ENHANCING MECHANICAL PROPERTIES AND WATER INTERACTIONS OF ARABINOXYLAN FILMS FROM CORN BRAN THROUGH ENZYMATIC-CHEMICAL MODIFICATION

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ABSTRACT

Due to the need to replace non-biodegradable plastics, arabinoxylan (AX) films were evaluated for potential use in food packaging. The mechanical properties, hydrophobicity, and biodegradability of AX films were evaluated after extracting AX from corn bran byproducts of dry-milling (DCB), wet-milling (WCB), and dried distiller's grains with solubles (DDGS) using acid-alkali procedure. Packaging materials were created using the three AX extracts combined with laccase and sorbitol as the basis for each film. The three AX films were then modified by immersing their surfaces in a lipase-acetate solution. Thickness of modified DCB AX and modified DDGS AX films was significantly increased (P<0.05) compared to their unmodified AX films. Tensile properties of the modified DCB AX films were significantly improved (P<0.05), as opposed to the unmodified DCB AX films. Tensile properties of the modified WCB AX and modified DDGS AX films were enhanced, but insignificantly (P>0.05), compared to their unmodified AX films counterparts. Significant increase (P<0.05) in tear resistance and insignificant increase (P>0.05) in puncture resistance were observed for all modified AX films. Moisture content of modified AX films created from DCB, WCB, and DDGS was significantly decreased (P<0.05) compared to unmodified AX films. Significant decrease (P<0.05) in water solubility and insignificant decrease (P>0.05) in water vapor permeability were found in modified DCB AX films compared to unmodified DCB AX films. Insignificant decrease (P>0.05) in water solubility resulted in modified WCB AX films compared to unmodified WCB AX films. Water vapor permeabilities of the modified AX films made from WCB and DDGS were significantly reduced (P<0.05) compared to their unmodified AX films. AX films were positively affected by suspension in the lipase-acetate mixture, making the modified films ductile, flexible, and more resistant to deformation when stretched compared to unmodified AX

films. WCB AX and DDGS AX films were more hydrophobic and biodegradable than DCB AX film with the modification of film surface suspension in the lipase-acetate solution. The modified DCB AX films showed better physical and mechanical properties, while the hydrophobicity and biodegradability of modified WCB AX films make it a safer packaging material which can also elongated shelf-life for food.

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LIST OF ABBREVIATIONS

AACC. .....American Association of Cereal Chemists.

ADM. .....Archer Daniels Midland Company.

AEAX. .....Alkaline Extracted Arabinoxylan.

ANOVA. .....Analysis of Variance.

AOAC. .....Association of Official Agricultural Chemists.

AOCS. .....American Oil Chemists Society.

Ara*f*. .....Arabinofuranose.

ASTM. .....American Society for Testing and Materials.

AX. .....Arabinoxylan.

A/X. .....Arabinose to Xylose Ratio.

CAX. .....Corn Arabinoxylan.

CB. .....Corn Bran.

DCB. .....Corn Bran from Dry-milling.

DDGS. .....Dried Distiller's Grains with Solubles.

DF. .....Dietary Fiber.

dn/dc. .....Specific Refractive Index Increment.

DWB. .....Dry Weight Basis.

FA. .....Ferulic Acid.

FTIR. .....Fourier Transform Infrared.

GH. .....Glycosidic Hydrolyses.

h. .....Hour.

HFA. .....High Ferulic Acid.

HPAEC-PAD. .....High Performance Anion Exchange Chromatography - Pulsed Amperometric Detection.

HPLC. .....High Performance Liquid Chromatography.

HPSEC-MALS-RI. .....High Performance Size Exclusion Chromatography - Multi Angle Laser Scatting - Refractive Index.

IDF. .....Insoluble Dietary Fiber.

LFA. .....Low Ferulic Acid.

LSD. .....Least Significant Difference.

min. .....Minutes.

M_W. .....Weight Average Molecular Weight.

NO. .....Number.

oz. .....Ounce.

PI. .....Polydispersity Index.

RH. .....Relative Humidity.

RI. .....Refractive Index.

rpm. .....Revolutions Per Minute.

s. .....Seconds.

SAS. .....Statistical Analysis Software.

SDF. .....Soluble Dietary Fiber.

SEM. .....Scanning Electron Microscope.

TDF. .....Total Dietary Fiber.

US. .....The United States.

vs. .....Versus.

WCB. .....Corn Bran from Wet-milling.

WD. .....Working Distances.

WEAX. .....Water Extractable Arabinoxylan.

WUAX. .....Water Unextractable Arabinoxylan.

WVP. .....Water Vapor Permeance.

WVTR.....Water Vapor Transmission Rate.

Xyl*p*.....Xylopyranose.

LIST OF SYMBOLS

α .....Alpha

C.....Carbon

cm.....Centimeter

cm^{-1} .....Reciprocal Centimeter

CO.....Carbonyl Group

CO_2.....Carbon Dioxide

C-CO_2.....Carbon and Carbon Dioxide Balance

^oC.....Celsius

Da.....Dalton

° .....Degree

ft^2.....Square Foot

g.....Grams

g kg^{-1} .....Grams per Kilogram

HCl.....Hydrochloric Acid

H_2O_2 .....Hydrogen Peroxide

in. .....Inch

kV.....Kilovolt

L .....Liter

lb/bu .....Pounds per Bushel

M.....Molarity

mg .....Milligrams

MJ/m^3.....Megajoule per Cubic Meter

mL.....Milliliter

mm .....Millimeter

mM.....Millimolar

mm Hg .....Millimeters of Mercury

mN.....Millinewton

MPa.....Megapascals

N.....Newton

1N.....Grams per Liter

$NaNO_3$.....Sodium Nitrate

NaOH .....Sodium Hydroxide

nm .....Nanometers

OH.....Hydroxyl Group

% .....Percentage

pH.....Power of Hydrogen

SO_2 .....Sulfur Dioxide

µL.....Microliter

µm .....Micrometer

v/v .....Volume per Volume

w/v.....Weight per Volume

w/w.....Weight per Weight

x.....Magnification Binoculars

1. INTRODUCTION

Corn (*Zea mays* L.) bran (CB) is a byproduct of the corn industry resulting from either dry-milling (DCB) or wet-milling (WCB) operation. In addition, dried distiller's grains with solubles (DDGS), including corn grains, are a coproduct of ethanol production (U.S. Grains Council, 2018). DCB, WCB, and DDGS have a low market value and are considered by some producers as a manufacturing waste, and thus need to be minimized to increase food industry sustainability (Anderson and Simsek, 2019a; Anderson and Simsek, 2019b; Tharanathan, 2003). The reduction of these byproducts and the improvement of profits for corn processing facilities means using arabinoxylan (AX) extracted from the DCB, WCB, or DDGS to create food packaging materials. However, films generated from AX with laccase and sorbitol are not highly hydrophobic materials used for food packaging; therefore, lipase and acetate must be added to increase hydrophobicity of the AX films (Stepan et al., 2013). Once hydrophobicity is improved, AX films can have high mechanical properties and low interactions with water, leading to the development of the biodegradable food packaging materials.

The CB is defined as a pericarp and is divided into the following layers: epidermis, mesocarp, cross cells, and tube cells (García-Lara et al., 2019). CB is approximately 7% of corn kernel mass at maturity and forms the outer and inner layers around the kernel, ranging from 35 to 200 µm (García-Lara et al., 2019). CB is rich in fibers; mainly are cellulose and hemicellulose, accounting for 200 and 500 g kg^{-1}, respectively (Agger et al., 2010; Carvajal-Millan et al., 2007). AX is about 350 g kg^{-1} of the hemicellulose present in the CB (Agger et al., 2010; Saeed et al., 2011). Unlike dry and wet milling operations, the DDGS procedure not only reduces the particle sizes of corn grain, it also comprises the specific ethanol production pathway in the conversion of corn carbohydrates to ethanol. DDGS are mostly utilized for animal feed

that include 30 g kg^{-1} protein, 15 g kg^{-1} cellulose, and 20 g kg^{-1} hemicellulose; half of the hemicellulose is AX (Xiang et al., 2014; Zarrinbakhsh et al., 2013).

AX is a non-starch polysaccharide comprising of xylose backbone residues linked by β-1,4-glycosidic bonds that contain arabinose substituents (Correia et al., 2011; Rumpagaporn et al., 2015; Zhang et al., 2014). The AX structure is that of a β-(1,4) linked xylose backbone at C (O)-2 with 4-O- methyl-D-glucuronic acid and at C (O)-2 and/or C (O)-3 locations of arabinose residues (Agger et al., 2010; Correia et al., 2011; Saeed et al., 2011). Ferulic acid (FA) is esterified to AX, but FA is localized at the C (O)-5 position of arabinose. Although FA is only a small portion of AX, it tends to have important impacts on the molecular weight, solubility, and gelation properties of the AX in CB and after separation (Kale et al., 2010; Kale et al., 2013; Rumpagaporn et al., 2015). AX is extracted from DCB, WCB, or DDGS by a variety of methods including acid-alkali extraction, which is an effective procedure for estimating AX and evaluating AX characteristics (Anderson and Simsek, 2019b; Xiang et al., 2014).

The use of AX as the basis for packaging materials would be one source of hydrophobic films that could be commercialized. However, food packaging must be suitable for the food being stored by matching the mechanical properties and water barrier of the food packaging material requirements. Mechanical properties of AX films consist of tensile strength, tear resistance, and puncture resistance, which must be high to improve the strength of food packaging materials (Briassoulis and Giannoulis, 2018; Ciannamea et al., 2018). The AX film water barriers must be low namely: moisture content, water solubility, and water vapor transmission rate, in order to elongate the packaged food shelf life (Anderson and Simsek, 2019a; Hansen and Plackett, 2008). In contrast to water barrier characteristics, contact angle and biodegradability have to be high in order to increase AX film hydrophobicity (Anderson and

Simsek, 2019a; Antoniou et al., 2014). Finally, although hydrophobic food packaging material can be created through the combinations of AX solution, using laccase, and sorbitol, the resulting material is not highly hydrophobic (Anderson and Simsek, 2019b). Thus, in order to address a high hydrophobicity, the surface of the casting AX film can be modified by immersing it in acetate reagent with lipase.

1.1. Need Statement

Currently, most food is packaged in plastics that are expensive, unhealthy, and unfriendly environment. In addition, many of the non-biodegradable materials (polyolefins, polyesters, and polyamides) used to package food for widespread spread consumption are also expensive and unnatural, with undesirable features. Thus, the enhancement of the crosslinking method is a creative strategy to improve the mechanical and physical characteristics of biopolymer films (Han et al., 2018). The creation in food packaging systems will help market needs and consumer preferences for healthy and safety food products and decrease the loss of the food industries. Green packaging can be developed to diminish the ecological influences of food packaging by using plant extracts and biodegradable materials, such as corn processing byproducts. The AX films made from DCB, WCB, or DDGS samples can lead to a green packaging approach with no negative interaction between components.

1.2. Overall Hypotheses

- Arabinoxylan acid-alkali extracted from the corn bran from dry-milling, corn bran from wet-milling, and dried distiller's grains with solubles will be a basis for food packaging materials, which could be commercialized, that increases the profits for corn processing industries

- The surface of the casting films made from arabinoxylan extracted will become more hydrophobic by suspending the film surfaces in acetate reagent with lipase
- There will be significant correlations between hydrophobicity of the arabinoxylan films and their properties (mechanical characterizations, water barrier features, and biodegradability of arabinoxylan films)

1.3. Overall Objectives

- To extract arabinoxylan and evaluate arabinoxylan characteristics coming from the samples including corn bran from dry-milling, corn bran from wet-milling, and dried distiller's grains with solubles
- To evaluate lipase-acetate application in the surface of arabinoxylan casting films and determine the physical and mechanical properties of the arabinoxylan films
- To produce hydrophobic arabinoxylan film materials and evaluate how the arabinoxylan films interact with water and soil in aerobic biodegradation

2. LITERATURE REVIEW

2.1. Corn

2.1.1. Production and Consumption

Corn (*Zea mays* L.) is one of the three most commonly produced grain crops in the world, including wheat and rice (Delcour and Hoseney, 2010d; García-Lara and Serna-Saldivar, 2019). The United States (US) harvests 38% of the world's total corn production, then China at 18% and Brazil at 8%; thus, the US is considered the top corn producer (García-Lara and Serna-Saldivar, 2019). Corn is grown in the US mid-western region and mainly found in the following eight states: Iowa, Illinois, Missouri, Indiana, Nebraska, Minnesota, Ohio, and Michigan. As a primarily agricultural crop, corn has a significant economic value and global impact on food supply, for both human nutrition and animal feed. Also known as maize, mature corn is referred to as dent maize.

Corn-based products specifically used for human consumption can be made from the entire kernels (popcorn) or large half-kernel parts (corn flakes). Popcorn quality is related to the popped volume, kernel shape, and snack flavor. It is produced by putting non-popped kernels into hot cooking oil. The popcorn kernels then expand outward, exposing the white inner endosperm of the kernel because the moisture inside the kernel rapidly vaporizes and causes the kernel to "pop" (Delcour and Hoseney, 2010b). The popcorn extents to a bigger shape as the corn pericarp operates as a pressure vessel, allowing the water to be high-heated and the endosperm to expand. Corn flakes are the most convenient ready-to-eat cereal. They are manufactured from corn grits by dry milling corn to remove the bran and germ. The large grits maintain their characteristics during the corn flake production process; thus, each grit can produce a single flake. The corn grits are cooked under pressure with a solution including sugar,

malt, and salt and then steamed to approximately 20% moisture content (Delcour and Hoseney, 2010a). After cooking, the grits are dried using hot air at 65 ^{o}C so that they will not be sticky.

2.1.2. Kernel Anatomy

Corn is a four-carbon compound plant with a large rate of photosynthetic activity and provides a high potential for carbohydrate production (Sema-Sldivar, 2010). There are various colors of corn kernels: yellow, white, red, dark brown, blue, or purple; however, by far the most common color is yellow, followed by white. The large, flattened corn is composed of four parts: tip cap, endosperm, germ, and bran (see Figure 2.1 below), accounting for nearly 1%, 82%, 10%, and 7% of the kernel mass at maturity, respectively (García-Lara et al., 2019). The tip cap of the corn kernel does not usually remain attached to the kernel after the processes of cleaning and milling.

Figure 2.1. The four major parts of corn kernel (Macke et al., 2016).

2.1.2.1. Endosperm

The endosperm makes up the majority of the corn kernel. This is where the kernel stores almost all the kernel's starch; it is constituted by the aleurone layer and starchy endosperm. The aleurone layer covers both the endosperm and germ, which are made up of proteins, oil bodies, and some fibers. The cells of the starchy endosperm are comprised of starch granules, protein bodies, and a thin cell wall. The two endosperm storage proteins are prolamins (zeins) and

globulins. The abundant zeins make up the majority of the storage proteins in the endosperm (Larkins, 2019).

Corn starch granules are most often polygonal or spherical (Delcour and Hoseney, 2010c). The outer portion, or hard endosperm, has vitreous and polygonal granules. In contrast, the inner portion, or soft endosperm, includes opaque and spherical granules. The hard, yellow endosperm of the corn kernel is filled with granules made of a polygonal starch attached to a protein matrix. The soft endosperm is comprised of ivory-colored granules, each comprised of a spherical starch covered by a protein matrix (Kumar and Singh, 2019). The dent corn starch is comprised of two polymers: 25% amylose and 75% amylopectin.

2.1.2.2. Germ

Most germ found in cereal crops is made up of two parts: the embryo (embryonic axis) and the scutellum (cotyledon). In corn, because the germ contains the information of hormones for tissues, leaves, stems, and roots, it is most metabolically active during germination. Germination activation initiates synthetic enzymes and develops hormones for growth (García-Lara et al., 2019). The germ is high in lipids, proteins, minerals, and vitamins, accounting for 85% of lipids and 26% of protein of total kernel contents (Larkins, 2019). The germ also includes triacylglycerols (a major component of corn oil) and significant amounts of albumin and globulin proteins. The scutellum is the food storage system in most corn germ cells, and the scutellum cell walls are likely to be hemicellulose. In addition, the scutellum polymer consists of xylose, arabinose, galactose, and glucuronic acid; these make up the xylose backbone of arabinoxylan (AX), which is a dietary fiber (García-Lara et al., 2019).

