plasticizer level	4	0.01	95	<.0001*
		Residual	36	0.00	-	-
D65	b	Material	2	31.40	400513	<.0001*
		Plasticizer	1	0.69	8767	<.0001*
		Plasticizer level	2	0.24	3075	<.0001*
		Material*plasticizer	2	0.22	2763	<.0001*
		Material*plasticizer level	4	0.37	4766	<.0001*
		Plasticizer* plasticizer level	2	0.72	9196	<.0001*
		Material*plasticizer* plasticizer level	4	0.13	1718	<.0001*
		Residual	36	0.00	-	-
A	L	Material	2	460.20	17760000	<.0001*
		Plasticizer	1	0.79	30676	<.0001*
		Plasticizer level	2	1.46	56188	<.0001*
		Material*plasticizer	2	1.11	43016	<.0001*
		Material*plasticizer level	4	0.83	32161	<.0001*
		Plasticizer* plasticizer level	2	0.99	38405	<.0001*
		Material*plasticizer* plasticizer level	4	0.73	28116	<.0001*
		Residual	36	0.00	-	-
A	a	Material	2	58.52	1122519	<.0001*
		Plasticizer	1	0.23	4424	<.0001*
		Plasticizer level	2	0.07	1328	<.0001*
		Material*plasticizer	2	0.13	2528	<.0001*
		Material*plasticizer level	4	0.11	2074	<.0001*
		Plasticizer* plasticizer level	2	0.08	1479	<.0001*
		Material*plasticizer* plasticizer level	4	0.02	352	<.0001*
		Residual	36	0.00	-	-
A	b	Material	2	43.23	634047	<.0001*
		Plasticizer	1	0.83	12232	<.0001*
		Plasticizer level	2	0.28	4120	<.0001*
		Material*plasticizer	2	0.26	3883	<.0001*
		Material*plasticizer level	4	0.41	5966	<.0001*
		Plasticizer* plasticizer level	2	0.80	11663	<.0001*
		Material*plasticizer* plasticizer level	4	0.14	2108	<.0001*
		Residual	36	0.00	-	-
CFW-2	L	Material	2	489.12	16500000	<.0001*
		Plasticizer	1	0.87	29214	<.0001*
		Plasticizer level	2	1.50	50554	<.0001*
		Material*plasticizer	2	1.04	35241	<.0001*
		Material*plasticizer level	4	0.87	29466	<.0001*
		Plasticizer* plasticizer level	2	1.03	34578	<.0001*
		Material*plasticizer* plasticizer level	4	0.74	24891	<.0001*
		Residual	36	0.00	-	-

Table A12. Analysis of variance for color of arabinoxylan films (smooth side) analyzed using Hunter Lab (continued).

Light	L, a, b	Source	DF	Mean Square	*F* Value	*Pr>F*
CFW-2	a	Material	2	25.63	446808	<.0001*
		Plasticizer	1	0.04	732	<.0001*
		Plasticizer level	2	0.02	324	<.0001*
		Material*plasticizer	2	0.03	483	<.0001*
		Material*plasticizer level	4	0.04	621	<.0001*
		Plasticizer* plasticizer level	2	0.00	84	<.0001*
		Material*plasticizer* plasticizer level	4	0.00	67	<.0001*
		Residual	36	0.00	-	-
CFW-2	b	Material	2	40.27	499536	<.0001*
		Plasticizer	1	0.86	10619	<.0001*
		Plasticizer level	2	0.32	3944	<.0001*
		Material*plasticizer	2	0.26	3250	<.0001*
		Material*plasticizer level	4	0.48	5967	<.0001*
		Plasticizer* plasticizer level	2	0.95	11743	<.0001*
		Material*plasticizer* plasticizer level	4	0.18	2171	<.0001*
		Residual	36	0.00	-	-

Table A13. Analysis of variance for color of arabinoxylan films (rough side) analyzed using CIE Lab.

Light	L, a, b	Source	DF	Mean Square	*F* Value	*Pr>F*
D65	L	Material	2	805.08	18720000	<.0001*
		Plasticizer	1	1.93	44921	<.0001*
		Plasticizer level	2	1.45	33620	<.0001*
		Material*plasticizer	2	0.73	17011	<.0001*
		Material*plasticizer level	4	0.62	14335	<.0001*
		Plasticizer* plasticizer level	2	1.18	27470	<.0001*
		Material*plasticizer* plasticizer level	4	1.15	26648	<.0001*
		Residual	36	0.00	-	-
D65	a	Material	2	138.41	1021187	<.0001*
		Plasticizer	1	0.14	1069	<.0001*
		Plasticizer level	2	0.04	283	<.0001*
		Material*plasticizer	2	0.06	419	<.0001*
		Material*plasticizer level	4	0.01	86	<.0001*
		Plasticizer* plasticizer level	2	0.08	623	<.0001*
		Material*plasticizer* plasticizer level	4	0.01	61	<.0001*
		Residual	36	0.00	-	-
D65	b	Material	2	96.71	529471	<.0001*
		Plasticizer	1	1.57	8623	<.0001*
		Plasticizer level	2	0.83	4553	<.0001*
		Material*plasticizer	2	0.08	427	<.0001*
		Material*plasticizer level	4	0.79	4346	<.0001*
		Plasticizer* plasticizer level	2	2.14	11743	<.0001*
		Material*plasticizer* plasticizer level	4	0.39	2130	<.0001*
		Residual	36	0.00	-	-
A	L	Material	2	710.89	18430000	<.0001*
		Plasticizer	1	1.62	42014	<.0001*
		Plasticizer level	2	1.33	34360	<.0001*
		Material*plasticizer	2	0.77	20027	<.0001*
		Material*plasticizer level	4	0.54	14080	<.0001*
		Plasticizer* plasticizer level	2	0.93	24026	<.0001*
		Material*plasticizer* plasticizer level	4	1.12	29099	<.0001*
		Residual	36	0.00	-	-

Table A13. Analysis of variance for color of arabinoxylan films (rough side) analyzed using CIE Lab (continued).