2.1.2.3. Corn Bran

Corn bran (CB) (see Figure 2.1 above) protects the grain from harsh environments and biotic stress. The CB is referred to as pericarp and is subdivided into the epidermis, mesocarp, cross cells, tube cells, and seed coat. The CB cells are hollow tubes with important channels for high water absorption entering from the tip cap (García-Lara et al., 2019). These cells make up fibrous cells in the corn bran. The CB has a variety of thicknesses around the kernel, ranging from 35 to 200 µm (García-Lara et al., 2019). The thin outer layer of CB is the epidermis, which limits the absorption of water. The next layer of CB is the mesocarp, which is the thickest layer. The last, or inner layer, of CB is made up of cross and tube cells, which play an important role in water distribution inside the kernel; it has thinner walls compared to the outer two layers.

The bran is rich in fibers; mainly cellulose and hemicellulose. Chemically, the content of CB ranges between 100 to 130 g kg^{-1} protein, 90 to 230 g kg^{-1} starch, 20 to 30 g kg^{-1} lipid, 20 g kg^{-1} ash, and 200 g kg^{-1} cellulose (Agger et al., 2010). The hemicellulose is mainly comprised of heteroxylan, which is present in the cell walls and accounts for 500 g kg^{-1} of the CB. AX is the main component of the heteroxylan found in the CB (Agger et al., 2010; Carvajal-Millan et al., 2007). Moreover, while AX is found in the corn bran, endosperm, and germ, the ratio of arabinose to xylose (A/X) in the arabinoxylan present in each part of the corn kernel differs. In other words, the two major fibers of AX found in the corn kernel, bran fiber and endosperm fiber, are characterized by their unique arabinose/xylose ratios.

2.1.3. Corn Processing

Pre-milling operations must address four key areas before milling the corn: a) the corn's physical properties, b) cleaning, c) tempering in dry milling, and d) steeping in wet milling. The first area addresses the optimal physical quality properties of the corn grain: maximum large size

and hard endosperm. In addition, the corn needs to be assessed for broken kernels, odors, and microbes. In other words, corn samples need to meet milling quality specifications, including specific measurements of test weight, kernel size, moisture content, broken kernels, stress cracks, and contamination. Table 2.1 below gives optimal and acceptable qualities of corn grains before milling can begin (Anderson and Almeida, 2019). The second key area to be addressed before milling is cleaning the corn kernels, which uses an aspiration or shaker screens to remove dust, fines, cob portions, coarse particles, shrunken grains, and metal. A destoner can also be used to discard stones, glass, and heavy materials. The aim of the cleaning step is to have a grinding process with clean and sound corn kernels without extraneous materials. The last two operations of tempering (in dry milling) and steeping (in wet milling) will be addressed in the dry milling and wet milling sections below.

Table 2.1. Corn kernel quality properties for milling processing.

Physical properties	Acceptable	Optimal
Test weight, lb/bu	60.72	58.16
Small kernels, %	15.0	10.0
Moisture content, %	16.0	15.5
Broken kernels, %	2.0	2.0
Stress cracks, %	5.0	None
Heat damage (black/carmel) kernels/500 g	3	None
Contamination (wood/metal)	None	None
Odor	None	None

(Anderson and Almeida, 2019).

2.1.3.1. Dry Milling

The corn dry milling process includes tempering, conditioning, heating, drying, grinding, and sifting to produce flour. Tempering consists of hydrating the cleaned corn kernels at 45-47 ^{o}C. Then, the kernel is left for conditioning until the moisture content increases to approximately 20% of the total kernel weight. These processes facilitate the easy separation of the germ from

the corn kernel when using a mill with a degerminator (Anderson and Lamsal, 2011). The tempering also aims to toughen the bran, making it resistant to fragmentation into small particles during germ removal. Moreover, tempering can soften the endosperm, leading to easier grinding. After tempering and conditioning, the corn is then dried in a convection oven at 49 ^{o}C and rinsed multiple times to decrease the corn kernel moisture amount to 14-15% of kernel weight. The heating process deactivates the fat-enzymes, resulting in a stable corn grain shelf life (Anderson and Almeida, 2019). After that, the corn is ground and sifted through multiple sieves by a mill, which rubs the corn particles to remove the germ and bran. Then, the endosperm is usually reduced to particle size < 50 μm, and corn flour can be transported and stored. Figure 2.2 below summarizes the processing steps for dry and wet corn milling, including dried distiller's grains.

2.1.3.2. Wet Milling

Corn wet milling aims to release different components than dry milling, which focuses on separating the main physical parts of the corn kernel: germ, bran, and endosperm. In contrast, the corn wet milling process separates the corn grain into the kernel's main chemical compositions of starch, protein, fiber, and oil. Cleaned corn kernels are first steeped in water containing 0.2% SO_2 (sulfur dioxide). This soaking process helps to swell each corn kernel, which helps to loosen the bran, so it comes easily off the kernel. This process also decreases the strain on grinding machines; hard kernels can affect both milling process and, ultimately, the final product. Having soft kernels increases the separation of germ, bran, and protein. Furthermore, SO_2 is used to stop the growth of contaminated organisms and to disrupt the disulfate bonds between proteins (Zhang et al., 2021). The breakdown of disulfate linkages can reduce the strength of endosperm and solubilize the protein matrix, leading to better starch separation.

Wet milling corn begins with steeping the kernels in a sulfurous acid solution consisting of 0.2% SO_2 and 0.5% lactic acid at 48-52 ^oC for 30-40 h (Rausch et al., 2019). After steeping, the kernels are dried and milled in a two-step operation: first the coarse ground at 900 rpm (germ separation) and fine ground (bran separation) via a mill. The purpose of the coarse action is to gently break the grains into large pieces so that the germ can be easily removed using liquid cyclones. The second step of this two-step operation is to screen the de-germed corn particles to 50 μm and ground into two categories: fiber-bran and fine slurry. The fiber-bran is made up of larger pieces than the very fine slurry, which is made up of starch and gluten. The fiber-bran is then separated from the fine slurry's starch and gluten by a metal sieve. The gluten is centrifuged at 6000 rpm for 20 min. The resulting fine starch flour is washed using deionized water and dried for 24 h at 50 ^oC. One final note: wet milling needs specialized machinery and expertise. Furthermore, it also requires large quantities of corn grain to make it feasible. Thus, wet milling is a technique usually employed by industry or in large scale manufacturing.

2.1.3.3. Dried Distiller's Grains with Solubles

Dried distiller's grains with solubles (DDGS) are those grains, including corn, that have a dense nutrient coproduct from dry-milled ethanol production (U.S. Grains Council, 2018). Unlike dry and wet milling, the DDGS process does not only reduce the grain particle size, it also includes the specific ethanol production pathway. Furthermore, ethanol production technologies have recently improved the conversion of starch to alcohol. DDGS production from maize grains includes the following steps: grinding, slurrification, liquefaction, fermentation, distillation, centrifuge, coarse solids, and drying (Figure 2.2). Using a hammermill, the corn is first milled (ground) to reduce the particle size from 3 to 5 mm. The second step, slurrification, is when the ground corn is conditioned by adding water and recycled stillage for leaching protein,

sugars, and some lipids (Chen et al., 1999). The slurrification mixture is pre-cooked at 40-60 ºC and is cooked at 90-165 ºC for 20 min. This cooking breaks down starch into glucose (starch gelatinization), and then amylolytic enzymes are added at 60-80 °C for 30 min in the liquefaction step (Lin and Tanaka, 2006).

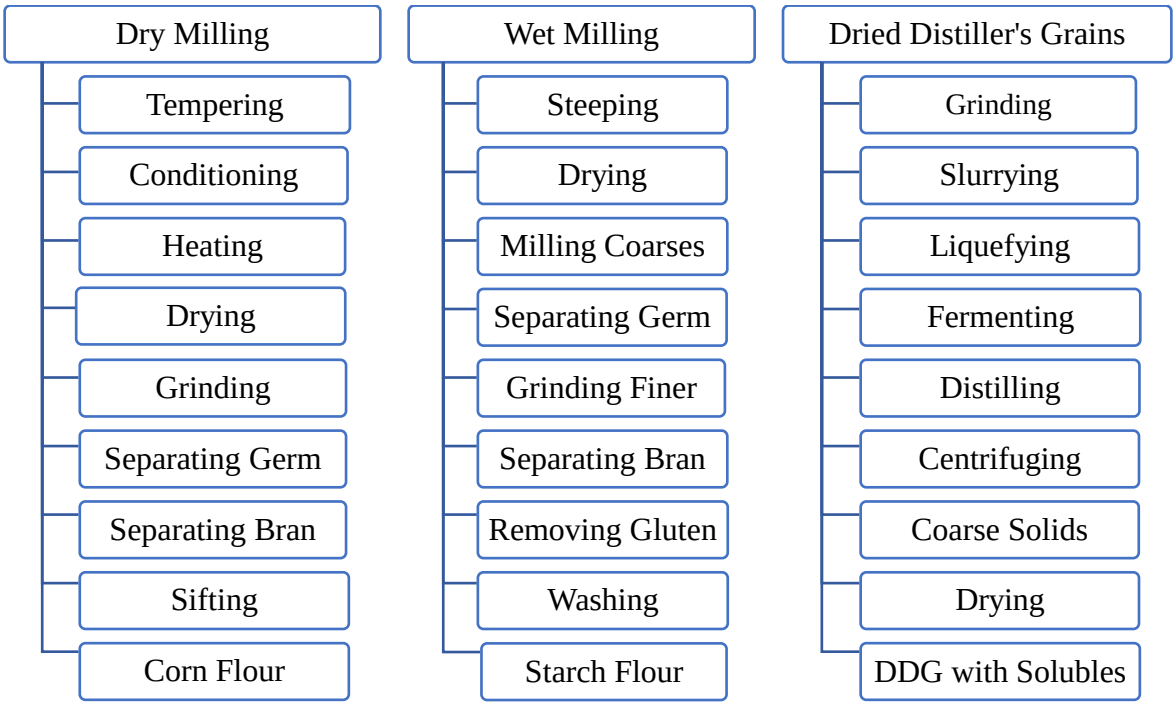

Figure 2.2. Dry and wet corn milling and dried distiller's grains processing adapted from (Zhang et al., 2021; U.S. Grains Council, 2018).

Next, the solution is fermented by the yeast, *Saccharomyces cerevisiae*, which converts sugars to ethanol (Pretorius, 2000). The main factor in efficient ethanol production is the yeast activity that depends on the optimum temperature for fermentation process at 32 ºC for 10-12 h (Torija et al., 2003). Ethanol is then collected by distillation columns and then purified using a molecular sieve system to produce pure ethanol. After distillation, the remaining fluids and solids is called whole stillage (containing water, fibers, sugars, protein, and oil), which is centrifuged to remove coarse solids from liquid (U.S. Grains Council, 2018). The liquid is called thin stillage and is required to have another centrifugation step to extract oil. The coarse solids

are called wet cake and are subjected to a drying process to produce dried distiller's grains with solubles, or DDGS.

2.1.3.4. Bran as a By-product and a Co-product

One of the major byproducts of both dry- and wet-milling operations is corn bran (CB), which has a low commercial value. However, the bran includes beneficial constituents for the creation of food additives containing protein, ferulic acid, and dietary fibers (Rose et al., 2010a). CB's chemical composition is mainly made up of dietary fibers, with a higher proportion of insoluble fiber and a much smaller proportion of soluble fiber (Dikeman et al., 2006). One of the main dietary fibers in CB is arabinoxylan (AX), with smaller quantities of the dietary fiber cellulose. In addition to CB as a byproduct of both dry- and wet-milling, DDGS are also coproduct of ethanol production. In fact, DDGS are the most important coproduct from ethanol production in both amount and value (U.S. Grains Council, 2018). DDGS are regularly utilized as an animal feed due to their high sugar and protein contents.

2.1.4. Milled Products and Proximate Compounds

Corn flour can significantly vary in its proximate compositions based on the kinds of processing (dry vs. wet millings) or the kernel type (hard vs. soft endosperm). The corn flour can also be differentiated via its attribute combinations. For example, two corn flours, both made from full-fat and de-germed millings, can have various ranges of oil, fiber, ash, and starch contents. More specifically, a milled product from the tempering-degermed process contains a lower-fat, higher-starch, and longer shelf life than the flour of full-fat milling process (Table 2.2). Furthermore, flour originating from the hard or vitreous endosperm portion provides the lowest fat and highest protein, whereas flour derived from the soft floury endosperm fraction has

a higher fat and lower protein content (Anderson and Lamsal, 2011). Moreover, corn flour particle sizes can affect the milled product's chemical compound quantity and characteristics.

Corn bran flour is made from corn bran (CB). CB, as discussed above, is made from the remainder of the kernel after the mechanical removal of the tip cap, germ, and endosperm. The outcome is CB, which is low in moisture, oil, protein, and starch but high in total dietary fibers. Table 2.2 shows the chemical composition of corn bran flour made from the dry milling process.

Table 2.2. Compositions of milled products from full-fat, de-germed millings, and bran.

Component	Corn flour (% As is)		
	Full-fat	Tempering-degermed	Bran
Moisture	13.0	12.0	9.6
Protein	7.8	7.0	3.8
Fat	3.5	0.7	2.4
Ash	1.3	0.4	1.1
Carbohydrate	72.9	79.4	13.0
Total fiber	1.5	0.5	70.1

(Anderson and Almeida, 2019).

These flour chemical composition parameters are determined using the official methods from the American Association of Cereal Chemists (AACC) International, the American Oil Chemists Society (AOCS), and the Association of Official Agricultural Chemists (AOAC). The flour particle sizes are determined in accordance with AACC International Method 55-60.01: Guideline for Determination of Particle Size Distribution (AACC International, 2011). The moisture content of flour is determined by following the AACC International Method 44-15.02: Moisture Air-Oven Method (AACC International, 1999d). The flour's ash content is estimated in following the AACC International Method 08-01.01: Ash-Basic Method (AACC International, 1999c). The protein content of flour is analyzed by estimating nitrogen content with the AACC International Method 46-30.01: Crude Protein-Combustion Method (AACC International,

1999b). The flour's lipid content is determined according to the AOCS Method Ba 3-38: Oil (AOCS, 2009). The starch content of the flour is analyzed by following the AACC International Method 76-13.01: a Megazyme Enzyme Assay Kit K-TSTA (AACC International, 1999a). The total dietary fibers of flour sample can be determined using IDF/SDF Method (AOAC, 2022.01) by the ANKOM Dietary Fiber Analyzer.

2.2. Arabinoxylan

Arabinoxylans are complex molecules consisting of a linear xylose backbone of D-xylanopyranosyl residues attached by β-1,4-glycosidic bonds that include arabinose substituents (Correia et al., 2011; Zhang et al., 2014). Arabinoxylan (AX) is a non-cellulose polysaccharide mainly localized in the cell walls and the aleurone layer surrounding the endosperm of many grains. AX is in the major group of hemicellulose presented in grains and is a heterogeneously branched polymer containing various side branches (Rumpagaporn et al., 2015; Zhang et al., 2014). The structural complexity of AX can vary based on the type of cereal crops: for example, rice AX has single sugar arabinose branches, whereas corn AX contains numerous branches (Rose et al., 2010b; Rumpagaporn et al., 2015). Furthermore, AX in corn is created by a combination of the monosaccharides: arabinose, xylose, galactose, and glucose; however, AX does not carry glucuronic acid units in other cereals such as wheat, barley, rye, and oats (Izydorczyk et al., 2005).

2.2.1. Arabinoxylan in Corn

In corn, AX is mainly made up of two monosaccharide sugars: xylose and arabinose, although glucose or galactose may also be found in the corn fiber (García-Lara et al., 2019; Rose et al., 2010b; Zhang et al., 2014). The two main monosaccharides, xylose and arabinose, can be localized as aldose or ketose in the L- or D-configuration, whereas D-xylose and L-arabinose are

the common forms of these two sugars in the AX. The structure of AX in corn is that of a β-(1,4) linked D-xylopyranose (Xyl*p*) backbone at C (O)-2 with 4-O- methyl-D-glucuronic acid and at C (O)-2 and/or C (O)-3 positions of α-L-arabinofuranose (Ara*f*) residues, as shown in Figure 2.3 below (Agger et al., 2010; Correia et al., 2011). Ara*f* and Xyl*p* create furanose and pyranose rings in solution. Additionally, the pyranose ring structure is more frequent because of a decrease in steric interactions between the hydroxyl groups, which leads to a more stable configuration. The ratio linkages of arabinose and xylose differ based on cereal grain type, where AX is localized within the grain, and the procedure use to extract the AX.