Light	L, a, b	Source	DF	Mean Square	*F* Value	*Pr>F*
A	a	Material	2	144.89	1332209	<.0001*
		Plasticizer	1	0.31	2816	<.0001*
		Plasticizer level	2	0.13	1232	<.0001*
		Material*plasticizer	2	0.06	528	<.0001*
		Material*plasticizer level	4	0.08	718	<.0001*
		Plasticizer* plasticizer level	2	0.31	2858	<.0001*
		Material*plasticizer* plasticizer level	4	0.03	279	<.0001*
		Residual	36	0.00	-	-
A	b	Material	2	150.69	909216	<.0001*
		Plasticizer	1	1.94	11732	<.0001*
		Plasticizer level	2	0.97	5834	<.0001*
		Material*plasticizer	2	0.11	656	<.0001*
		Material*plasticizer level	4	0.87	5271	<.0001*
		Plasticizer* plasticizer level	2	2.53	15249	<.0001*
		Material*plasticizer* plasticizer level	4	0.40	2433	<.0001*
		Residual	36	0.00	-	-
CFW-2	L	Material	2	761.68	17070000	<.0001*
		Plasticizer	1	1.72	38504	<.0001*
		Plasticizer level	2	1.37	30670	<.0001*
		Material*plasticizer	2	0.75	16744	<.0001*
		Material*plasticizer level	4	0.57	12825	<.0001*
		Plasticizer* plasticizer level	2	1.01	22602	<.0001*
		Material*plasticizer* plasticizer level	4	1.14	25509	<.0001*
		Residual	36	0.00	-	-
CFW-2	a	Material	2	62.37	595978	<.0001*
		Plasticizer	1	0.06	569	<.0001*
		Plasticizer level	2	0.01	128	<.0001*
		Material*plasticizer	2	0.03	243	<.0001*
		Material*plasticizer level	4	0.00	40	<.0001*
		Plasticizer* plasticizer level	2	0.03	297	<.0001*
		Material*plasticizer* plasticizer level	4	0.00	40	<.0001*
		Residual	36	0.00	-	-
CFW-2	b	Material	2	126.96	334913	<.0001*
		Plasticizer	1	2.09	5516	<.0001*
		Plasticizer level	2	1.08	2858	<.0001*
		Material*plasticizer	2	0.10	257	<.0001*
		Material*plasticizer level	4	1.01	2675	<.0001*
		Plasticizer* plasticizer level	2	2.80	7387	<.0001*
		Material*plasticizer* plasticizer level	4	0.49	1299	<.0001*
		Residual	36	0.00	-	-

Table A14. Analysis of variance for color of arabinoxylan films (rough side) analyzed using Hunter Lab.

Light	L, a, b	Source	DF	Mean Square	*F* Value	*Pr>F*
D65	L	Material	2	580.96	17730000	<.0001*
		Plasticizer	1	1.54	47028	<.0001*
		Plasticizer level	2	1.11	34002	<.0001*
		Material*plasticizer	2	0.56	17145	<.0001*
		Material*plasticizer level	4	0.48	14652	<.0001*
		Plasticizer* plasticizer level	2	1.00	30523	<.0001*
		Material*plasticizer* plasticizer level	4	0.86	26352	<.0001*
		Residual	36	0.00	-	-
D65	a	Material	2	59.55	852979	<.0001*
		Plasticizer	1	0.07	952	<.0001*
		Plasticizer level	2	0.02	312	<.0001*
		Material*plasticizer	2	0.03	406	<.0001*
		Material*plasticizer level	4	0.01	78	<.0001*
		Plasticizer* plasticizer level	2	0.04	630	<.0001*
		Material*plasticizer* plasticizer level	4	0.00	66	<.0001*
		Residual	36	0.00	-	-
D65	b	Material	2	32.00	478016	<.0001*
		Plasticizer	1	0.45	6661	<.0001*
		Plasticizer level	2	0.32	4721	<.0001*
		Material*plasticizer	2	0.02	274	<.0001*
		Material*plasticizer level	4	0.34	5061	<.0001*
		Plasticizer* plasticizer level	2	0.77	11495	<.0001*
		Material*plasticizer* plasticizer level	4	0.19	2874	<.0001*
		Residual	36	0.00	-	-
A	L	Material	2	521.00	16630000	<.0001*
		Plasticizer	1	1.32	42060	<.0001*
		Plasticizer level	2	1.03	32716	<.0001*
		Material*plasticizer	2	0.59	18955	<.0001*
		Material*plasticizer level	4	0.42	13472	<.0001*
		Plasticizer* plasticizer level	2	0.80	25549	<.0001*
		Material*plasticizer* plasticizer level	4	0.85	27022	<.0001*
		Residual	36	0.00	-	-
A	a	Material	2	61.79	1057173	<.0001*
		Plasticizer	1	0.14	2440	<.0001*
		Plasticizer level	2	0.07	1211	<.0001*
		Material*plasticizer	2	0.03	572	<.0001*
		Material*plasticizer level	4	0.04	710	<.0001*
		Plasticizer* plasticizer level	2	0.16	2787	<.0001*
		Material*plasticizer* plasticizer level	4	0.02	295	<.0001*
		Residual	36	0.00	-	-
A	b	Material	2	45.45	740521	<.0001*
		Plasticizer	1	0.54	8851	<.0001*
		Plasticizer level	2	0.37	5963	<.0001*
		Material*plasticizer	2	0.03	419	<.0001*
		Material*plasticizer level	4	0.37	6088	<.0001*
		Plasticizer* plasticizer level	2	0.90	14659	<.0001*
		Material*plasticizer* plasticizer level	4	0.20	3283	<.0001*
		Residual	36	0.00	-	-
CFW-2	L	Material	2	552.41	16100000	<.0001*
		Plasticizer	1	1.38	40295	<.0001*
		Plasticizer level	2	1.05	30694	<.0001*
		Material*plasticizer	2	0.57	16684	<.0001*
		Material*plasticizer level	4	0.44	12905	<.0001*
		Plasticizer* plasticizer level	2	0.86	25197	<.0001*
		Material*plasticizer* plasticizer level	4	0.85	24897	<.0001*
		Residual	36	0.00	-	-

Table A14. Analysis of variance for color of arabinoxylan films (rough side) analyzed using Hunter Lab (continued).

Light	L, a, b	Source	DF	Mean Square	*F* Value	*Pr>F*
CFW-2	a	Material	2	26.74	507097	<.0001*
		Plasticizer	1	0.03	519	<.0001*
		Plasticizer level	2	0.01	146	<.0001*
		Material*plasticizer	2	0.01	235	<.0001*
		Material*plasticizer level	4	0.00	36	<.0001*
		Plasticizer* plasticizer level	2	0.02	310	<.0001*
		Material*plasticizer* plasticizer level	4	0.00	42	<.0001*
		Residual	36	0.00	-	-
CFW-2	b	Material	2	41.23	349655	<.0001*
		Plasticizer	1	0.58	4941	<.0001*
		Plasticizer level	2	0.41	3446	<.0001*
		Material*plasticizer	2	0.02	183	<.0001*
		Material*plasticizer level	4	0.43	3633	<.0001*
		Plasticizer* plasticizer level	2	0.99	8399	<.0001*
		Material*plasticizer* plasticizer level	4	0.24	2054	<.0001*
		Residual	36	0.00	-	-

Table A15. Analysis of variance for contact angle and wetting tension of arabinoxylan films (smooth side).