Hydro-cinnamic acids (p-coumaric and ferulic acids) and acetic acid are often attached to the xylose backbone, and are probably removable during AX extraction (Saeed et al., 2011). The acetic acid substance can be directly ester-linked to the Xyl*p* backbone in the C (O)-2 or C (O)-3 position, while ferulic acid (FA) is esterified to AX, but FA is localized at the C (O)-5 position of Ara*f* (Figure 2.3).

2.2.1.1. Phenolics

Most of the phenolic acid in CB is ferulic acid (FA); in addition, it plays a significant role in the cross-linkages between polysaccharides containing AX and AX, AX and protein, and AX and lignin (Acosta-Estrada et al., 2019). Moreover, although FA is proportionally the highest phenolic acid in AX, overall it is only a small portion of the AX; nevertheless, FA tends to have significant effects on the molecular weight, solubility, viscosity, and gelation properties of the AX in CB (Kale et al., 2013).

Although FA is found in small amounts, FA has an important impact on the functionality of AX in the cell wall and after separation (Kale et al., 2010; Rumpagaporn et al., 2015). The FA contains crosslink AX molecules: dimers and trimers, which are commonly presented in the wall.

Because of this, AX is subdivided into water extractable (WEAX) and water unextractable (WUAX) fractions. WUAX is naturally crosslinked within the cell wall. However, WEAX is not crosslinked because its ester bonds can be broken with an alkaline treatment, leading it to be soluble in water and resulting in a viscous solution (Rumpagaporn et al., 2015). Thus, in turn, WEAX can also exhibit strong gelatinous characteristics because of the oxidative crosslinking of FA moieties (Kale et al., 2013). Finally, AX's structural features affect how its various properties and characteristics are influenced by processing conditions, and thus affect the quality of the end-use product; more specifically, AX's various properties and characteristics as influenced by processing conditions can affect how AX from corn in the human diet affects human health.

Figure 2.3. The structure of corn bran arabinoxylan adapted from (Correia et al., 2011).

2.2.2. Extraction

AX is extracted from cereal grains from the various anatomical portions of the kernels using several techniques: water extraction, acidic treatment, enzymatic treatment, alkaline treatment, or a combination of these techniques.

2.2.2.1. Water Extraction

Water extraction of AX is the simplest method and least aggressive procedure, preserving most of the AX original structure (Zannini et al., 2022). Using water extraction alone to measure AX has some benefits: water is safe, available, edible, and inexpensive (Fadel et al., 2018). Wheat or corn flour is constantly stirred into water at room temperature for 90 min. During

mixing, AX is solubilized in the water with a 1:10 w/v mixture. Then, starch is removed using the amyloglucosidase enzyme (Zhang et al., 2014). After that, 65% ethanol can be utilized to purify and precipitate the AX, which is then dried and measured.

A modification of water extraction was recently performed by adding more steps and using centrifugation (Zannini et al., 2022). Similarly, wheat or corn flour is stirred within a 1:10 w/v mixture for 90 min at 25 ^{o}C to solubilize AX (Zannini et al., 2022; Zhang et al., 2014) Next, the solution is precipitated using an organic solvent to deactivate endogenous enzymes, which removes any free sugars. Then, the sample is centrifuged to release insoluble and soluble polymers (Zannini et al., 2022). The insoluble polymers are dried and the pure concentrated AX measured. The water-soluble (supernatant) polymer in AX is filtered with celite to remove proteins. The starch is removed using amyloglucosidase. After the removal of these solubles, the supernatant can be lyophilized and stored as pellets that are high in AX.

The disadvantage of water treatment, including its modification, is that water with conditions below 100 ^{o}C cannot break the cross-links in AX, making it lower water extractability than WEAX estimated by other methods (Izydorczyk and Biliaderis, 2007). In order to improve the solubility of AX, mechanical treatments can be used before water extraction. These mechanical treatments include steam, microwave, ultrasound, or milling, all of which can increase the extractability of AX.

2.2.2.2. Acid Extraction

Using only an acid solution is not always the best option for AX extraction from cereals because it is neither as simple nor as convenient as water extraction. A few of the reagents used to extract the AX from wheat include acetic acid, formic acid, or other chemicals that include acids, methanol or ethanol (Xu et al., 2006). For example, Xu et al. (2006) in a study comparing

acid extraction used formic acid, acetic acid, methanol, and ethanol in three treatments. In the first treatment, they used formic acid, acetic acid, and water (30/60/10, v/v/v) to extract AX from 10.0 g of dewaxed wheat flour. In the second treatment, they used methanol and water (60/40, v/v) for 10.0 g of dewaxed wheat flour. The third treatment consisted of ethanol and water (60/40, v/v) with 0.1% HCl for 10.0 g of dewaxed wheat flour. Then, all three treatments were filtered through a nylon cloth and precipitated in 95% ethanol at 22 ^{o}C for 12 h. Then, the resulting pellets high in AX were washed using 70% ethanol and dried in hot air.

The best acid combination for high extraction of AX resulted from the formic acid, acetic acid, and water treatment, which released 296 g kg^{-1} AX on a dry weight basis (Xu et al., 2006). Although this was the best result from among the three acid extractions tested, it was still considered a minimal AX extraction as compared to alkaline or enzymatic extractions because of the extreme acid hydrolysis in cleaving the Xyl*p* from the AX backbone. In addition, methanol and ethanol treatments were used to extract AX instead of organic acids, which resulted in lower yields of 55 and 86 g kg^{-1}, respectively (Xu et al., 2006). Thus, a small portion of AX was extracted and degraded on low molecular weight due to excessive acid hydrolysis.

2.2.2.3. Enzymatic Extraction

The enzymatic method is also used to extract AX from cereals. Xylanase and endo-xylanase are utilized to collapse the glycosidic bond between β-(1,4) linked D-xylopyranose units, indicating to oligosaccharides with different molecular weights (Escarnot et al., 2011; Walker et al., 2017). Endo-xylanase can break down the Xyl*p* backbone and can cleave β-(1,4) linkages, making WUXA soluble and extractable (Li et al., 2013). Xylanase can act partially in extracting and solubilizing WUAX and the depolymerization of WEAX portion. Xylanase is mainly classified as either glycosidic hydrolyses (GH) 10 or (GH) 11. GH10 xylanase hydrolyzes

closely to side chain substitutions, while GH11 xylanase cleaves at sites far away from chain substitutions (Maslen et al., 2007). Thus, AX is more effectively solubilized from de-starched wheat bran GH11 xylanase than GH10 xylanase (Escarnot et al., 2011). GH11 is more selective for insoluble species and has a greater ability to penetrate the grain cell wall.

The alkaline extraction shows a higher extraction of AX than the enzymatic method. For instance, AX extraction from de-starched wheat bran using xylanase was 124 g kg^{-1}, which is lower than 343 g kg^{-1} with alkaline sodium hydroxide (Fadel et al., 2018; Zhou et al., 2010). The low extraction of AX from the enzymatic procedure is probably because of the existence of enzyme inhibitors, leading to the limitation of hydrolysis (Zhang et al., 2014). However, AX extracted using enzymes has a greater FA content than AX extracted with alkaline. Alkaline solutions could produce FA and collapse the ester bond between AX and FA side chain, causing a loss of antioxidant functionality (Zhou et al., 2010). Thus, combining enzymatic and alkaline AX extractions is a more standard approach and provides better outcomes.

Enzymatic extraction of AX from corn bran was applied by Rose et al. (2010b), and its modification was done by Zhang et al. (2016). In the Zhang et al. (2016) procedure, hexane was used to de-fat the CB flour, with a CB flour to hexane ratio 1:7 w/v. Then, 300.0 g of the defatted flour was blended in 2000 g of distilled water 1.5:10 w/w for 45 s, and the pH of the flour-water solution was adjusted to 7 before incubating enzymes. Next, 780 μL of α-amylase was added, and the solution was stirred in a 90 ^{o}C water bath for 1 h to hydrolyze starch. The solution was boiled for 15 min to inactivate the enzyme and centrifuged with 4000 rpm for 20 min. Residue was dried overnight at 45 ^{o}C and de-starched samples were placed in distilled water at 1:10 w/w including 8% NaOH concentration. The sample was again stirred for 1 h in the boiling water bath, and then centrifuged (4000 rpm, 20 min) for the last time (Zhang et al., 2016). To remove

protein, Zhang et al. (2016) then mixed 600 mL of the supernatant with 400 µL of proteinase at 60 ^{o}C and then stirred in a water bath for 1 h. The solution was then boiled for 15 min to inactivate the enzyme and centrifuged with 4000 rpm for 20 min. The de-starched and de-proteinized sample was blended with 1.4 L of 70% ethanol followed by storage at 4 ^{o}C overnight, and then centrifuged with 4000 rpm for 20 min. The precipitate was washed twice using 20 mL of ethanol. After washing, the precipitate was desiccated in an oven at 45 ^{o}C to get the alkaline extracted AX (AEAX) sample (Zhang et al., 2016).

Finally, these researchers added 3.0 g of the AEAX to 72 g of distilled water, and then xylanase was used to modify the AEAX sample with an incubation time of 24 h. Then, 0.03 g of xylanase enzyme was added and mixed well in the AEAX-water mixture. The enzyme condition was set as pH 4.0 at 50 ^{o}C for 24 h incubation, followed by boiling the mixture in the water bath for 15 min to inactivate the enzyme. The moisture content of AEAX sample was removed by rotary vacuum evaporation and desiccated at 45 ^{o}C in an oven overnight (Zhang et al., 2016).

2.2.2.4. Alkaline Extraction

Alkaline extraction disrupts covalent and hydrogen bonds in the carbohydrate matrix to liberate the polysaccharides hemicellulose and cellulose, among others, from the cell wall; in turn this also releases other polysaccharides (Li et al., 2017). During alkaline extraction, hydroxyl ions interrupt the hydrogen bonds between hemicellulose and cellulose, hydrolyzing the ester linkages. This hydrolysis of the ester linkages solubilizes the WUAX in the AX (Cyran et al., 2004). Then, alkaline conditions assist the uronic acids to change their negative charges, causing repulsion among the molecules that make up the polysaccharides, resulting in improving the extractability of AX (Hollmann and Lindhauer, 2005). Thus, this extraction procedure affects

the structure of AX because it disrupts the cross-linkages and allows for access to the WUAX molecular structures, in order to investigate their functional characteristics.

Furthermore, the efficacy of the alkaline extraction procedure has been improved over time. For example, Yadav et al. (2008) reported that under alkaline conditions the small modification of a diluted alkaline solution of hydrogen peroxide (H_2O_2) can extract a high amount of AX in corn bran. In this study, a sequential alkaline extraction with alkaline hydrogen peroxide was used to extract hemicellulose, including nearly 840 g kg^{-1} of AX (Yadav et al., 2008). Interestingly, the addition of H_2O_2 improved the AX extraction by 8.8% compared to extraction without H_2O_2. Alkaline extraction with hydrogen peroxide is an effective method for estimating AX in corn bran developed by adding water to wash the alkaline (Yadav et al., 2008).

In addition, the alkaline extraction of corn bran AX was improved by Kala et al. (2013); then the alkaline extraction procedure was slightly modified for even better yields by Zhang et al. (2019). Zhang et al. (2019) defatted CB flour using a flour to hexane ratio of 1:10 w/v. Next, the defatted CB flour was de-starched for 1 h using the α-amylase and hydrolyzed starch. Proteinase was then added to the defatted and de-starched CB flour and incubated for 4 h at 50 ^{o}C to remove protein. This process results in a higher percentage of AX in the CB flour sample. Then, 50.0 g of this CB flour with a higher percentage of AX was added to 500 mL sodium hydroxide (NaOH) solution and stirred at 50 ^{o}C. After stirring, the solution was centrifuged at 10,000 rpm for 10 min. The pH of the supernatant resulting from the centrifuging was then adjusted to 4 using concentrated hydrochloric acid (HCl). This mixture was again centrifuged and the resulting AX solid was washed using ethanol, and then dried using a hot air oven at 50 ^{o}C then freeze-dried (Zhang et al., 2019). As first pointed out in the enzymatic extraction portion of this section, alkaline extraction results in higher yields of AX when compared to the enzymatic extraction.

2.2.3. Characteristics

2.2.3.1. Molecular Weight

In addition to AX's structure, the corn AX properties of solubility and gelling are closely associated with molecular weight (M_W) and concentration; in turn, these properties affect AX's functionality and nutraceutical attributes (Acosta-Estrada et al., 2019). AX molecular weight is determined by the concentration and extraction of AX. A high concentration of AX can lead to higher M_W compared with AX's low content. More extraction yields of AX can result in a greater AX M_W, leading to a higher AX viscosity. For example, a study showed that corn bran hemicellulose had 323700 Da, whereas hemicellulose hydrolysate contained 190800 Da (Kale et al., 2010).

The determination of AX M_W is impacted by the techniques used to extract AX. For example, AX M_W from alkaline extraction accounted for 300000 to 500000 Da, as determined by HPSEC-MALS-RI (Kala et al., 2013); this level of determination for the M_W would not have been possible using an enzymatic extraction. Furthermore, the concentration and type of enzymes used can affect the determination of AX M_W. For instance, the enzyme concentration during extraction led to a decrease in M_W, allowing the AX to form a clear solution and high AX solubility (Kala et al., 2018). Moreover, Zhang et al. 2015 reported that using endo-xylanases to extract WUAX increased the solubility of the fiber because of the Xyl*p* backbone degradation, resulting in a reduction in M_W of extracted AX.

2.2.3.2. Solubility

The solubility of AX is influenced by two main factors: chemical structure and M_W. AX is considerably branched (see Figure 2.3 for an illustration of AX's branched structure) since the dissolved AX flexibility is improved when highly substituted (Liu et al., 2020). Furthermore, low

substitutions of AX lead to a low A/X ratio, which results in low AX solubility. For example, AX treated with arabinofuranosidase had an A/X ratio of 0.43 and was approximately 70% water soluble, whereas the unmodified AX contained an A/X ratio of 0.50 and the percentage of solubility accounted for 95-98% (Zannini et al., 2022). This kind of corn bran AX structure tends to be more crystalline, resulting in a decrease AX solubility. An increase in crystallinity results in less arabinose interacting with water, which reduces the accessible surface area of AX to act with water (Anderson and Simsek, 2019a). In addition, this kind of corn bran AX structure can also present as a soluble polymer because arabinose residues are linked to the xylose chain. In this situation, the A/X ratio is reduced by enzyme action, and arabinose units are removed from disubstituted xylose, leading to high AX solubility (Zannini et al., 2022). This means AX with high arabinose substitutions can increase AX solubility in water.

Although the branching structure of AX affects its solubility, M_W also impacts AX solubility. In corn bran, AX has a higher proportion of the WUAX molecule than the WEAX molecule (Zhang et al., 2016). Thus, increasing the WUAX component has been a very significant area of study for researchers who are interested in transforming WUAX to WEAX. This interaction between the WUAX and the WEAX components AX in corn bran is key because of the cross-linking degrees with other components of the corn cell wall (Beaugrand et al., 2004; Saulnier et al., 2007). These interactions between WUAX and WEAX are through linkages of covalent ester bonds between either the carboxylic acid group of uronic acids and AX hydroxyl groups or di-ferulic acid links between two AX chains. The links between AX molecules and bonds of both hydrogen and covalent in the cell wall are collapsed when WEAX is treated with alkaline. This is because AX has a large M_W and high FA acid content. One of the characteristics of AX is that as the M_W of AX increases, the solubility of the fiber decreases.

2.2.3.3. Gelling

As AX forms a viscous solution, AX creates a strong gel via an oxidizing agent. AX gels have a neutral taste and high-water absorption capacity. These gels are also stable and strong under these conditions. In the AX structure, the covalent bonds facilitate interactions between AX and other components, like FA, which contribute to AX's strong gelling capacity (Vansteenkiste et al., 2004).

AX gel strength relies on multiple factors, including: high ferulic acid (HFA) and low ferulic acid (LFA) levels; alkaline concentration; treatment time; testing temperature; pH during testing; and laccase amount. AX gelation diminishes with increasing both treatment time and NaOH concentration. This is because FA content reduces when the NaOH amount and treatment time increase. Thus, FA content is related to the gel structural characteristics of density of crosslinking and pore size (Kala et al., 2013; Zhang et al., 2019). In contrast, a low alkaline concentration and HFA content resulted in a strong AX gel strength (Ayala-Soto et al., 2016). The extraction yield of AX was increased at a higher temperature, indicating a strong gel formation (Ayala-Soto et al., 2016).

Furthermore, adding laccase to the crosslinked network of FA leads to a better AX gel formation (Ayala-Soto et al., 2016; Kala et al., 2013). Laccase is a glycoprotein found everywhere in nature that has been reported in greater plants and almost every fungus. The physiological function of laccase is to catalyze polymerization or depolymerization processes (Riva, 2006). For example, the same amount of laccase was added to corn arabinoxylan (CAX) high in FA and CAX that was low in FA (Kala et al., 2013). The CAX that was high in FA led to crosslinking and increased gelation while the CAX that was low in FA had lower crosslinking and decreased gelation (Kala et al., 2013). Hence, CAX gels were made at a higher ester-linked

FA, which contained higher amount of FA and formed stronger gels along with increased values of viscosity (Ayala-Soto et al., 2016).

Two studies previously reported that the extracted AX with low NaOH concentration had formed gels at pH=5 when treated by a laccase (Carvajal-Millan et al., 2005; Kala et al., 2013). In contrast, no gels were formed at pH=5 for soluble crosslinked CAX with either LFA or HFA because of low A/X ratio used 0.22 (Zhang et al., 2019). Both weak and strong gels were generated by pH=2 of soluble crosslinked CAX solutions with less and more FA, whereas no gels were generated at the low pH for the crosslinked CAX (Figure 2.4). This crosslinked CAX by laccase formed low pH gels were caused by hydrogen bond and hydrophobic interactions.

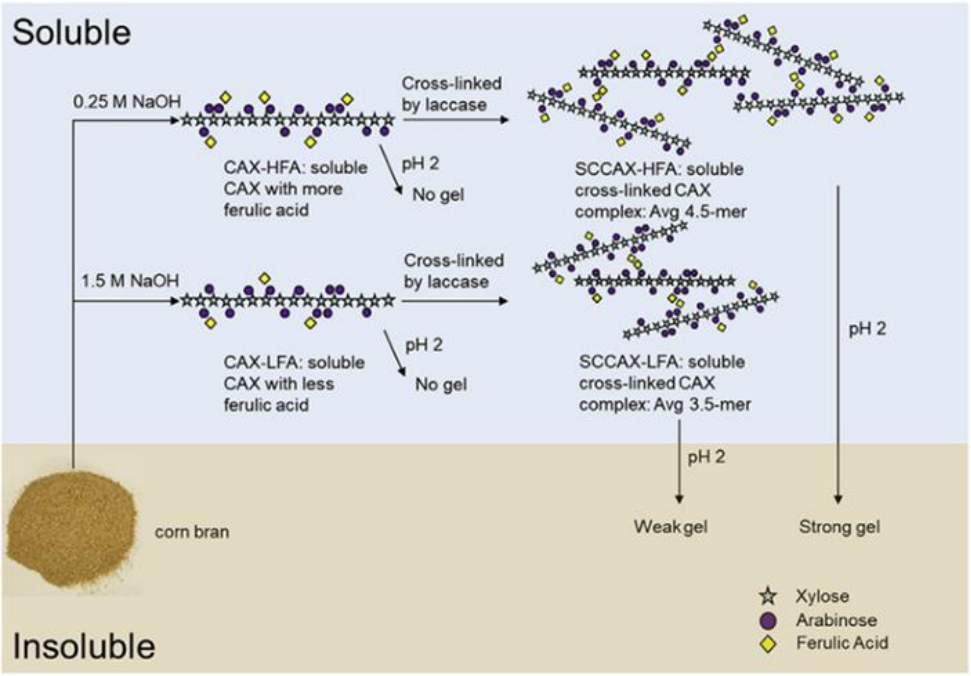

Figure 2.4. Gel formation of corn bran arabinoxylan using a laccase (Zhang et al., 2019).

2.2.4. Dietary Fiber

Arabinoxylan is one of many dietary fibers (DF), including β-glucan, oligofructose, and inulin. Naturally present in food, DF refers to carbohydrate compounds formed from multiple monomeric units that are not hydrolyzed by the endogenous enzymes of the human small

intestine (McCleary et al., 2011). DF can be also defined as non-starch polysaccharides obtained from raw food by physical, enzymatic, or chemical procedures; DF has known physiological health benefits (McCleary et al., 2011).

Based on extraction, AX has been well-studied with respect to the physicochemical properties of the fiber, which affect the quality of the kernel. Compared to alkaline extracted AX, AX extracted by enzymes has a lower average M_W, fewer arabinose substitutions, and a higher FA content. Furthermore, an increase in FA provides a better immunomodulatory effect because FA is a polyphenol, which contains antioxidant activity related to improved immune cell functions (Alvarez et al., 2006; Mpofu et al., 2006; Zhou et al., 2010). DF, such as wheat AX, corn AX, and oat β-glucan, is becoming increasingly popular in the food application and feed industry as the interest in healthy diets also increases (Mendis and Simsek, 2014).

2.2.5. Human Health

Increasing daily DF consumption has been shown to reduce the risk of diabetes, cancer, and cardiovascular-related issues. Furthermore, growing evidence has demonstrated that DF consumption patterns are associated with improved mental health (Kim et al., 2020; Xu et al., 2018). In addition, research has shown the relevance of AX solubility for human health due to its prebiotic nature; for example, it can control diabetes mellitus, common cardiovascular disorders, and improve colon function (Rose et al., 2010b; Saeed et al., 2011). Active AX acts as a prebiotic, which leads to improved immunomodulatory function, lower cholesterol levels, regulated blood glucose levels, and enhanced mineral absorption.

Thus, it is very important that to gain a better understanding of AX mechanisms and their relationships to human health. For example, a positive prebiotic effect in human health has been reported for AX. These health advantages are believed to be linked to the fermented microbes of

DF in the large intestine (Wang et al., 2010). The intestinal microbes, *Lactobacillus* and *Bifidobacteria*, are beneficial species, which generate short chain fatty acids through the fermentation of DF (Wang et al., 2010). These short chain fatty acids cause an acidity in the intestine (low pH), which positively withers the bacteria of potential pathogens. Furthermore, *Lactobacillus* and *Bifidobacteria* are significantly related to immunostimulation, postprandial blood glucose responses, and a cholesterol lowering effect (Wang et al., 2010). Thus, AX reduces gut infections and colon cancer by increasing the levels of intestinal short chain fatty acids (Rose et al., 2010b). Moreover, research has shown that CAX was more efficient in the conversion to short chain fatty acids by the intestinal microbes as compared to both wheat AX and rice AX (Rose et al., 2010a).

2.3. Food Packaging

2.3.1. Functions of Packaging

Packaging includes preparing foods for storage, distribution, transport, retail, and the end-use. Food packaging ensures safe delivery to the final customer at optimum condition and minimum cost (Lindh et al., 2016; Robertson, 2013a). There are four important functions of food packaging that have been recognized: containment, protection, convenience, and communication (Robertson, 2013a). These primary functions are interrelated and need to be evaluated and considered in the development of packaging materials. The containment of food protects the environment from the many products, which are transported from one place to another. Consequently, loss of product and pollution can be widespread if containment is not considered in the packaging industries (Chávez-Dulanto et al., 2021; Robertson, 2013a).

Protection is regarded as the primary function to protect food contents from outside ecological effects such as water vapor, gases, odors, dust, microorganisms, light, and physical

damage to expand food shelf-life and decrease the food losses (Arrieta et al., 2015; Narayanan et al., 2017). Product packaging is designed to increase the convenience that foods are pre-prepared and can be ready to consume or re-heated in a short time. Therefore, the packaging would meet the demands of consumers for convenience, promoting sales. Communication on the packaging is equally significant and serves as a silent salesman (Judd et al., 1989). For instance, nutritional labels on the outside of food packages have become mandatory in many countries.

2.3.2. Biodegradable Materials

The packaging market has provided a nearly 50% of the packaging that is utilized in food service (Kochańska et al., 2021). Food packaging material is generated through non-biodegradable plastics: polyolefins, polyesters, polycarbonates, and polyamides, which are created from unprocessed plant material (Kochańska et al., 2021; Robertson, 2013b). The use of these products has been increased because of their barrier properties such as, low oxygen and water vapor transmission rates. In contrast, it can be valuable to make biodegradable packaging materials from the corn processing byproducts, such as CB and DDGS (Anderson and Simsek, 2019b). The U.S. is the top corn producer in the world, and Indiana is considered the fifth top producer of corn in this country (García-Lara and Serna-Saldivar, 2019).

There are some reasons why the demand for high-quality biodegradable packaging is growing in several countries. First, new substances of packaging could be developed using corn processing byproducts, leading to improving the worth of corn and then increasing farmers' income. Second, plastic packaging for food supply or services is usually one-time use that results in a large amount of non-biodegradable material waste being sent to garbage, resulting in ecological problems (Lindh et al., 2016). Third, biodegradable material can be consumed with the packaged product, leaving no residual packaging for food packaged. Fourth, biodegradable

material can improve or maintain the organoleptic properties of foods inside packaging such as flavorings, colorings, and sweeteners that are incorporated into the packaged foods (Robertson, 2013c). During the COVID-19 pandemic, utilization of single-use plastic packaging in the hotel, restaurant, and catering sector for take-out increased due to ensuring lockdowns (Kochańska et al., 2021). Using CB and DDGS to modernize biodegradable packaging material could globally and locally increase the need for corns.

2.3.3. Plasticizers

Plasticizers refer to a substance added to change the thermal and mechanical properties of material (Robertson, 2013e). These changes provide the polymer pliability, flexibility, possible extension at room temperature, and tensile strength, reducing brittleness. There are four main requirements for a plasticizer to be added: solvent power, efficiency, permanence, and agreement condition (Antoniou et al., 2014; Robertson, 2013b). Plasticizers have to be powerful solvents to penetrate the crystalline regions of polymers. When plasticizers are non-solvent, they will only enter the amorphous regions due to acting as a softener.

Efficiency of plasticizer-polymer relationship can be defined by the balance between the amount of plasticizer used and polymer needed to create a desirable polymer (Antoniou et al., 2014). This can be evaluated through plasticizer size, molecular weight, and diffusion rate into the amorphous and crystalline regions of polymer. For example, smaller particles of a molecule will diffuse more rapidly than larger particles, making them more effective. Another requirement is that volatility of the plasticizer is the major factor in permanence. A bigger molecule leads to lower volatility and longer permanence. The diffusion of plasticizer into the polymer can influence the permanence, so that higher diffusion rate results in greater permanence. Lastly, the

optimal temperature range has to match between the plasticizer and polymer. This confirms that the use of temperature ranges will ideally process all chemical species involved.

2.3.3.1. Sorbitol and Glycerol

A film created from AX is usually brittle, causing the film to split when handled so that the film has low extensibility and flexibility. Plasticizers are used to reduce brittleness and to increase AX film's flexibility (Antoniou et al., 2014; Robertson, 2013b). Sorbitol and glycerol are polyols, a type of plasticizer, but sorbitol is used more than glycerol. Sorbitol has a high solubility in crystalline forms and utilizes as a plasticizer in polysaccharide-based films. It gives desirable barrier properties, a high hydrophobicity, and low water vapor permeability (Anderson and Simsek, 2019a). Sorbitol is recognized as a safe compound that is a humectant and non-cariogenic. Glycerol contains a lower molecular weight compared to sorbitol and a higher hydroxyl group ratio than sorbitol (Antoniou et al., 2014). Sorbitol or glycerol used can significantly impact the material characteristics. For instance, films containing glycerol as a plasticizer have excellent mechanical properties such as an extension and resistance (Antoniou et al., 2014). Nevertheless, films made with sorbitol have higher hydrophobicity and lower water vapor transmission rates than films made with glycerol.

2.3.4. Desirable Food Packaging

Food packaging has to be high in both tear resistance and tensile strength, while it must be low in aroma compound transmission, water vapor permeability, and oxygen transmission rate (Briassoulis and Giannoulis, 2018; Ciannamea et al., 2018). People prefer for food packaging to be transparent so that they can directly see what item can be purchased or not (Arrieta et al., 2015). Research focuses on plant-based (biodegradable) food packaging made from hydrocolloids such as AX, which is an option for transparent food packaging. Therefore,

biodegradable food packaging could be created using hydrocolloids and lipids, which are combined to increase mechanical strength and generate a good barrier for oxygen and water vapor. Additionally, polysaccharides such as AX can be applied as the basis for films, resulting in high mechanical strengths and pliability when AX is combined with plasticizers (Anderson and Simsek, 2019b). Plasticizers are added to biopolymeric packaging for high flexibility and pliability. Moreover, food packaging films have to be hydrophobic to increase the shelf-life of a product (Anderson and Simsek, 2019a). In the future, it would be great to replace synthetic food packaging with AX packaging because of the ecological advantages.

2.4. Arabinoxylan Films

2.4.1. Casting

A combination of AX extracted and a plasticizer, sorbitol or glycerol, can be processed for the creation of film, making it biodegradable packaging (Anderson and Simsek, 2019b). Food packaging has to be a high tensile strength, high tear resistance, low water vapor transmission rate, and low oxygen permeability to increase the shelf life of the foods. Importantly. The small ratio of AX generates suitable properties to make a film, indicating a decrease in water absorption and increase in crystallinity (Zhang et al., 2011). When the Xyl*p* backbone of AX is vastly substituted, the motion of the local chain decreases and then the chain stability increases. AX is a polar molecule thus it is a good barrier to nonpolar volatile compounds such as oxygen and aromatics. The wet method is the common procedure used for casting biodegradable films. This can be made by dissolving AX yield in water, stirring, adding plasticizer, heating, and drying (Anderson and Simsek, 2019b). Casting and drying condition will directly affect the characteristics of the product because of their influences on the molecular structure of the film being created.

2.4.2. Tensile Properties

The tensile strength, elongation, and modulus of elasticity are considered under the tensile properties of films. The principle of tensile is the capability of being stretched where a material can sustain the maximum load applied (Robertson, 2013d). Tensile strength of a film sheeting refers to an evaluation of the material's resistance to deformation when under tension. Elongation is measured at the point where the film breaks down and is expressed as the percentage of the original length of material being changed (Robertson, 2013d). Significantly, the ability of films to stretch can be measured by an elongation whose high value indicates a high energy consumption by the materials before breaking.

The modulus of elasticity is the material's tendency to undergo distortion when under tension. This modulus is a stiffness index of thin film sheeting and can be determined as the ratio of stress to strain under compression (ASTM International, 2018b). Food packaging is usually formed to be opened under tension or pressure; thus, the packaging has to be highly strong to resist these forces. Procedures for testing tensile properties features of films are pronounced in the American Society for Testing and Materials (ASTM) method D882-18 (ASTM International, 2018b). The tensile strength of AX film is based on film characteristics, which involve A/X, plasticizer type, and the amount of plasticizer (Antoniou et al., 2014). In wheat AX films, when the A/X increases, the tensile strength reduces (Ying et al., 2015).

2.4.3. Tear Resistance

Tear resistance measures how material resists the propagation of a tear, which is evaluated by measuring the force needed to initiate tearing (Robertson, 2013d; ASTM International, 2021). Tear resistance or tear strength is an essential characteristic of packaging films to protect food from small imperfections if they are present. A right-angle notch can be cut

into a film and the resistance to tension forces measured using ASTM method D1004-21 (ASTM International, 2021). The type of plasticizer can affect the tear strength, and using glycerol instead of sorbitol leads to a lower tear resistance but greater elongation (Hong-rui et al., 2014). This is because glycerol is smaller than sorbitol, allowing it to insert into the polymer chains more easily. When a polymer chain is disrupted, tear strength reduces but flexibility increases.

2.4.4. Puncture Resistance

Puncture resistance refers to a resistant material to a stressful or considerable force over a small region. It is a critical parameter for packaging of foods because of the tension placed upon food packaging throughout the transportation of food from industries to consumers. The puncture resistance of film material is measured using a modified version of ASTM method D7192-20 (ASTM International, 2020). Glycerol is utilized instead of sorbitol, resulting in a higher resistant puncture of packaging material along with smaller distortion (Thomazine et al., 2005). As the quantity of plasticizer increases, maximum force before puncture declines; however, the maximum deformation rises. High purity and extraction of AX could obtain a high tensile strength and high tear and puncture resistances of AX film (Anderson and Simsek, 2019b).