Parameter	Solvent	Source	DF	Mean Square	*F* Value	*Pr>F*
Contact angle	Water	Material	2	1810.80	852.52	<.0001*
		Plasticizer	1	719.40	338.69	<.0001*
		Plasticizer level	2	64.67	30.45	<.0001*
		Material*plasticizer	2	900.60	424.00	<.0001*
		Material*plasticizer level	4	171.95	80.95	<.0001*
		Plasticizer* plasticizer level	2	60.64	28.55	<.0001*
		Material*plasticizer* plasticizer level	4	141.24	66.50	<.0001*
		Residual	18	2.12	-	-
Wetting tension	Water	Material	2	2419.62	841.75	<.0001*
		Plasticizer	1	1160.81	403.83	<.0001*
		Plasticizer level	2	108.49	37.74	<.0001*
		Material*plasticizer	2	1358.67	472.66	<.0001*
		Material*plasticizer level	4	234.94	81.73	<.0001*
		Plasticizer* plasticizer level	2	97.70	33.99	<.0001*
		Material*plasticizer* plasticizer level	4	211.72	73.66	<.0001*
		Residual	18	2.87	-	-
Contact angle	Mineral oil	Material	2	128.95	238.93	<.0001*
		Plasticizer	1	291.90	540.87	<.0001*
		Plasticizer level	2	167.18	309.76	<.0001*
		Material*plasticizer	2	100.80	186.78	<.0001*
		Material*plasticizer level	4	47.28	87.61	<.0001*
		Plasticizer* plasticizer level	2	39.08	72.41	<.0001*
		Material*plasticizer* plasticizer level	4	7.46	13.83	<.0001*
		Residual	36	0.54	-	-
Wetting tension	Mineral oil	Material	2	24.47	215.24	<.0001*
		Plasticizer	1	63.72	560.41	<.0001*
		Plasticizer level	2	40.92	359.92	<.0001*
		Material*plasticizer	2	15.19	133.61	<.0001*
		Material*plasticizer level	4	13.00	114.34	<.0001*
		Plasticizer* plasticizer level	2	13.13	115.48	<.0001*
		Material*plasticizer* plasticizer level	4	2.08	18.27	<.0001*
		Residual	36	0.11	-	-

Table A16. Analysis of variance for contact angle and wetting tension of arabinoxylan films (rough side).

Parameter	Solvent	Source	DF	Mean Square	*F* Value	*Pr>F*
Contact angle	Water	Material	2	3062.31	558.17	<.0001*
		Plasticizer	1	24.09	4.39	0.0505^{NS}
		Plasticizer level	2	576.92	105.16	<.0001*
		Material*plasticizer	2	606.27	110.51	<.0001*
		Material*plasticizer level	4	431.94	78.73	<.0001*
		Plasticizer* plasticizer level	2	55.91	10.19	0.0011*
		Material*plasticizer* plasticizer level	4	357.19	65.11	<.0001*
		Residual	18	5.49	-	-
Wetting tension	Water	Material	2	4606.44	617.27	<.0001*
		Plasticizer	1	19.00	2.55	0.128^{NS}
		Plasticizer level	2	823.79	110.39	<.0001*
		Material*plasticizer	2	901.81	120.84	<.0001*
		Material*plasticizer level	4	639.93	85.75	<.0001*
		Plasticizer* plasticizer level	2	101.38	13.58	0.0003*
		Material*plasticizer* plasticizer level	4	520.17	69.70	<.0001*
		Residual	18	7.46	-	-
Contact angle	Mineral oil	Material	2	63.62	116.35	<.0001*
		Plasticizer	1	1.50	2.75	0.106^{NS}
		Plasticizer level	2	13.17	24.08	<.0001*
		Material*plasticizer	2	31.08	56.83	<.0001*
		Material*plasticizer level	4	30.41	55.61	<.0001*
		Plasticizer* plasticizer level	2	31.24	57.12	<.0001*
		Material*plasticizer* plasticizer level	4	12.13	22.17	<.0001*
		Residual	36	0.55	-	-
Wetting tension	Mineral oil	Material	2	14.06	130.38	<.0001*
		Plasticizer	1	0.27	2.52	0.1213^{NS}
		Plasticizer level	2	2.57	23.86	<.0001*
		Material*plasticizer	2	7.16	66.40	<.0001*
		Material*plasticizer level	4	6.73	62.35	<.0001*
		Plasticizer* plasticizer level	2	7.11	65.87	<.0001*
		Material*plasticizer* plasticizer level	4	2.15	19.92	<.0001*
		Residual	36	0.11	-	-

Table A17. Analysis of variance for atomic force microscopic analysis of arabinoxylan films (smooth side).

Parameter	Image Element	Source	DF	Mean Square	*F* Value	*Pr>F*
Height	Z Range	Material	2	8519718	366.84	<.0001*
		Plasticizer	1	231485	9.97	0.0032*
		Plasticizer level	2	3696791	159.18	<.0001*
		Material*plasticizer	2	2306465	99.31	<.0001*
		Material*plasticizer level	4	2890955	124.48	<.0001*
		Plasticizer* plasticizer level	2	487839	21.01	<.0001*
		Material*plasticizer* plasticizer level	4	1557286	67.05	<.0001*
		Residual	36	23225	-	-
Height	Root mean square	Material	2	208438	275.21	<.0001*
		Plasticizer	1	12673	16.73	0.0002*
		Plasticizer level	2	85120	112.39	<.0001*
		Material*plasticizer	2	46529	61.43	<.0001*
		Material*plasticizer level	4	69858	92.24	<.0001*
		Plasticizer* plasticizer level	2	15893	20.98	<.0001*
		Material*plasticizer* plasticizer level	4	31790	41.97	<.0001*
		Residual	36	757	-	-
Height	Arithmetic average	Material	2	133701	237.65	<.0001*
		Plasticizer	1	8486	15.08	0.0004*
		Plasticizer level	2	53584	95.24	<.0001*
		Material*plasticizer	2	29754	52.89	<.0001*
		Material*plasticizer level	4	43941	78.10	<.0001*
		Plasticizer* plasticizer level	2	10358	18.41	<.0001*
		Material*plasticizer* plasticizer level	4	20702	36.80	<.0001*
		Residual	36	563	-	-
Height	Surface area	Material	2	1094216	1323.32	<.0001*
		Plasticizer	1	3859950	4668.15	<.0001*
		Plasticizer level	2	3765243	4553.62	<.0001*
		Material*plasticizer	2	1188518	1437.37	<.0001*
		Material*plasticizer level	4	1107660	1339.58	<.0001*
		Plasticizer* plasticizer level	2	3696368	4470.32	<.0001*
		Material*plasticizer* plasticizer level	4	1056459	1277.66	<.0001*
		Residual	36	827	-	-
Phase	Z Range	Material	2	756	0.69	0.5103^{NS}
		Plasticizer	1	14954	13.56	0.0008*
		Plasticizer level	2	355	0.32	0.7266^{NS}
		Material*plasticizer	2	1305	1.18	0.3179^{NS}
		Material*plasticizer level	4	1951	1.77	0.1565^{NS}
		Plasticizer* plasticizer level	2	1685	1.53	0.2306^{NS}
		Material*plasticizer* plasticizer level	4	4940	4.48	0.0048*
		Residual	36	1103	-	-
Phase	Root mean square	Material	2	52	7.27	0.0022*
		Plasticizer	1	120	16.62	0.0002*
		Plasticizer level	2	36	5.04	0.0118*
		Material*plasticizer	2	1	0.10	0.9023^{NS}
		Material*plasticizer level	4	67	9.23	<.0001*
		Plasticizer* plasticizer level	2	127	17.59	<.0001*
		Material*plasticizer* plasticizer level	4	23	3.24	0.0226*
		Residual	36	7	-	-
Phase	Arithmetic average	Material	2	55	9.59	0.0005*
		Plasticizer	1	93	16.31	0.0003*
		Plasticizer level	2	24	4.24	0.0223*
		Material*plasticizer	2	1	0.17	0.847^{NS}
		Material*plasticizer level	4	45	7.87	0.0001*
		Plasticizer* plasticizer level	2	109	19.07	<.0001*
		Material*plasticizer* plasticizer level	4	17	3.03	0.0297*
		Residual	36	6	-	-

Table A17. Analysis of variance for atomic force microscopic analysis of arabinoxylan films (smooth side) (continued).