2.4.5. Scanning Electron Microscope

Scanning electron microscope (SEM) utilizes electrons for imaging that is reflected the surface region of a sample to create an image. Electrons are interacted with the sample, which can create multiple types of electrons, photons, or irradiations. According to Zhou et al. 2007, the beam of electrons scans the surface of the sample from side to side and from top to bottom with very small spot on the sample. Higher Magnification corresponds the smaller the size of the scanned surface area of the solid sample (Zhou et al., 2007). SEM is used to create high-resolution images of shapes, which come from a short wavelength from 400 to 700 nanometers

(nm). Shorter working distances (WD) of the order of 3-12 mm improve image resolution compared to longer WD (Zhou et al., 2007). Drying of the solid samples is required for the sample preparation for this method that high temperature can destroy the surface samples. Images from SEM show up differences in atomic numbers, which display as brighter or darker (Zhou et al., 2007). SEM images include information on topography and morphology; topography provides the features on the surface of sample while morphology gives the form or shape of the sample.

2.4.6. Moisture Content of Film

The mechanical properties and physical characterizations of a film can be affected by the moisture content. The moisture can be determined by taking the weight of the film and placing the film in an oven at 110 ℃ for 24 h, then the weight of the film after heating can be determined (Anderson and Simsek, 2019a; Garcia et al., 2004). In the former study, films with glycerol contained higher moisture contents than films made with sorbitol due to highly hydrophilicity of glycerol (Antoniou et al., 2014). It is feasible that films made with glycerol might take more water out of the material.

2.4.7. Film Water Solubility

The amount of water-soluble existing in a film will identify what its end-uses could be, and this quantity can be measured by dissolving pieces of film in water (Anderson and Simsek, 2019a; García et al., 2004). Food already contains a high moisture content that is unacceptable to be preserved in a package with water-soluble material. This is because the material can touch the food, making it dissolving. Water-soluble material depends upon several chemical features, such as the ratio of AX. When A/X reduces, the AX structure can be more crystalline as well as its film becomes less water soluble (Anderson and Simsek, 2019a; Heikkinen et al., 2013). An

increase in crystallinity results in less arabinose interacting with water, which reduces the accessible surface area of AX to act with water.

2.4.8. Contact Angle

A contact angle is an angle formed by a drop of liquid at a solid surface (ASTM International, 2022a). It is called an inverse measure of wettability. Water is mostly used to determine contact angle as it is a highly polar liquid; however, non-polar liquid can be utilized in some cases. The ASTM method D7490-13(2022) can be used to determine the surface properties of solid coatings using contact angle measurements (ASTM International, 2022a). The contact angles determine the hydrophobicity or hydrophilicity of the solid surface being analyzed. Plant-based films have high degrees of content angles that can be more hydrophobic, leading to better biodegradability of packaging materials. Glycerol is being more hydrophilic than sorbitol; thus, the contact angle degree of water on film made by glycerol is less than sorbitol film (Antoniou et al., 2014). That explains a film plasticized with glycerol has a higher wettability than a film generated with sorbitol. Because of this, the film structure using sorbitol as a plasticizer contains a more compact structure than film utilizing glycerol, leading to fewer interactions with water.

2.4.9. Water Vapor Transmission Rate

Due to the shelf life of the food, the water vapor permeability is significantly considered in the production of packaging material. Water vapor permeability is the time of water vapor transmission rate through area of the flat thickness material induced by vapor pressure difference between two specific surfaces. Thus, foods contain water in the packaging that must be controlled during storage. The water vapor permeability of food packaging can be evaluated by following the ASTM method E96/E96M-22a$^{\varepsilon 1}$ (ASTM International, 2022b). The type of plasticizer and the plasticizer level used can affect the water vapor permeability of food

packaging material (Anderson and Simsek, 2019a). When the volume of glycerol increases, the water vapor permeability of the film has observed because of glycerol's hygroscopic (Hansen and Plackett, 2008). Glycerol is being hydrophilic allowing a high level of water retention in the film (Antoniou et al., 2014). As a result, films plasticized with sorbitol are better than those generated by glycerol depending on water vapor transmission rate. Plant-based films are low in water vapor transmission rates, which are hydrophobic substances, making the films competent materials of food packaging.

2.4.10. Biodegradability

Utilization of biodegradable materials is increasingly becoming preferable in the production of food packaging. Making biodegradable packaging materials from the corn processing co-products, such as CB and DDGS, can have a friendly environment. After making AX films, the shelf life of the films must be maintained. However, there is a need for maintaining AX films because of the hydrophilic nature of AX, which can lead to poor moisture barrier properties. In the previous study, the biodegradability analysis of AX films was completed using soil under alkaline condition according to the ASTM method D5988-18 (Anderson and Simsek, 2019a; ASTM International, 2018a). Arabinoxylan films contained a high M_W of AX that films postulated to produce a biodegradable material used for packaging (Rosicka-Kaczmarek et al., 2016). More research on biodegradable AX films from corn processing co-products will be done to have better water barrier properties of packaging materials.

3. ENHANCEMENT OF MECHANICAL PROPERTIES OF BIODEGRADABLE FILMS FROM CORN BYPRODUCTS BY ENZYMATIC-CHEMICAL MODIFICATION

3.1. Abstract

AX based-films can enhance mechanical characteristics of biodegradable materials when the films are used for food packaging. However, the mechanical properties of AX films for use in the food packaging industry need to be properly explored to demonstrate the viability of film use for food packaging materials. Thus, AX was extracted from the corn bran byproducts of DCB, WCB, and DDGS via acid-alkali method. Packaging materials were created using each of these three AX extracts combined with laccase and sorbitol as the basis for each of the three films; the three films were modified by suspending the surface of the film in a lipase-acetate solution. Then, the following mechanical characteristics were evaluated: thickness, tensile properties, tear resistance, and puncture resistance. Thickness and tensile properties of the modified AX films made from DCB and DDGS were significantly ($P<0.05$) increased compared to the unmodified AX films. The modified AX films made from WCB had insignificant ($P>0.05$) results for both decreased thickness and improved tensile properties, as compared to the unmodified WCB AX films. A significant ($P<0.05$) increase in tear resistance was observed when all modified AX film surfaces were submerged in the lipase-acetate mixture. Puncture resistance was enhanced, but not significantly ($P>0.05$), for the modified AX films compared to unmodified AX films. The group presences of OH and CO in the surfaces of AX films made from DCB and DDGS and suspended in the lipase-acetate solution suggested that these two modified AX films may have excellent biodegradability properties. The AX films were positively affected by suspension in a lipase-acetate solution, making modified films bendable, flexible, and more resistant to deformation when stretched, as opposed to unmodified AX films.

3.2. Introduction

Corn is a cereal known also as maize, and mature corn is referred to as dent maize. One of the major byproducts of both dry-milling and wet-milling operations is corn bran (CB), which has a low commercial value. The bran (DCB) obtained from dry-milling and the bran (WCB) from wet-milling have different appearances, such as color and various chemical compounds. The corn dry milling process includes tempering, conditioning in warm water, heating, drying, grinding, and then sifting through multiple sieves by a mill, which rubs the corn particles to remove the germ and bran, resulting in endosperm-flour (Anderson and Almeida, 2019; Anderson and Lamsal, 2011). In contrast, corn wet milling process separates the corn grain into the kernel's main chemical compositions of starch, protein, oil, and fiber-bran. This operation comprises several steps: steeping in 0.2% SO_2, drying, milling into coarse grounds, separating germ, grinding into finer grounds, separating the WCB, removing gluten, washing, and sifting into starch flour (Rausch et al., 2019; Zhang et al., 2021).

The bran is rich in fibers; mainly cellulose and hemicellulose. Chemically, the content of CB ranges between 100 to 130 g kg^{-1} protein, 90 to 230 g kg^{-1} starch, 20 to 30 g kg^{-1} lipid, 20-15 g kg^{-1} ash, and 200 g kg^{-1} cellulose (Agger et al., 2010). The hemicellulose is mainly comprised of heteroxylan, which is present in the cell walls and accounts for 500 g kg^{-1} of the CB (Agger et al., 2010; Carvajal-Millan et al., 2007). Arabinoxylan (AX) is the main component of the heteroxylan found in the CB. Moreover, while AX is found in the corn bran, endosperm, and germ, the ratio of arabinose to xylose (A/X) in the AX present in each part of the corn kernel differs (Carvajal-Millan et al., 2007; Correia et al., 2011; García-Lara et al., 2019). In other words, the two major fibers of AX found in the corn kernel, bran fiber and endosperm fiber, are characterized by their unique arabinose/xylose ratios.

Dried distiller's grains with solubles (DDGS) are those grains, including corn, that have a dense nutrient coproduct from dry-milled ethanol production (U.S. Grains Council, 2018). Unlike dry and wet milling, the DDGS process does not only reduce the grain particle size, it also includes the specific ethanol production pathway in the conversion of starch to ethanol. DDGS production from maize grains includes the following steps: grinding from 3 to 5 mm, slurrying with adding water, liquefying by amylolytic enzymes, fermenting, distilling, centrifuging, and drying (Chen et al., 1999; Lin and Tanaka, 2006; Torija et al., 2003). Most importantly, the solution is fermented by the yeast, *Saccharomyces cerevisiae*, which converts sugars to ethanol at 32 ^{o}C for 12 h (Pretorius, 2000; Torija et al., 2003). Ethanol is then collected by distillation columns and the remaining fluids and solids is called whole stillage, which is centrifuged to remove coarse solids from the liquid (U.S. Grains Council, 2018). The coarse solids are called wet cake and are subjected to a drying process to produce DDGS. The DDGS is mostly used for animal feed that contains 30 g kg^{-1} protein, 15 g kg^{-1} cellulose, and 20 g kg^{-1} hemicellulose; half of the hemicellulose is AX (Xiang et al., 2014; Zarrinbakhsh et al., 2013).

AX is a non-cellulose polysaccharide mainly localized in the cell walls and the aleurone layer surrounding the endosperm of corn grains (Correia et al., 2011; Zhang et al., 2014). AX is mainly made up of two monosaccharide sugars: xylose and arabinose, although glucose or galactose may also be found in the corn fiber (García-Lara et al., 2019; Rose et al., 2010b; Zhang et al., 2014). The structure of AX in corn is that of a β-(1,4) linked D-xylopyranose (Xyl*p*) backbone at C (O)-2 with 4-O- methyl-D-glucuronic acid and at C (O)-2 and/or C (O)-3 positions of α-L-arabinofuranose (Ara*f*) residues (Agger et al., 2010; Correia et al., 2011). Most of the phenolic acid in CB is ferulic acid (FA), which is esterified to AX, but FA is localized at the C (O)-5 position of Ara*f*. Additionally, FA plays a significant role in the cross-linkages

between polysaccharides containing AX and AX, or AX and protein (Acosta-Estrada et al., 2019). Moreover, although FA is proportionally the highest phenolic acid in AX, overall it is only a small portion of the AX; nevertheless, FA tends to have significant effects on the molecular weight, solubility, and gelation properties of the AX in CB (Kale et al., 2010; Kale et al., 2013; Rumpagaporn et al., 2015).

While CB and DDGS are used for animal feed, AX extracted from the CB and DDGS can be utilized as the basis for materials in food packaging. People prefer for food packaging to be transparent so that they can directly see what item can be bought or not (Arrieta et al., 2015). A film created from AX is typically brittle, causing the film to split when handled; consequently, the film contains low extensibility and flexibility (Antoniou et al., 2014; Robertson, 2013d). Plasticizers, often sorbitol or glycerol, are typically used to reduce brittleness and to increase AX film's flexibility, and both are recognized as safe compounds, humectants, and non-carcinogenic (Antoniou et al., 2014; Robertson, 2013b). Sorbitol or glycerol used can significantly influence the characteristics of packaging materials. Whereas films containing glycerol have excellent mechanical properties such as an extension and resistance, films made with sorbitol include a high hydrophobicity for food packaging materials that can improve the shelf-life of a packaged product (Anderson and Simsek, 2019a; Antoniou et al., 2014).

Mechanical properties of AX films include tensile strength, tear resistance, and puncture resistance, which must be high to increase the strength of food packaging materials (Briassoulis and Giannoulis, 2018; Ciannamea et al., 2018). These mechanical characteristics of AX film are depended on the AX source, plasticizer type and the amount of plasticizer used (Anderson and Simsek, 2019b; Antoniou et al., 2014; Hong-rui et al., 2014). The mechanical characterizations of AX films made from DCB, WCB, and DDGS for use in the food packaging industry have to

be properly investigated to demonstrate the feasibility of film use for food packaging. Others properties of biomaterial films that must be considered are how it interacts with water, such as moisture content, water solubility, contact angle, water vapor transmission rate, and biodegradability. All the mechanical characteristics and water interaction properties of the AX films determine their usability as a material in food packaging.

AX casting film can be modified with a lipase to increase film hydrophobicity, with the lipase suspended in vinyl acetate (Stepan et al., 2013). Thus, the use of lipase decreases the amount of acetate required for the esterification reaction (Bengtsson et al., 2003; Stepan et al., 2013). Lipase has a high level of reaction specificity in hydrolyzing ester bonds, resulting in the esterification of AX (Kontkanen et al., 2004; Sakai et al., 2008; Utsugi et al., 2009). The activity of lipase can demonstrate an esterified monosaccharide or oligosaccharide of AX, indicating a biomaterial film could be developed (Stepan et al., 2013).

The objectives of this research were to extract AX and analyze AX characteristics from the three sources including DCB, WCB, and DDGS, to include lipase-acetate application in the surface of AX casting films and to evaluate the mechanical properties of AX films. Biodegradable food packaging material was created using the combinations of AX solution, laccase, and sorbitol (Anderson and Simsek, 2019b); the surface of the casting AX film was modified by suspending it in acetate reagent with lipase in this research.

3.3. Materials and Methods

3.3.1. Collecting and Milling

Milled DCB was purchased from Archer Daniels Midland (ADM) Company, Decatur, IL. Corn bran produced through wet-milling, WCB operation was also bought from ADM, but this sample needed to be ground in the laboratory. The un-milled third specimen, DDGS, was

purchased from Elk Mound Seed Company, Elk Mound, WI. Each DCB, WCB, or DDGS sample contained three replications. The DCB specimen had brown flour while the WCB sample was milled to yellow flour using a UDY miller, cyclone sample mill from UDY corporation. The specimen of DDGS was ground to sticky flour using the UDY miller. Then, the three flour samples were placed in plastic bags and stored in a freezer until use for polymer extraction.

3.3.2. Proximate Composition

The proximate compositions of the DCB, WCB, or DDGS flour, and their extracted AX were determined as follows: The moisture content of the DCB, WCB, DDGS, and three AX samples was determined by following the AACC International Method 44-15.02: Moisture Air-Oven Method (AACC International, 1999d). The flour's ash content of the DCB, WCB, DDGS, and their AX specimens was estimated in following the AACC International Method 08-01.01: Ash-Basic Method (AACC International, 1999c). The protein content of DCB, WCB, DDGS, and their AX flours was analyzed by estimating nitrogen content with the AACC International Method 46-30.01: Crude Protein-Combustion Method (AACC International, 1999b). The flour's lipid content of DCB, WCB, or DDGS was determined according to the non-official method using a hexane. The starch content of the DCB, WCB, DDGS, and three AX specimens was analyzed by following the AACC International Method 76-13.01: a Megazyme Enzyme Assay Kit K-TSTA (AACC International, 1999a). The total dietary fibers (TDF) of the DCB, WCB, or DDGS samples were determined using IDF/SDF Method (AOAC, 2022.01) by the ANKOM Dietary Fiber Analyzer.

3.3.3. Extraction of Arabinoxylan

3.3.3.1. Defatting

Once the dry milling of the DCB, WCB and DDGS samples was complete, the three specimens were defatted using hexane. To start this procedure, DCB, WCB, or DDGS flour was mixed with hexane 1:10 w/v (Anderson and Simsek, 2019b). The solution was stirred at 900 rpm for 1 h at 25 °C using a LED Digital Stirrer. Then, the hexane was poured into a waste bottle, and the new hexane was placed and allowed to stir for another 1 h (Figure 3.1). After a second time mixing, hexane was entirely poured into the waste bottle, and the defatted flour of DCB, WCB, or DDGS was dried at 23-25 °C in a fume hood overnight. After drying, these defatted flour specimens were spread on a glass tray to ensure the drying process.

3.3.3.2. Acid-Alkali Extraction

Extraction was conducted using sequential acid-alkali extraction. The defatted DCB, WCB, or DDGS (50 g) were added to 500 mL of 0.25M HCl and stirred at 450 rpm on a hot plate set to 75 °C, holding the slurry temperature at 45 °C for 2 h. Next, 100 mL of 3.0M NaOH was added to the slurry, while maintaining stirring and heating for another 2 h (Figure 3.1). Then, the pH was adjusted to 7.0 using concentrated HCl. The slurry was then centrifuged at 4945 rpm for 10 min to remove the solids. The arabinoxylan fraction was then precipitated with ethanol.

3.3.3.3. Ethanol Fractionation

Fractionation with ethanol was completed using a modified method by Xiang et al. (2014). After acid-alkali treatment, the AX solution of DCB, WCB, or DDGS was fractionated using analytical grade 95% ethanol. The ethanol was added to the AX solution in a 2:1 v/v and stirred for 1 h at 25 °C in a Lab-Line Mistral Multi-Stirrer. After fractionation, the solution was

centrifuged at 6600 rpm for 10 min (Figure 3.1). Thus, the pellet-solid was allowed to dry overnight at 50 °C in oven. Recording a weight of the pellet AX sample (from DCB, WCB, or DDGS) after each extraction was addressed and stored it in the freezer.

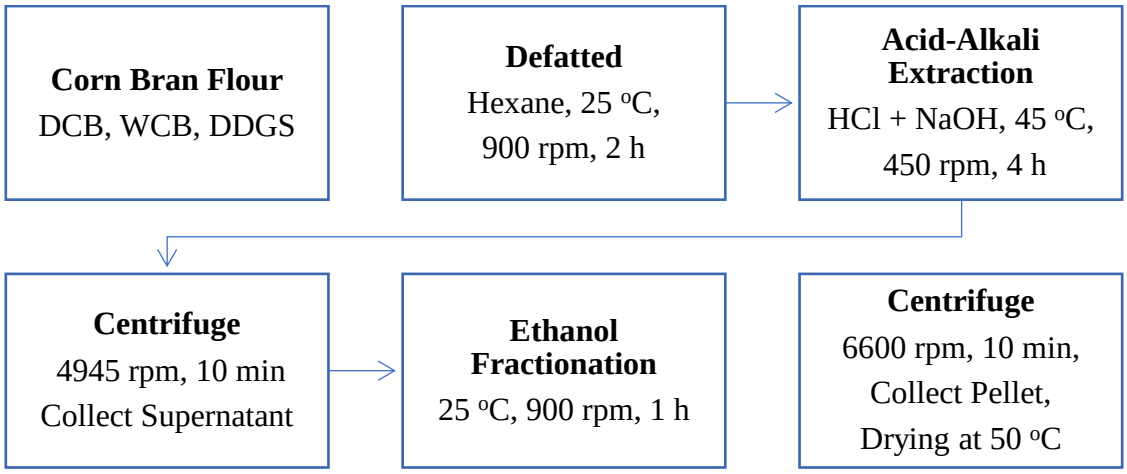

Figure 3.1. Flow procedure of arabinoxylan extraction.

3.3.4. Arabinoxylan Characteristics

3.3.4.1. Arabinoxylan Polymers

The sugar compositions of DCB, WCB, DDGS, or their AX were determined by High Performance Anion Exchange Chromatography-Pulsed Amperometric Detection (HPAEC-PAD). 1 mL aliquot of 1M HCl was separately added to 5-6 mg of each sample to make sure an enough time for the removal of the acidic solvents by evaporation (Nagel et al., 2014). The samples were incubated in heating blocks at 100 °C for 1 h. The samples were cooled and neutralized using 1 mL of 1M NaOH to facilitate the separation of Ara*f* and Xyl*p*. After that, the solutions were filtered through a 0.2 µm nylon syringe filter, and the sugar compounds of filtered samples were analyzed using HPAEC-PAD with a CarboPac PA20 column according to the method of Nagel et al. (2014). Sugar compositions of DCB, WCB, DDGS, or AX samples were calculated according to the following formula 1:

$$AX\ (\%) = 0.88 \times (\%\ Xylp + \%\ Araf) \quad (1)$$

3.3.4.2. Molecular Weight

Weight average molecular weights (M_W) and polydispersity index (PI) were determined by HPSEC-MALS-RI (Nishitsuji et al., 2022). The system was Shimadzu HPLC coupled to a Wyatt Opti lab RI detector and Wyatt MALS Dawn Heleos II. Separation was done using two columns (PL Aquagel-OH 40 and 60) connected in series at a flow rate of 0.5 mL/min. The mobile phase used was 0.05% sodium azide in double distilled water. The extracted AX of DCB, WCB, or DDGS was dissolved in 50mM $NaNO_3$ (2 mg/mL) for 16 h, and the solution was filtered through a 0.45-μm filter. The 100 μm of sample was injected to the HPSEC-MALS-RI system. The dn/dc value was 0.146 that M_W and PI were calculated using Astra 6.1.6 software. The light scattering model was Zimm with a fit degree of 1.

3.3.5. AX Film Casting and its Modification

Film casting was completed in deionized water including a D-sorbitol (Anderson and Simsek, 2019b) with a slight modification (Figure 3.2). The film solutions were created by making a 2.7% (w/v) of AX extraction from DCB, WCB, and DDGS samples in deionized water with laccase from *Aspergillus sp.* Laccase (130 μL/2.7% AX film solution) was added to produce cross-linked gel formations (Kala et al., 2013). The AX solution was stirred for 24 h at 25 ^{o}C using a Cimarec i Poly 15 Stirrer. Then, the solution was heated for 15 min at 90 °C in a water-shaking bath. D-Sorbitol (98% powder hygroscopic) was added as a plasticizer to the AX solution at 25% (w/v), 25% D-sorbitol/2.7% AX extraction. After adding D-sorbitol, the solution was heated for 15 min at 90 °C in the water-shaking bath. Then, the AX film solutions made from DCB, WCB, and DDGS were casted onto a Nunc Square BioAssay Dish and dried at 60 °C overnight in an air oven (VWR® Forced Air Ovens) (Figure 3.2). Once dried, all AX films were stored at Boveda 50-60% relative humidity (RH) and at 25 ^{o}C in a dry keeper desiccator cabinet.

Unmodified AX film from per sample had three replications. Modified AX film also included three replications.

In order to modify the AX film, the surface of the AX film from the DCB, WCB, and DDGS samples was constantly incubated into a lipase-acetate mixture to increase film hydrophobicity (Stepan et al., 2013). The lipase was suspended in vinyl acetate and the modified AX films were suspended in the reaction mixture at 40 ^{o}C for 24 h in a closed system (Stepan et al., 2013). On the following day, the surfaces of the modified films were washed several times using methanol and hexane and dried in the fume hood for 10 min (Figure 3.2). Finally, the treated AX films were maintained at 50-60% RH, 25 ^{o}C in the dry keeper desiccator cabinet before the determination of their mechanical properties and compared to unmodified AX films.

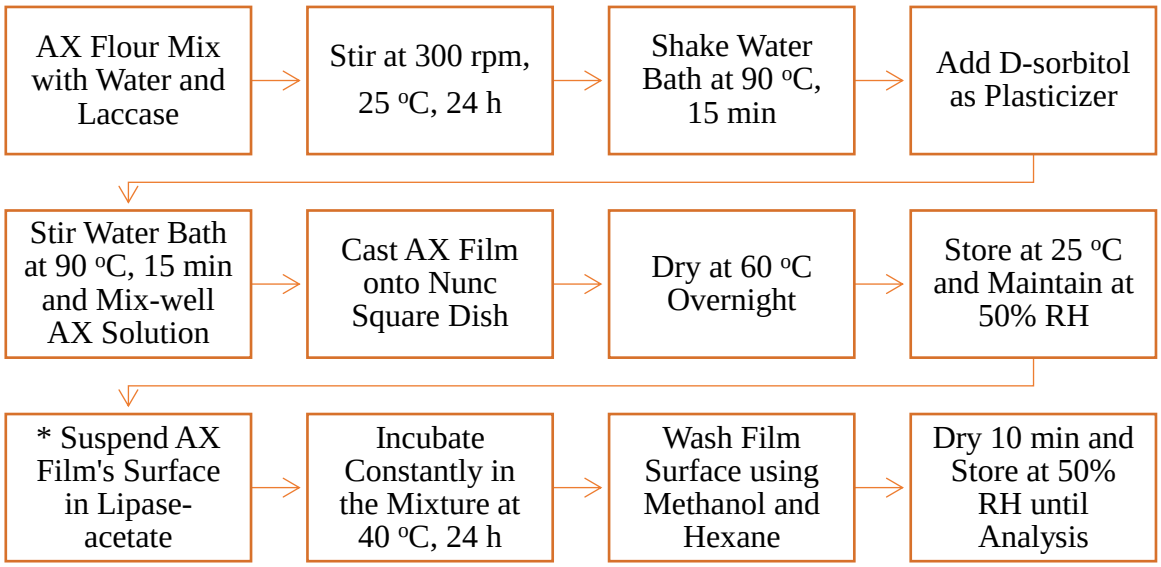

Figure 3.2. Flow chart of AX film casting and its modification steps. *Start modifying AX films.

3.3.6. AX Film Morphology

The modified and unmodified AX films generated from DCB, WCB, and DDGS samples were dried in an oven at 105 °C for 1 h (Zhou et al., 2007). The dried AX film samples were cut into very small pieces (0.4 cm × 0.8 cm) and attached to the scanning electron microscope

(SEM) stub with adhesive tape. A Nova nano SEM was utilized to obtain the images at 7.00 kV. Six morphology images were taken at magnifications of 2022-2078x.

3.3.7. AX Film Thickness

The thickness of each modified or unmodified AX film was measured by evaluating five points of the AX film's surface using a Mitutoyo Dial Thickness Gauge, NO. 7304A. The surface of the AX film was inserted into the thickness gauge clipper and the digital display was recorded in Inch (in.).

3.3.8. Tensile Strength

The maximum tensile strength, percentage of tensile elongation, and modulus of elasticity were determined three times for all films of DCB, WCB, or DDGS specimen by a texture analyzer (Texture Technologies Group TA-XT2i). The peak tensile strength is the ratio between the maximum load and the minimum cross-sectional region of the film prior to testing. Percentage of tensile elongation indicates the ratio between the extension at break and the initial gage length multiplied by 100. Modulus of elasticity is the relationship between stress and strain in the load-extension curve. This procedure was completed by following the ASTM D882-18 (ASTM International, 2018b). Modified and unmodified AX film was cut by ASTM Specimen Die Pioneer Dietecs and VEVOR Leather Cutting Machine Manual Black (Figure 3.3.A) (ASTM International, 2018b).

The width of the breaking part (center) of the cutting film was 3.02 mm, and break sensitivity was 750.2 mN with tension test mode. The test temperature and RH of the cutting film were at 23-25 °C and 50-60%, respectively. The test speed and post-test speed were 8.30 and 10 mm/sec, respectively. The testing condition of tensile strength also included a strain rate of 51 mm and an initial grip separation of 25.4 mm. The AX film was held between initial grip

separation settings: flat grip inserts and line grip inserts. The two grips completely cut the AX film to two pieces.

3.3.9. Tear Resistance

Tear resistance of the modified and unmodified AX films made from DCB, WCB, and DDGS samples was determined three times using a texture analyzer (Texture Technologies Group TA-XT2i). This testing was performed by following ASTM D1004-21 (ASTM International, 2021). Each AX film was cut using an ASTM D-1004 Tear Die and a VEVOR Leather Cutting Machine Manual Black (Figure 3.3.B) (ASTM International, 2021). The testing condition was at 23-25 °C and 50-60% RH of cutting film. The test mode was tension, with test speed and post-test speed 1 and 10 mm/sec, respectively. Trigger force was 49.0 mN, including a strain rate of 51 mm and an initial grip separation of 25.4 mm. The AX film was held between initial grip separation settings: flat grip inserts and line grip inserts. Once the test was completed, the two grips cut the film to two pieces. The tear resistance and tear extension outcomes were automatically calculated through the computer system.

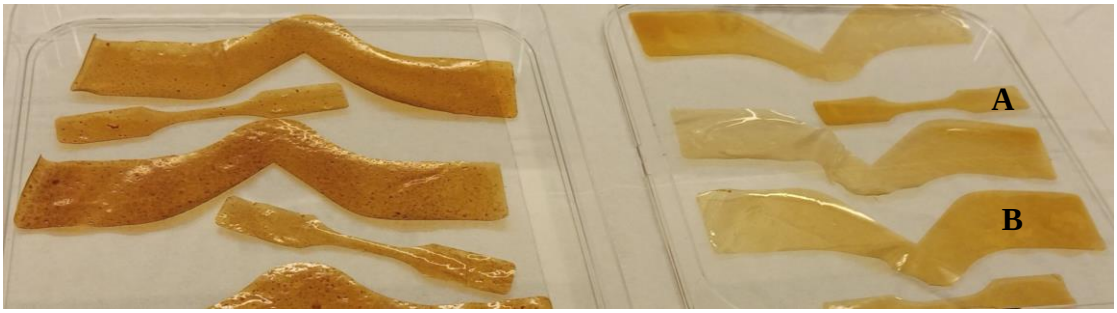

Figure 3.3. AX Films cutting by tear and tensile dies. A: tensile shape. B: tear shape.

3.3.10. Puncture Resistance

Puncture resistance was done in three replications for each modified or unmodified AX film per sample using a texture analyzer (Texture Technologies Group TA-XT2i) with a 2 mm diameter stainless probe with a flat head. The puncture resistance of each modified or

unmodified AX film was determined by a modified version of ASTM D7192-20 (ASTM International, 2020). The test mode, test temperature, and RH of the 4 cm x 5 cm film piece were compression, at 23-25 °C, and 50-60%, respectively. The pre-test speed, test speed, and post-test speed were 2, 33, and 10 mm/sec, respectively, with a 49.0 mN trigger force. To hold the AX films, a tortilla extensibility platform of the texture analyzer (Texture Technologies Group TA-XT2i) was utilized in conjunction via two CD disks with an outer diameter of 120 mm and an inner diameter of 15 mm (Anderson and Simsek, 2019b). The puncture resistance and puncture extensibility results were automatically calculated through Texture Exponent 32 software (Texture Technologies Corporation, 2016).

3.3.11. Structural Analysis

Fourier Transform Infrared (FTIR) spectroscopy was used to determine the interaction in structures between modified and unmodified AX films (Leon-Bejarano et al., 2020). The testing condition was at 4 cm^{-1} resolution and 10 Aperture, with the range of wavenumber between 4000 and 700 cm^{-1} (Thermo Scientific, Nicolet 8700, Wilmington, DE, USA).

3.3.12. Statistical Analysis

Statistical analysis was performed with SAS 9.4 for Windows, SAS Institute, Cary, NC. The source (DCB, WCB, and DDGS) was the first factor, and the modification of AX films was the second factor. For each dependent variable, we used two-way effects Model ANOVA to assess the main effects of source and modification of AX films as well as their interaction. In all cases, the interaction was either not significant or if significant, it was of a diverging nature such that the main effects were still interpretable. Fisher's least significant difference (LSD) was used for mean separation of the levels of the main effects. A Cell Means model was used to assess

differences among the interaction means, and LSD was used for mean separation. All tests were assessed using the significant level of 0.05.

3.4. Results and Discussion

3.4.1. Proximate Composition of CB

3.4.1.1. Moisture and Ash

The milled samples from DCB, WCB, and DDGS were analyzed for proximate compositions, which exhibited large variations among the three samples due to processing as dry-milling, wet-milling, or ethanol production (Table 3.1). Moisture was the first analysis completed upon receiving the pre-milled DCB and after the WCB and DDGS were milled by the researcher. The moisture content is usually utilized as a baseline for determining other compositions such as ash, protein, oil, starch, and fibers (Haynes et al., 2009; McCleary et al., 2012). The DCB had the highest percentage of moisture content at 9.96%, followed by DDGS at 7.96% and WCB at 1.33% (Table 3.1). Hence, the moisture content of the three samples was significantly (P<0.05) different from one sample to another. These DCB, WCB, and DDGS samples varied in moisture content because of processing methods and storage conditions.