Parameter	Image Element	Source	DF	Mean Square	*F* Value	*Pr>F*
Amplitude	Z Range	Material	2	12450	0.97	0.3894^{NS}
		Plasticizer	1	12380	0.96	0.3330^{NS}
		Plasticizer level	2	12974	1.01	0.3747^{NS}
		Material*plasticizer	2	13176	1.02	0.3691^{NS}
		Material*plasticizer level	4	12822	1.00	0.4217^{NS}
		Plasticizer* plasticizer level	2	12788	0.99	0.3798^{NS}
		Material*plasticizer* plasticizer level	4	12880	1.00	0.4194^{NS}
		Residual	36	12859	-	-
Amplitude	Root mean square	Material	2	0	15.42	<.0001*
		Plasticizer	1	0	1.95	0.1716^{NS}
		Plasticizer level	2	0	3.44	0.0431*
		Material*plasticizer	2	0	18.12	<.0001*
		Material*plasticizer level	4	0	3.15	0.0254*
		Plasticizer* plasticizer level	2	0	1.22	0.3063^{NS}
		Material*plasticizer* plasticizer level	4	0	3.49	0.0166*
		Residual	36	0	-	-
Amplitude	Arithmetic average	Material	2	0	4.57	0.0171*
		Plasticizer	1	0	0.58	0.4495^{NS}
		Plasticizer level	2	0	3.45	0.0426*
		Material*plasticizer	2	0	0.39	0.6810^{NS}
		Material*plasticizer level	4	0	1.64	0.1847^{NS}
		Plasticizer* plasticizer level	2	0	0.85	0.4360^{NS}
		Material*plasticizer* plasticizer level	4	0	0.65	0.6276^{NS}
		Residual	36	0	-	-

Table A18. Analysis of variance for atomic force microscopic analysis of arabinoxylan films (rough side).

Parameter	Image Element	Source	DF	Mean Square	*F* Value	*Pr>F*
Height	Z Range	Material	2	91600440	105.87	<.0001*
		Plasticizer	1	4020543	4.65	0.0379*
		Plasticizer level	2	2049707	2.37	0.1080^{NS}
		Material*plasticizer	2	54372	0.06	0.9392^{NS}
		Material*plasticizer level	4	1327566	1.53	0.2130^{NS}
		Plasticizer* plasticizer level	2	1020642	1.18	0.3190^{NS}
		Material*plasticizer* plasticizer level	4	177476	0.21	0.9339^{NS}
		Residual	36	865193	-	-
Height	Root mean square	Material	2	3205808	89.68	<.0001*
		Plasticizer	1	184265	5.15	0.0293*
		Plasticizer level	2	40671	1.14	0.3318^{NS}
		Material*plasticizer	2	7434	0.21	0.8132^{NS}
		Material*plasticizer level	4	34867	0.98	0.4331^{NS}
		Plasticizer* plasticizer level	2	4055	0.11	0.8931^{NS}
		Material*plasticizer* plasticizer level	4	10840	0.30	0.8739^{NS}
		Residual	36	35748	-	-
Height	Arithmetic average	Material	2	2094690	86.60	<.0001*
		Plasticizer	1	109829	4.54	0.0400*
		Plasticizer level	2	25573	1.06	0.3579^{NS}
		Material*plasticizer	2	6223	0.26	0.7745^{NS}
		Material*plasticizer level	4	23444	0.97	0.4363^{NS}
		Plasticizer* plasticizer level	2	1147	0.05	0.9537^{NS}
		Material*plasticizer* plasticizer level	4	7308	0.30	0.8746^{NS}
		Residual	36	24187	-	-

Table A18. Analysis of variance for atomic force microscopic analysis of arabinoxylan films (rough side) (continued).

Parameter	Image Element	Source	DF	Mean Square	*F* Value	*Pr>F*
Height	Surface area	Material	2	723139	3.28	0.0490*
		Plasticizer	1	6000993	27.24	<.0001*
		Plasticizer level	2	6454039	29.30	<.0001*
		Material*plasticizer	2	104540	0.47	0.6260^{NS}
		Material*plasticizer level	4	168735	0.77	0.5545^{NS}
		Plasticizer* plasticizer level	2	7943692	36.06	<.0001*
		Material*plasticizer* plasticizer level	4	207777	0.94	0.4503^{NS}
		Residual	36	220311	-	-
Phase	Z Range	Material	2	50970	10.29	0.0003*
		Plasticizer	1	14404	2.91	0.0968^{NS}
		Plasticizer level	2	5437	1.10	0.3446^{NS}
		Material*plasticizer	2	13422	2.71	0.0801^{NS}
		Material*plasticizer level	4	11676	2.36	0.0719^{NS}
		Plasticizer* plasticizer level	2	13004	2.63	0.0862^{NS}
		Material*plasticizer* plasticizer level	4	15672	3.16	0.0251*
		Residual	36	4953	-	-
Phase	Root mean square	Material	2	267	4.90	0.0131*
		Plasticizer	1	0	0.00	0.9726^{NS}
		Plasticizer level	2	78	1.43	0.2523^{NS}
		Material*plasticizer	2	143	2.62	0.0863^{NS}
		Material*plasticizer level	4	174	3.19	0.0243*
		Plasticizer* plasticizer level	2	262	4.81	0.0141*
		Material*plasticizer* plasticizer level	4	76	1.41	0.2518^{NS}
		Residual	36	54	-	-
Phase	Arithmetic average	Material	2	101	3.12	0.0565^{NS}
		Plasticizer	1	3	0.08	0.7793^{NS}
		Plasticizer level	2	31	0.95	0.3945^{NS}
		Material*plasticizer	2	83	2.56	0.0911^{NS}
		Material*plasticizer level	4	92	2.87	0.0369*
		Plasticizer* plasticizer level	2	148	4.59	0.0168*
		Material*plasticizer* plasticizer level	4	47	1.47	0.2325^{NS}
		Residual	36	32	-	-
Amplitude	Z Range	Material	2	16	49.48	<.0001*
		Plasticizer	1	0	0.13	0.7237^{NS}
		Plasticizer level	2	2	7.15	0.0024*
		Material*plasticizer	2	0	0.05	0.9510^{NS}
		Material*plasticizer level	4	2	5.68	0.0012*
		Plasticizer* plasticizer level	2	3	8.92	0.0007*
		Material*plasticizer* plasticizer level	4	1	4.16	0.0072*
		Residual	36	0	-	-
Amplitude	Root mean square	Material	2	0	14.93	<.0001*
		Plasticizer	1	0	1.85	0.1822^{NS}
		Plasticizer level	2	0	7.00	0.0027*
		Material*plasticizer	2	0	0.35	0.7057^{NS}
		Material*plasticizer level	4	0	6.13	0.0007*
		Plasticizer* plasticizer level	2	0	2.35	0.1094^{NS}
		Material*plasticizer* plasticizer level	4	0	0.62	0.6527^{NS}
		Residual	36	0	-	-
Amplitude	Arithmetic average	Material	2	0	11.52	0.0001*
		Plasticizer	1	0	3.85	0.0576^{NS}
		Plasticizer level	2	0	11.40	0.0001*
		Material*plasticizer	2	0	0.14	0.8704^{NS}
		Material*plasticizer level	4	0	7.81	0.0001*
		Plasticizer* plasticizer level	2	0	6.09	0.0053*
		Material*plasticizer* plasticizer level	4	0	1.13	0.3594^{NS}
		Residual	36	0	-	-

Table A19. Analysis of variance for biodegradability of arabinoxylan films.

Source	Numerator DF	Denominator DF	*F* Value	*Pr>F*
Day	6	216	20224	<.0001*
Treatment	17	36	85.01	<.0001*
Treatment*Day	102	216	152.87	<.0001*

APPENDIX B. HIGH PERFORMANCE SIZE EXCLUSION CHROMATOGRAMS

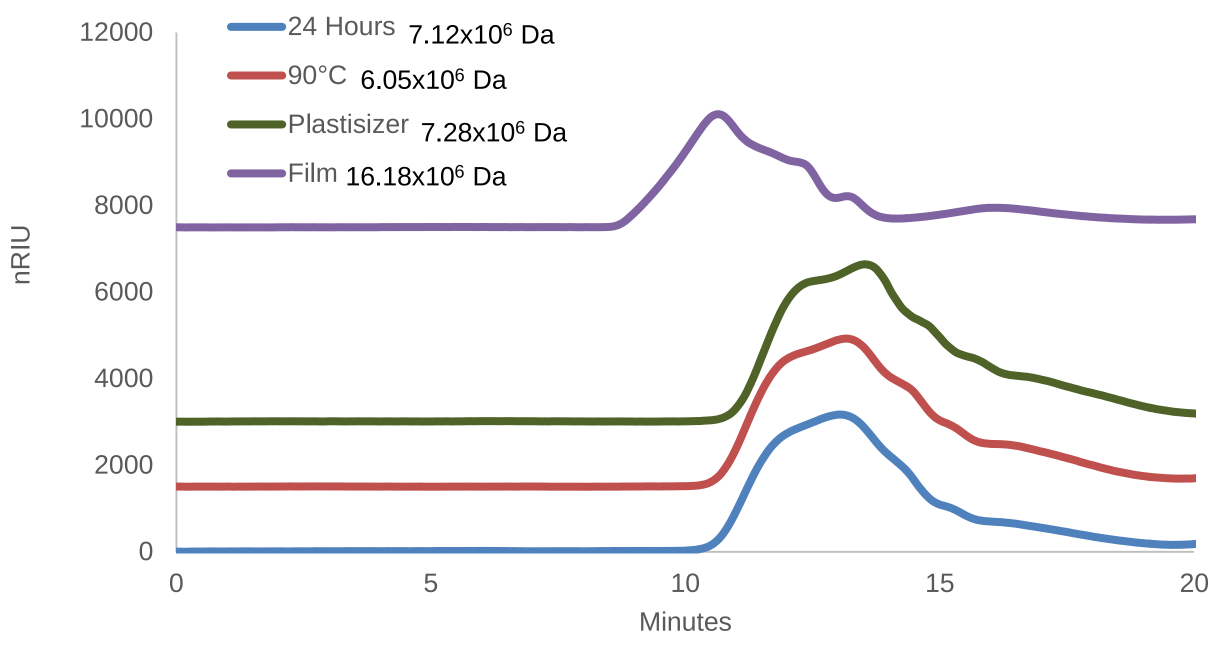

Figure B1. Chromatograms of wheat bran arabinoxylan and films.

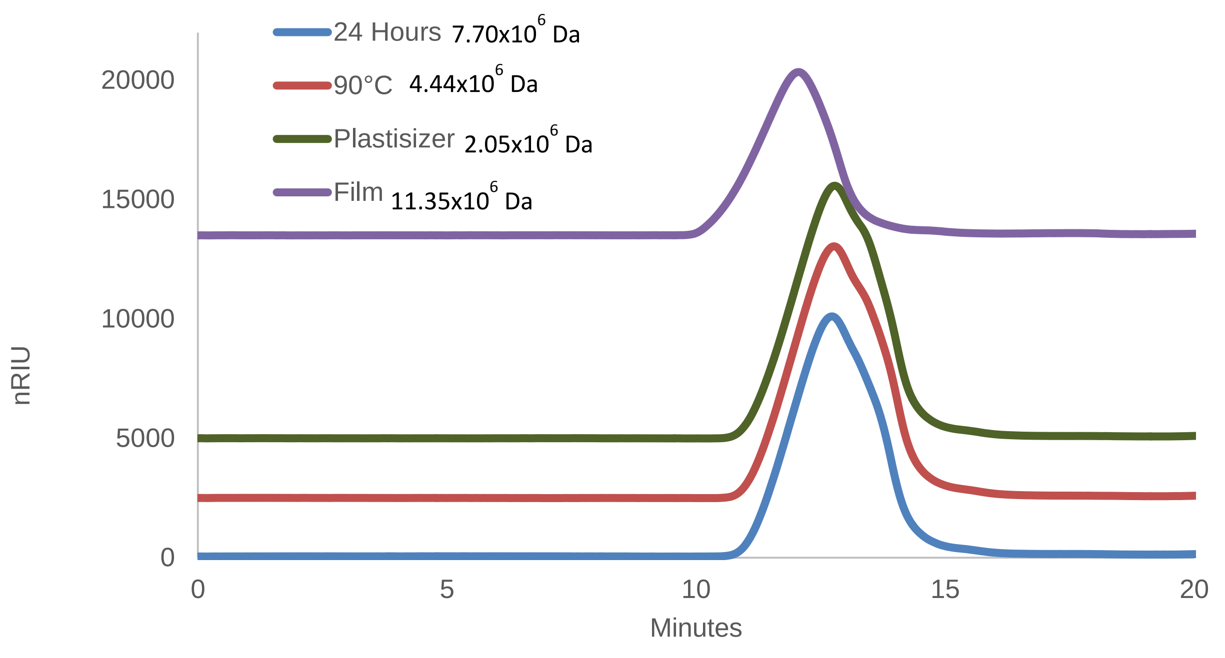

Figure B2. Chromatograms of maize bran arabinoxylan and films.