Ash is mostly found in the CB, the location of the inorganic, mineral compounds present in the grain (Anderson and Lamsal, 2011). However, ash content is a good indicator of bran contamination in white flour or flour extraction rate (Anderson and Almeida, 2019). The DDGS contained the largest amount of ash content with 4.29% compared to DCB and WCB samples with 0.49 and 0.31%, respectively (Table 3.1). Therefore, the ash content of all specimens was significantly (P<0.05) different from one sample to another. DDGS had a higher ash content because DDGS processing does not include a bran separation step (U.S. Grains Council, 2018; Zarrinbakhsh et al., 2013). As the rate of flour extraction is increased, the amount of

contamination with non-endosperm increases and the ash content increases (Anderson and Almeida, 2019).

3.4.1.2. Protein, Oil, and Starch

Protein facilitates in building structure and support for the corn kernel cell walls (Anderson and Almeida, 2019; Larkins, 2019). Processing influences the protein content of DCB, WCB, and DDGS, as observed in the significant differences of the protein contents in this experiment. The amounts of protein in the DCB and WCB were 4.89% and 9.93%, respectively, much lower than the percentage of protein in the DDGS with 34.45% (Table 3.1). These protein percents were all significantly (P<0.05) different amongst the three samples. It is possible that DCB and WCB included lower proteins because of separating germ in dry-milling and removing gluten in wet-milling (Anderson and Lamsal, 2011; Zhang et al., 2021).

Oils are present in small amounts in the CB when the CB flour is contaminated by germ particles (Anderson and Almeida, 2019). Although the second centrifugation step in the ethanol production process extracts oil, the DDGS has a much higher oil content compared to DCB and WCB (U.S. Grains Council, 2018). The results of lipid analysis in this research bears this out. Thus, the oil contents of these byproduct materials followed this trend: 1.32% oil in DCB, 4.66% oil in WCB, 8.82% lipid in DDGS (Table 3.1). These percentages of lipid contents had significant (P<0.05) differences from one specimen to another. In the milling process, the heating step might be applied to deactivate the lipid-enzymes, thus maintaining the oil content (Anderson and Almeida, 2019; Rausch et al., 2019).

The endosperm is where the kernel stores almost all the kernel's starch, which is relatively low amounts in the CB byproducts (Anderson and Almeida, 2019). The total amounts of starch in corn grains can differ based on harvesting conditions and milling procedures (Zhang

et al., 2021). The contents of total starch in WCB and DDGS were 5.42% and 6.05%, respectively, both significantly (P<0.05) lower than the 7.03% starch for DCB sample (Table 3.1). It is likely that the CB made from wet-milling contained the lower percentage of starch due to the removing the prolamin-starch matrix (Larkins, 2019). The DDGS process requires enzymatic and yeast treatments, leading to more free sugars and less starch. Even though the starch content of DCB was higher compared to WCB and DDGS, it should be in the range of 9-20% as reported in the previous research (Anderson and Almeida, 2019; Zhang et al., 2021).

Table 3.1. Proximate composition and arabinoxylan contents of corn bran samples.

	Dry weight basis (DWB) (%)									
Sample	Moisture	Ash	Protein	Oil	Starch	IDF	SDF	TDF	AX	A/X
DCB	9.96a	0.49a	4.89a	1.32a	7.03a	88.81a	3.07a	91.88a	62.26a	0.80a
WCB	1.33b	0.31b	9.93b	4.66b	5.42b	69.66b	2.97a	72.64b	54.25b	0.85b
DDGS	7.96c	4.92c	34.45c	8.82c	6.05b	39.55c	4.96b	44.51c	35.49c	1.06c

Means with the same letter in the same column for the same sample type are not significantly different (P<0.05). The data are the means of three independent replicate experiments (n = 3).

3.4.1.3. Dietary Fibers

The CB is generally low in moisture, lipid, protein, and starch but rich in TDF, with a greater proportion of IDF and a much fewer SDF (Dikeman et al., 2006). In this study, the results of TDF, IDF, and SDF matched these compositional trends in the three samples. The DCB included the highest TDF content, accounting for 91.88%, followed by WCB with 72.64% and DDGS with 44.51% (see above Table 3.1). Similarly, DCB had the largest amount of IDF content at 88.81% compared to WCB and DDGS samples with 69.66% and 39.55%, respectively (see above Table 3.1). Thus, the TDF and IDF percentages of the three samples were significantly (P<0.05) different from one specimen to another. In contrast, SDF amounts in the DCB and WCB were not significantly (P>0.05) different, both estimated at 3% (see above Table 3.1). However, the SDF in the DDGS was significantly (P<0.05) higher at approximately 5%

(see above Table 3.1). It seems that CB as a by-product of dry-milling has more DF than WCB or DDGS procedure, leading to the highest DF in DCB. The tempering step of dry-milling process toughens the bran and facilitates the easy separation of the CB from the corn kernel (Anderson and Lamsal, 2011). In addition, CB is a co-product of ethanol production process, which has converted the compositions (protein, sugars and non-starch bound lipids) to ethanol, resulting in the lowest DF in DDGS material.

One of the main DF in CB is AX, determined using HPAEC-PAD. Then, the Ara*f*/Xyl*p* ratio (A/X) was measured. AX is a non-cellulose polysaccharide chiefly found in the cell walls and is mainly made up of two monosaccharides: Ara*f* and Xyl*p* in the fiber (García-Lara et al., 2019; Rose et al., 2010b; Zhang et al., 2014). The AX concentration with its A/X are different and dependent upon the growing condition and corn processing. The total AX contents of the DCB, WCB, and DDGS samples were as follows: 62.26%, 54.25%, and 35.49%, respectively (see above Table 3.1). In addition, the A/X for the samples were varied at 0.80 for DCB, 0.85 for WCB, and 1.06 for DDGS (see above Table 3.1). These AX concentrations and A/X values were all significantly different ($P<0.05$) amongst three samples. The estimated percentages of AX content in the DCB, WCB, and DDGS samples were in accordance with the values of TDF (see above Table 3.1). Thus, TDF increased in the CB as AX increased, in line with previously published study (Agger et al., 2010; Carvajal-Millan et al., 2007). It is likely that the low A/X in the AX of the DCB and WCB samples was due to their low SDF (Zannini et al., 2022).

3.4.2. Composition and Characterization of AX

3.4.2.1. Yields

Acid-Alkali extraction method was used to yield AX from each sample (DCB, WCB, and DDGS) that chemical extraction of AX is shown in the Figure 3.1. Extracted AX was analyzed

for proximate composition, AX polymer, A/X, and M_W which resulted in significant variances based on the three samples (Table 3.2). These compositions and characterizations of AX obtained in this research has provided the information about the effectiveness of the acid-alkali extraction method used. The AX yields of DCB and DDGS were around 23.30% and 23.01%, respectively, while the WCB AX accounted for 25.39% (Table 3.2). The yields of AX were not significantly (P>0.05) different from DCB to DDGS, but significantly (P<0.05) different from the AX yield of WCB. AX is extracted from CB where the greatest concentration of AX is in the ranging 25-35% of the total bran (Saeed et al., 2011; Zannini et al., 2022). The higher yield in the WCB AX as compared to the DCB AX and DDGS AX was likely because of the difference in the processing level. Corn dry milling separates the main physical portions of the kernel: germ, bran, and endosperm (Anderson and Almeida, 2019). In contrast, the corn wet milling process separates the grain's main chemical compositions of starch, protein, fiber, and oil (Zhang et al., 2021). Ethanol production procedure needs more steps, such as fermenting, distilling, and centrifuging, which make the process difficult to extract AX from DDGS (Xiang et al., 2014).

3.4.2.2. Moisture and Ash

Moisture of AX extracted from DCB, WCB, and DDGS samples was also analyzed. The moisture content is frequently used as a baseline for measuring other proximate compositions (Haynes et al., 2009; McCleary et al., 2012). The DCB AX contained the greatest moisture percentage of 14.14%, followed by the DDGS AX with 13.85% and the WCB AX with 13.15% (Table 3.2). Thus, the moisture contents of the AX yields were significantly (P<0.05) different amongst three AX extracts. The moisture contents of these AX extracts increased from the starting flour materials (see above Table 3.1 and see below Table 3.2). Therefore, these AX

yields from DCB, WCB, and DDGS samples probably changed in moisture content due to material sources and storage conditions.

The AX concentration is influenced by the mill stream, and a high relationship between ash and AX contents has been found that a sample had low ash content with low AX content (Ramseyer et al., 2011). The DDGS AX had the highest percentage of ash content, 18.36%, compared to DCB AX and WCB AX samples with 5.36% and 5.86%, respectively (Table 3.2). Hence, the ash contents of all AX extracts were significantly (P<0.05) different from one AX to another. The ash remaining in the AX fractions increased from the starting flour materials (see above Table 3.1 and see below Table 3.2). Therefore, ash remaining in the AX yields from DCB, WCB, and DDGS followed the relationship that high ash content led to high AX content (Ramseyer et al., 2011). After AX extraction, DDGS AX increased in ash content as it increased in AX polymer.

3.4.2.3. Protein and Starch

Protein supports in building structure of the cell walls in the corn kernel (Anderson and Almeida, 2019; Larkins, 2019). However, acid-alkali extraction process of AX consists of disrupting covalent and hydrogen bonds and reducing the CB matrixes, making it low in protein and starch (Cyran et al., 2004). The protein contents of DCB AX and DDGS AX were 6.67% and 6.51%, respectively, which both were significantly (P<0.05) lower than 9.33% protein for the WCB AX extract (Table 3.2). The protein percentages of the extracted AX decreased from both WCB and DDGS flours except for the AX yield of DCB, which increased (see above Table 3.1 and see below Table 3.2). It seems that the use of acid-alkali extraction of AX decreased in protein contents of WCB AX and DDGS AX because of the collapsing protein matrixes in the chemical extraction.

Both the protein and the starch percentages found in the WCB AX and DDGS AX were reduced. However, these two compositions in the AX yield of DCB increased compared to the starting flour materials (see above Table 3.1 and see below Table 3.2). The starch content of WCB AX and DDGS AX was about 2.5% each, which was significantly (P<0.05) smaller than 8.83% starch for AX yield of DCB (Table 3.2). As the total starch contents of WCB and DDGS were relatively low in percentage, their AX extracts contained a lower percentage of starch. It is possible that chemical extraction method of AX decreased in starch contents of WCB AX and DDGS AX because of hydrolyzing effect on the starch and releasing more non-polysaccharides using the acid-alkali extraction (Cyran et al., 2004; Zhang et al., 2014).

Table 3.2. Proximate composition and characteristics of the arabinoxylan extractions.

Extracted AX	DWB (%)								
	Yield	Moisture	Ash	Protein	Starch	AX	A/X	M_W	PI
DCB	23.30a	14.14a	5.36a	6.67a	8.83a	76.60a	1.02a	4063667a	3.05a
WCB	25.39b	13.15b	5.86b	9.33b	2.53b	77.64b	1.20b	1876333b	2.37b
DDGS	23.01a	13.85c	18.36c	6.51a	2.73b	66.68c	1.32c	879133c	1.80c

Means with the same letter in the same column for the same sample type are not significantly different (P>0.05). The data are the means of three independent replicate experiments (n = 3).

3.4.2.4. Arabinoxylan Polymers and Molecular Weight

After acid-alkali extraction of AX, the polymer and its A/X were also evaluated by HPAEC-PAD. The AX concentration with its A/X varies based on the byproduct or coproduct sources and extraction procedure of AX yields. The AX polymers for the extracted AX of the DCB, WCB, and DDGS samples were different as follows: 76.60%, 77.64%, and 66.68%, respectively (see above Table 3.2). The A/X were also dissimilar from one to another at 1.02 for DCB AX, 1.20 for WCB AX, and 1.32 for DDGS AX (see above Table 3.2). The polymer of AX and A/X were significantly different (P<0.05) amongst all three types of extracted AX. The AX polymer and its A/X in the AX fractions increased from the DCB, WCB, and DDGS flours (see

above Table 3.1 and Table 3.2). This growth of AX polymers is due to the higher yields after acid-alkali extraction, indicating a successful chemical method to extract AX from the bran byproducts (Cyran et al., 2004).

The M_W and PI of the AX yields from all samples were determined by HPSEC-MALS-RI. From the highest to lowest, the M_W of the three AX extracts were 4063667 for DCB AX, 1876333 for WCB AX, and 879133 for DDGS AX (see above Table 3.2). These AX M_W were significantly different (P<0.05) amongst all three yields of AX. This is most likely because of the high concentration of AX, resulting in higher M_W compared with AX's low content (Kale et al., 2010). The M_W obtained in this research matched this concept when compared to AX contents and their M_W of the AX yields from WCB and DDGS (see above Table 3.2). Additionally, the low soluble DF of DCB and WCB had low A/X of AX extracts (see above Table 3.1 and Table 3.2). This is probably due to the higher M_W of DCB AX and WCB AX (Acosta-Estrada et al., 2019).

The PI is a measure of the width of the variation in the M_W polymer distribution, which is a ratio between weight average molecular weight and number average molecular weight. Also, PI is a measure of the heterogeneity of M_W polymer as its size functions (Chang, 2005). Same as the M_W, the PI for all AX extracts was significantly (P<0.05) different from one AX yield to another. Thus, the PI of these AX extracts followed this trend from the greatest to the smallest: DCB AX (3.05), WCB AX (2.37), and DDGS AX (1.80) (see above Table 3.2). As all PI of AX yields were higher than 1, their M_W were greater than their number average molecular weights (Chang, 2005). These trends indicated that there was more heterogeneity in the three AX extracts from DCB, WCB, and DDGS.

3.4.3. AX Film Morphology

The surface morphology of modified and unmodified AX films from DCB, WCB, and DDGS samples was analyzed using SEM. The evaporated surface images of AX films revealed differences in topographical features and morphological structures, which were displayed as brighter or darker color (Figure 3.4). The bright spots indicated the presence of nickel in the images of unmodified and modified AX films from DCB and WCB; notably, the modified AX film from DDGS had the biggest bright spots (Figure 3.4). The cracks appeared in these AX films were due to drying in the oven, which is a required step for solid surface of sample preparation, before the SEM procedure (Zhou et al., 2007). The modified AX films from DCB and WCB depicted lower surface cracks compared to their unmodified AX films. It is possible that lower cracking can demonstrate a smooth surface or impermeable film, while higher cracking tends to lead to a rough surface or porous film (Salvada et al., 2022). The unmodified AX films from DDGS had no visible cracks but contained rough surface of the film, which can be more permeable material. The modified AX films from DDGS detected small cracks and included large spots, which might provide impermeable material.