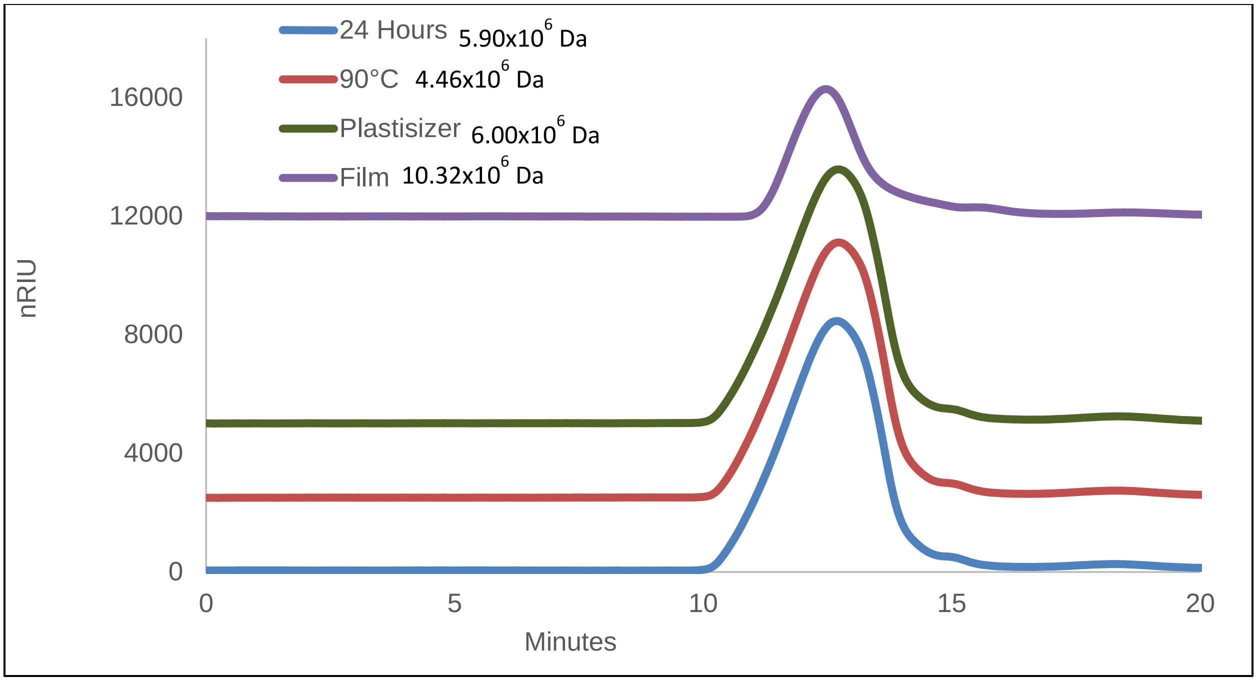

Figure B3. Chromatograms of dried distillers grain arabinoxylan and films.

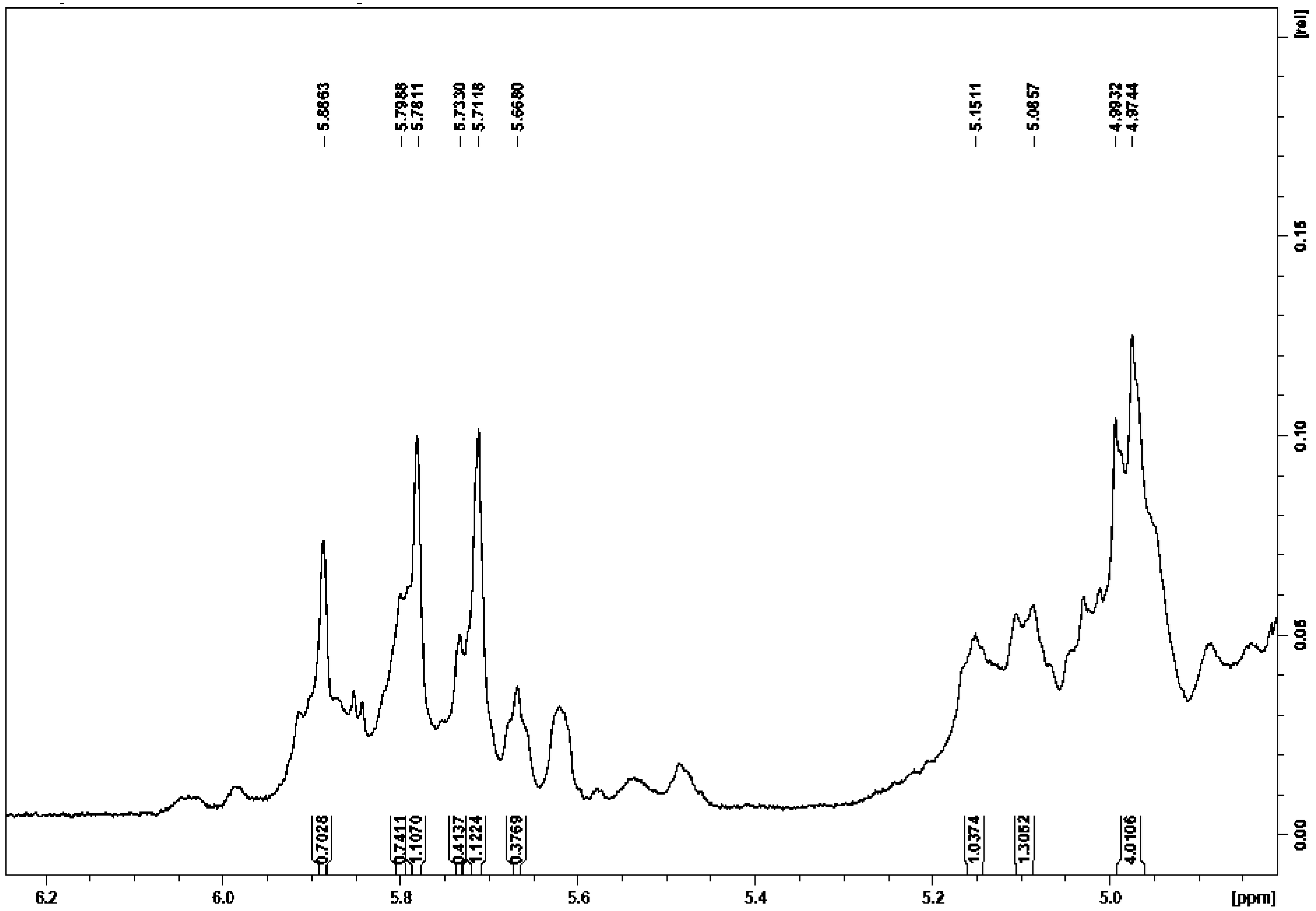

Figure C1. Wheat bran arabinoxylan nuclear magnetic resonance spectroscopy spectrum.

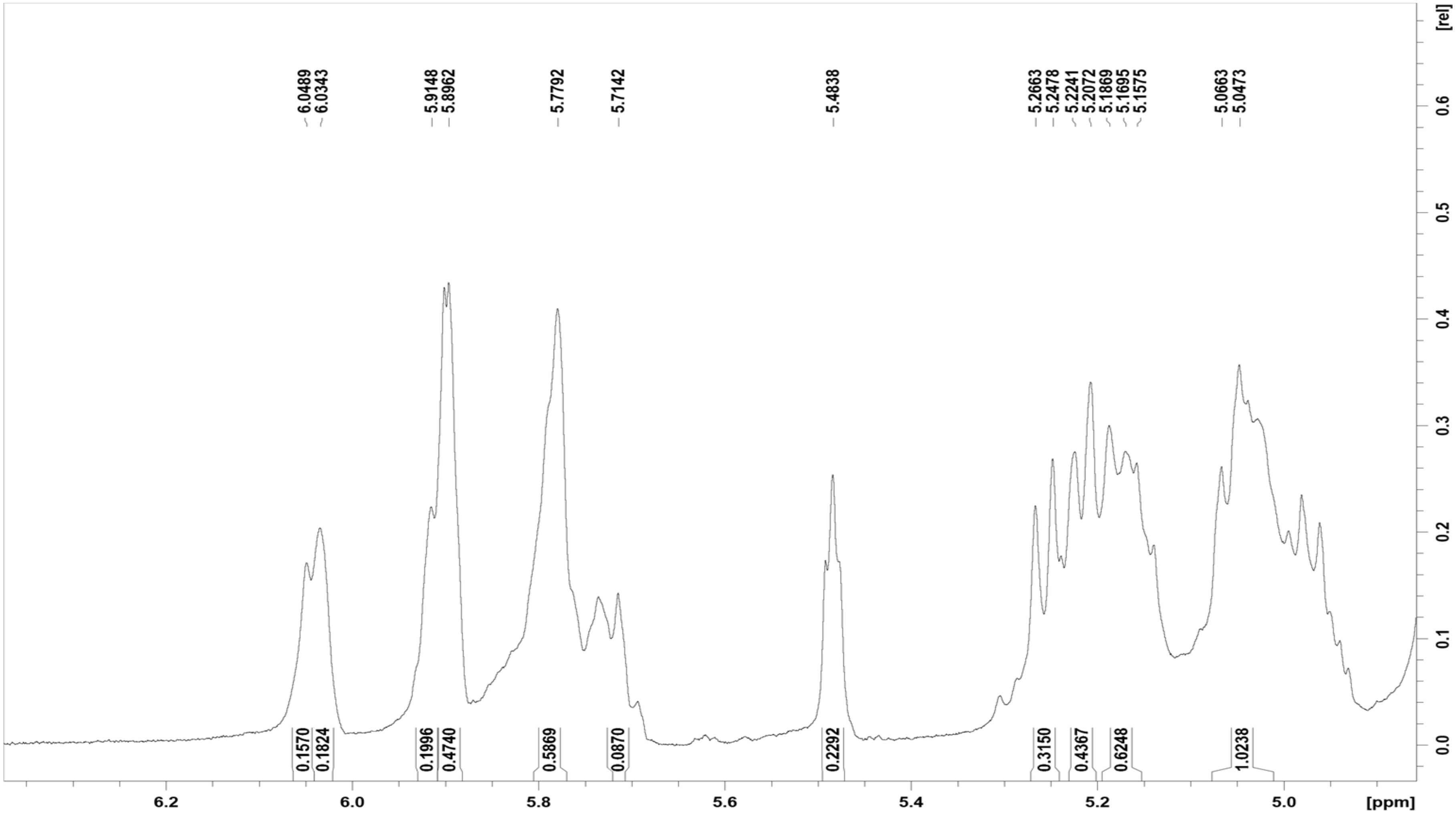

Figure C2. Maize bran arabinoxylan nuclear magnetic resonance spectroscopy spectrum.

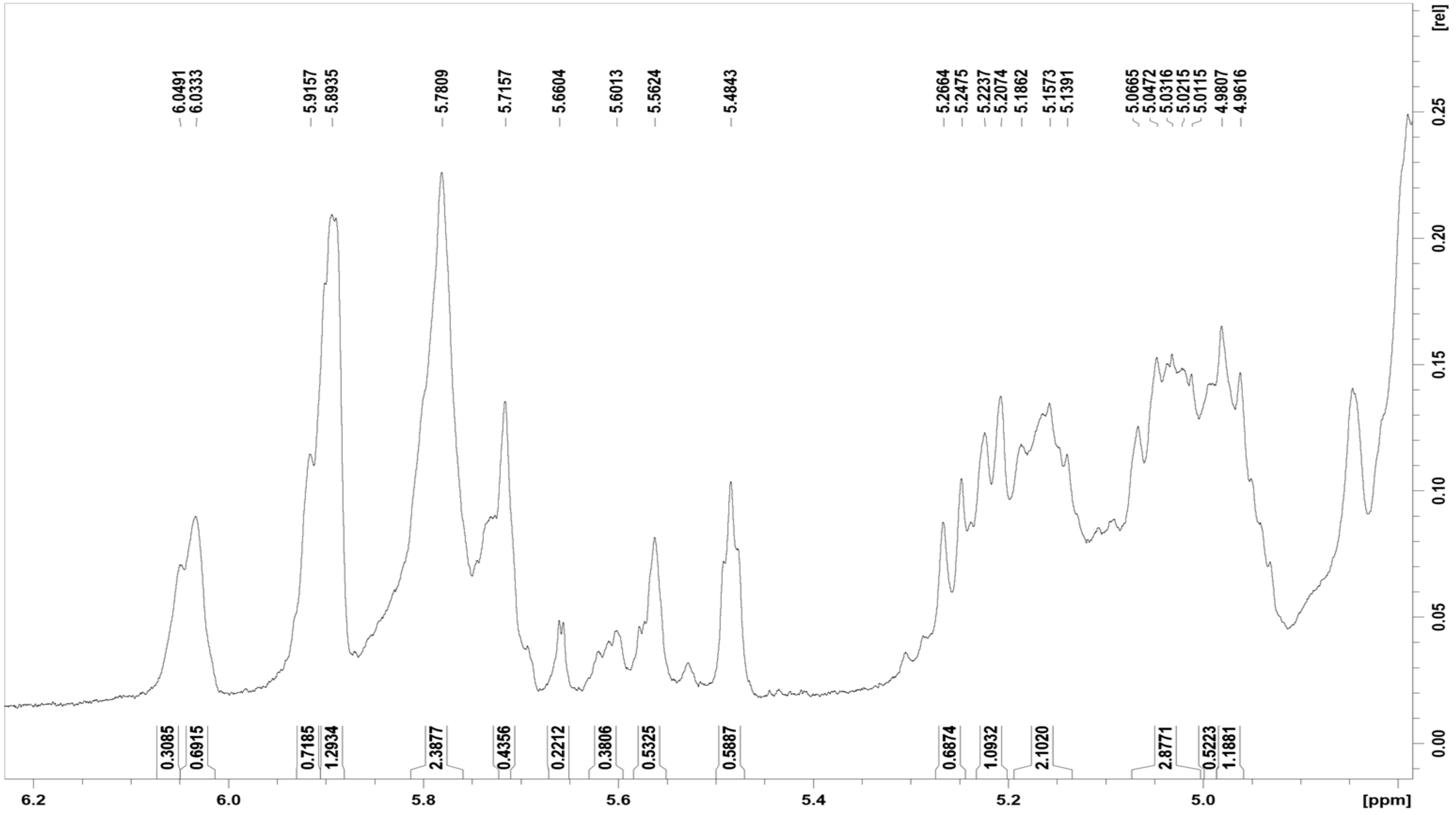

Figure C3. Dried distillers grain arabinoxylan nuclear magnetic resonance spectroscopy spectrum.

APPENDIX D. BIODEGRADABILITY PROFILES

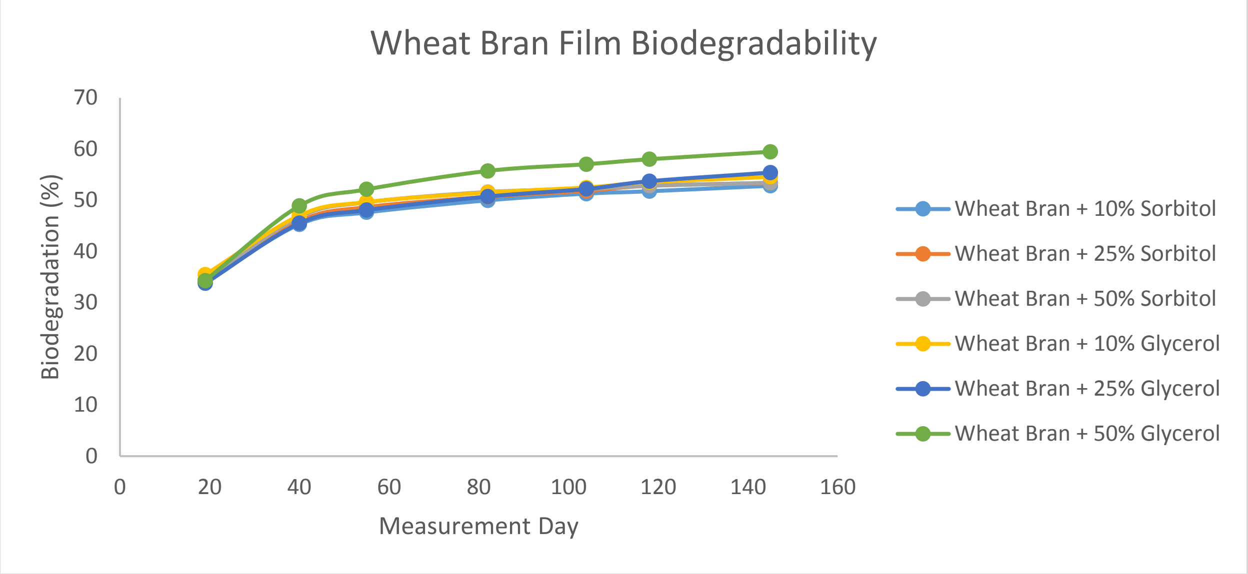

Figure D1. Biodegradability profile for films made from wheat bran arabinoxylan.

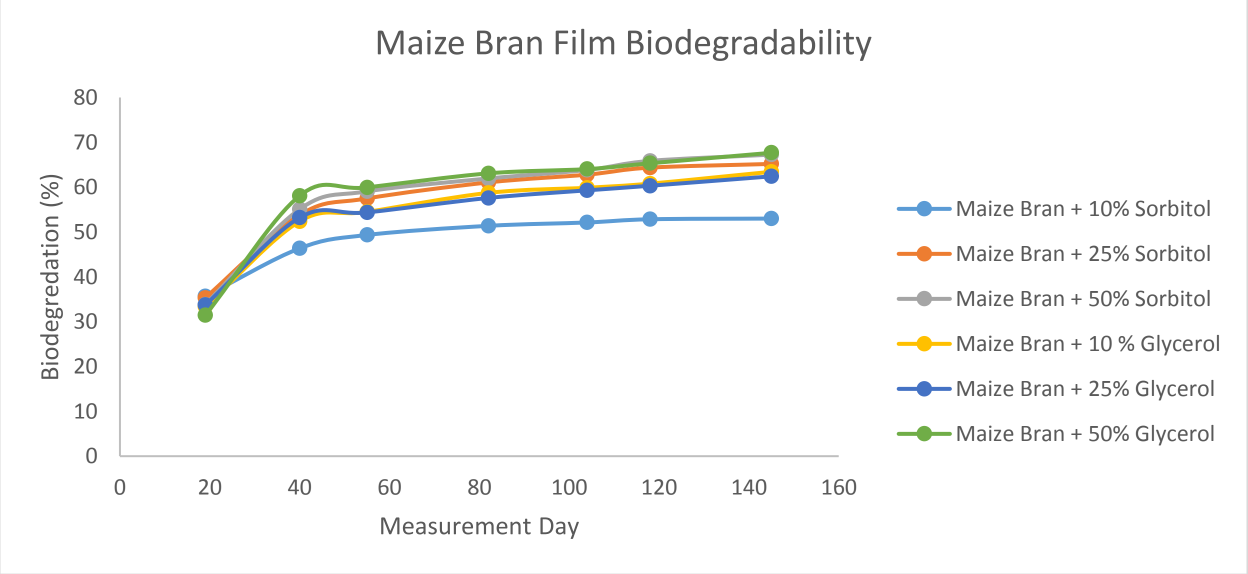

Figure D2. Biodegradability profile for films made from maize bran arabinoxylan.

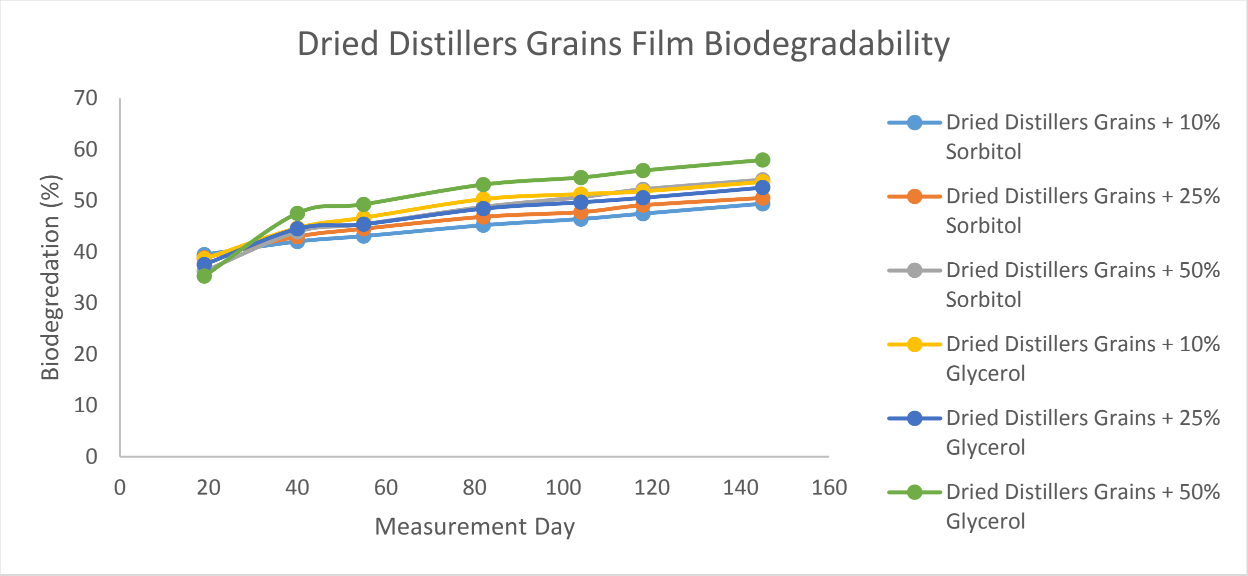

Figure D3. Biodegradability profile for films made from dried distillers grain arabinoxylan.