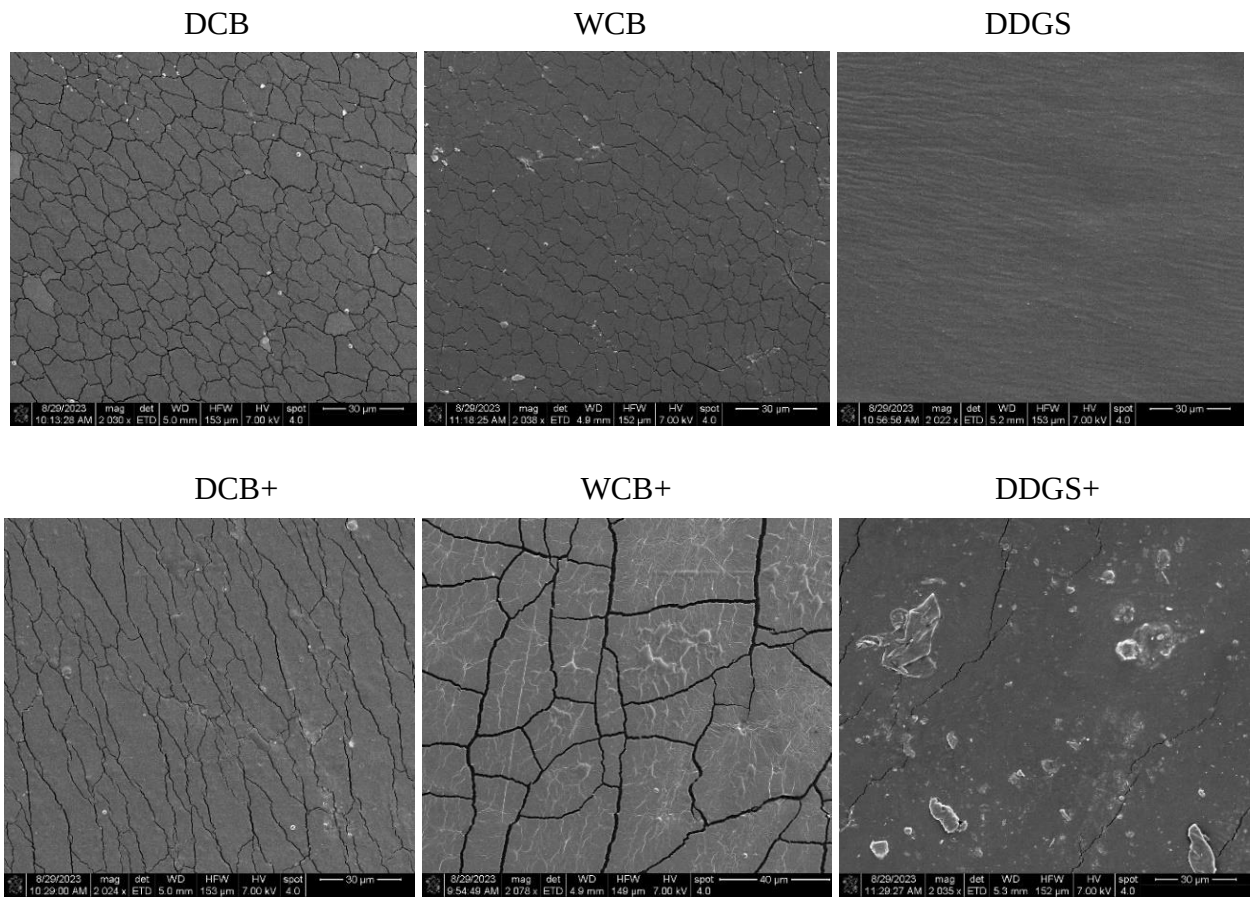

Figure 3.4. Scanning electron microscopy of unmodified and modified AX films. +: AX film that suspended lipase-acetate.

3.4.4. AX Film Thickness

The AX film thickness was different among DCB, WCB, and DDGS samples due to sample sources and AX heterogeneity (Salvada et al., 2022). The AX films made from DCB was the thickest at 0.0041 in., much higher than the AX films thicknesses from films made from WCB and DDGS, 0.0036 in. and 0.0037 in., respectively (Table 3.3). This range of AX-based film thickness values matches those of previous studies (Salvada et al., 2022; Weng et al., 2021). Furthermore, thickness for the three modified AX film samples was 0.0041 in., significantly (P<0.05) higher than the 0.0035 in. for the unmodified AX films (Table 3.3). Specifically, the thickness in the modified AX films made from DCB and DDGS was significantly (P<0.05)

increased compared to their unmodified AX films (Figure 3.5. A). Thus, the addition of the of lipase-acetate mixture increased the AX film thickness of modified AX films made from DCB and DDGS. The modified AX films made from WCB showed a similar thickness to its unmodified AX films, with no statistically significant differences between modified and unmodified WCB AX film samples (P>0.05) (Figure 3.5. A). This probably indicated that composite AX films made of WCB are thin, with the thickness difficult to measure (Salvada et al., 2022).

3.4.5. Tensile Strength

Tensile properties of film sheeting are mostly dependent on film preparation method, testing speed, and film thickness. The tensile properties of AX films contain tensile strength, tensile elongation, and modulus of elasticity, which are relevant parameters to evaluation of food packaging. In addition, packaging materials need to be high in both tensile strength and modulus of elasticity to enhance their tensile properties (Salvada et al., 2022). AX films must have a good balance of tensile strength and tensile elongation to develop the necessary strength for food packaging materials (Péroval et al., 2002).

The WCB AX films had the highest tensile strength at 0.144 MPa, followed by the DDGS AX films at 0.120 MPa and the DCB AX films at 0.111 MPa (as seen in Table 3.3). Similarly, the WCB AX films had the greatest modulus of elasticity at 0.020 MJ/m^3, followed by the DDGS AX films at 0.017 MJ/m^3 and the DCB AX films at 0.015 MJ/m^3 (Table 3.3). There was no significant (P>0.05) difference between the AX film sources and both elastic modulus and tensile strength of the AX films. However, the results from the WCB AX films indicated that the WCB AX films was ductile, bendable, and more unsusceptible to deformation when stretched compared to either the DCB AX films or the DDGS AX films. This result was

comparable to the ductile, bendable, and more unsusceptible to deformation packaging material descriptions (Salvada et al., 2022; Weng et al., 2021). The tensile elongation exhibited the lowest percentage at 77.01% for the DCB AX films, significantly (P<0.05) much lower than the 85.47% of tensile elongation for the DDGS AX films (Table 3.3). These results were notable because a high percentage of tensile elongation makes the film more deformable elastically when stretched (Salvada et al., 2022).

A significant (P<0.05) increase in tensile strength was observed when the surfaces of modified AX films were submerged in lipase-acetate solution. The tensile strength of modified AX films was significantly (P<0.05) increased at 0.148 MPa compared to 0.102 MPa for the unmodified films counterparts (Table 3.3). The modulus of elasticity was increased, but not significantly (P>0.05), for the modified AX films to 0.021 MJ/m^3 when compared to 0.015 MJ/m^3 for the unmodified AX films made from DCB, WCB, and DDGS (Table 3.3). The percentage of tensile elongation was 83.28% for the unmodified AX films, decreasing to 78.31% for the modified AX films (Table 3.3). There was no significant (P>0.05) difference between the unmodified AX films and both modulus of elasticity and tensile elongation of the modified AX films. Nevertheless, the modification for AX film surfaces led to improved tensile properties by making the AX film sheeting ductile and more resistant to deformation.

Table 3.3. Evaluation of thickness and tensile properties of unmodified and modified AX films.

Factors	AX films	Thickness (in.)	Tensile strength (MPa)	Tensile elongation (%)	Modulus of elasticity (MJ/m^3)
Sources	DCB	0.0041a	0.111a	77.01a	0.015a
	WCB	0.0036b	0.144a	79.91ab	0.020a
	DDGS	0.0037ab	0.120a	85.47b	0.017a
Modification	Unmodified	0.0035a	0.102a	83.28a	0.015a
	Modified	0.0041b	0.148b	78.31a	0.021a

Means with the same letter in the same column for the same sample type are not significantly different (P>0.05). The data are the means of three independent replicate experiments (n = 3).

The statistical interaction between the tensile strength of modified and unmodified DCB AX films was significantly (P<0.05) increased in the modified DCB AX films (Figure 3.5. B). The modified WCB AX and DDGS AX films showed improved tensile strength, but not significantly (P>0.05) different from their unmodified AX films (Figure 3.5. B). Thus, tensile strength of modified AX films was affected by suspending in lipase-acetate mixture, leading to better flexible films. This result is in line with other current research (Salvada et al., 2022; Weng et al., 2021). The elastic modulus of the modified DCB AX films was significantly (P<0.05) increased compared to the unmodified DCB AX films (Figure 3.5. D), which confirmed the improved tensile strength of the modified DCB AX films. Additionally, the modified WCB AX film also had enhanced its elasticity modulus, but not significantly (P>0.05) different from its unmodified WCB AX film (Figure 3.5. D). This increase of tensile strength and elastic modulus for the modified AX films resulted in flexible and bendable materials for food packaging materials. The modified DCB AX and WCB AX films exhibited a similar tensile elongation to their unmodified AX film samples, having no statistically significant differences between them (P>0.05) (Figure 3.5. C). In contrast, the percentage of elongation was significantly (P<0.05)

reduced for the modified DDGS AX films compared to unmodified DDGS AX films (Figure 3.5. C). This decrease in the percentage of tensile elongation can result in non-deformable films used for food packaging materials, as also shown in the literature (Salvada et al., 2022).

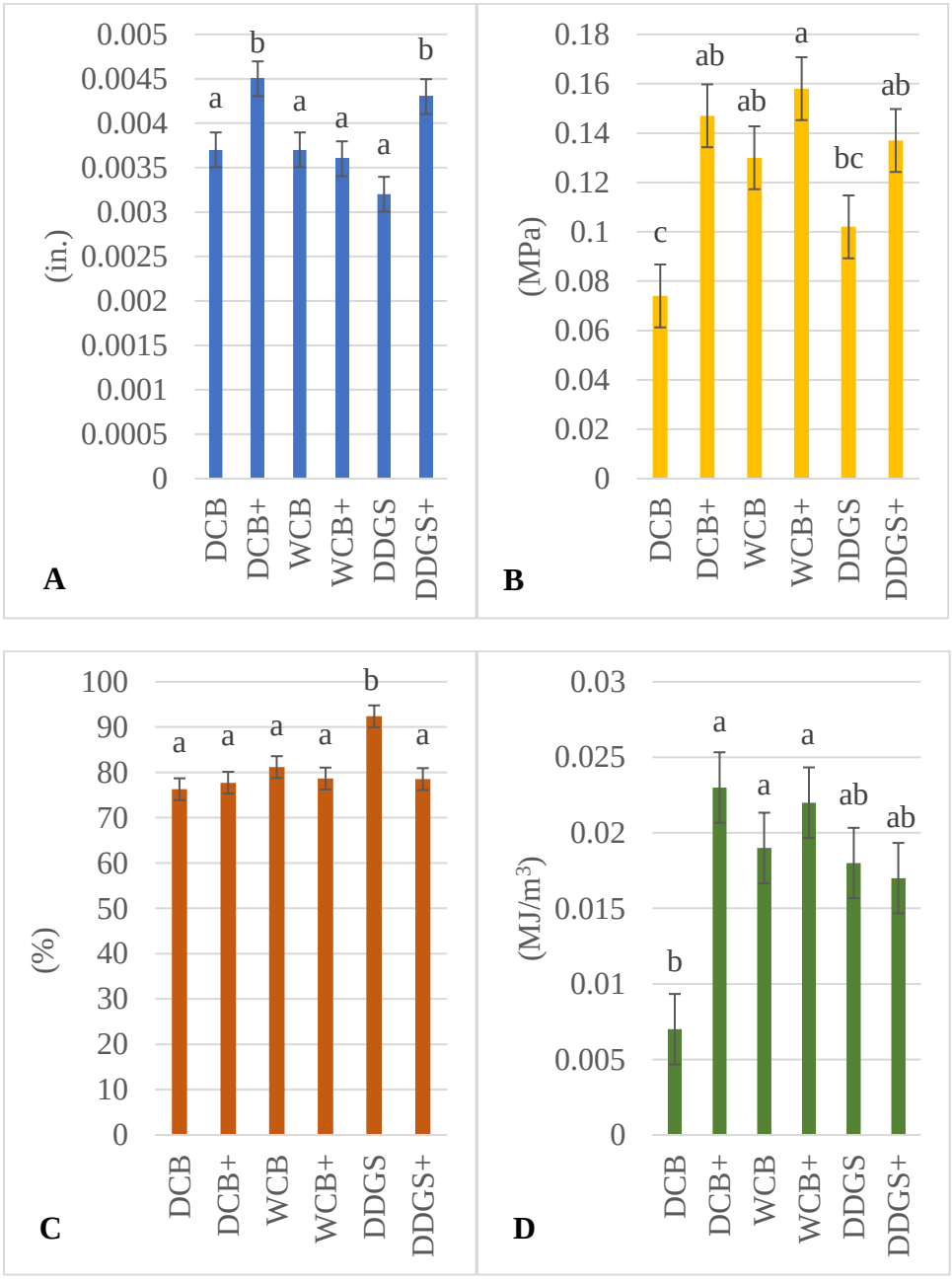

Figure 3.5. Interactions of thickness and tensile properties of unmodified and modified AX films. A: Thickness. B: Tensile strength. C: Tensile elongation. D: Modulus of elasticity. Means with the same letter in the same box are not significantly different (P>0.05). The data are the means of three independent replicate experiments (n = 3). +: AX film that suspended lipase-acetate.

3.4.6. Tear Resistance

Film thickness, film geometry, and speed of testing are controlled in the tear resistance method. This method determines the force to initiate tearing across a specific film geometry; the maximum stress is measured as the tear resistance (Robertson, 2013b). Then, the maximum tear resistance and the maximum tear extension are calculated for the film specimens tested at a particular speed. The food packaging materials must be high in tear resistance and low in tear extension to improve their tear characteristics (Ciannamea et al., 2018). The load required before a tear propagation is also referred to as film sheeting resistance to rupturing. A higher load results in a higher tear resistance and lower tear extension.

The DCB AX films contained the largest tear resistance at 1.05 N, followed by the WCB AX films at 0.85 N and the DDGS AX films at 0.40 N (Table 3.4). The difference between the tear resistances of the DCB AX films and the WCB AX films was not significant ($P>0.05$); however, both were significantly ($P<0.05$) different from the tear resistance of the DDGS AX films (Table 3.4). As a result, the DCB AX films had more flexible packaging film sheeting, while the DDGS AX films had less packaging material flexibility. When bio-based films characterize as higher tear resistance and lower extensible behavior, they can be flexible and pliable food packaging materials (Briassoulis and Giannoulis, 2018). Furthermore, the DDGS AX films had the most extensible tearing with 57.69 mm, followed by 56.31 mm for the DCB AX films and 55.90 mm for the WCB AX films (Table 3.4). There was no significant ($P>0.05$) difference between the AX film sources and tear extension of the AX films. The decrease in the extensible behavior of the WCB AX films led to an increase in the material flexibility.

The tear resistances of both modified and unmodified AX films showed an increase in the tear resistance when the surfaces of AX films were suspended in a lipase-acetate solution; the

unmodified AX films tear resistance was 0.39 N, significantly (P<0.05) increased to 1.14 N as compared to the modified AX films (Table 3.4). Similarly, tear extensions of both modified and unmodified AX films showed a decrease in the tear extension when the surfaces of the AX films were suspended in a lipase-acetate mixture. Hence, the tear extension of the unmodified AX films was 57.82 mm, which was significantly (P<0.05) decreased to 55.45 mm for the modified AX films (Table 3.4). It is most likely that high tear resistance and low extensible behavior of bio-based films can produce flexible and pliable food packaging materials (Briassoulis and Giannoulis, 2018; Ciannamea et al., 2018). This study's modifications of AX film surfaces enhanced the tear strength, indicating an increase in the flexibility and pliability of the AX film materials.

Table 3.4. Determination of tear and puncture properties of unmodified and modified AX films.

Factors	AX films	Tear resistance	Tear extension	Puncture resistance	Puncture extensibility
		(N)	(mm)	(N)	(mm)
Sources	DCB	1.05a	56.31a	6.78a	34.43a
	WCB	0.85a	55.90a	6.75a	33.78ab
	DDGS	0.40b	57.69a	4.21b	33.17b
Modification	Unmodified	0.39a	57.82a	5.29a	33.29a
	Modified	1.14b	55.45b	6.54a	34.29b

Means with the same letter in the same column for the same sample type are not significantly different (P>0.05). The data are the means of three independent replicate experiments (n = 3).

It is important to have a narrow range of film thicknesses within the same sample; therefore, tear resistance is more valid when comparing thicknesses (Briassoulis and Giannoulis, 2018). The unmodified and modified WCB AX films did not show significant (P>0.05) differences in thickness (see above Figure 3.5. A). Thus, there were significant (P<0.05) interactions for the tear resistance and extensible behavior of the modified and unmodified WCB

AX films. The tear resistance of the modified WCB AX films was significantly (P<0.05) increased compared to the unmodified WCB AX films (Figure 3.6. A). Also, the modified WCB AX films significantly (P<0.05) decreased in tear extension (Figure 3.6. B). These three outcomes of modified WCB AX films furnished data for an accurate and reasonable tear strength, leading to more flexible films for packaging materials (Briassoulis and Giannoulis, 2018). The modified DCB AX and DDGS AX films significantly (P<0.05) improved in tear resistance, but remained steady (P>0.05) in tear extension, compared to the unmodified DCB AX and DDGS AX films (Figure 3.6. A and B). Hence, tear resistance of all modified AX films was influenced by immersing their surfaces in lipase-acetate solution, leading to better flexible films.

3.4.7. Puncture Resistance

Puncture resistance of materials utilized in food packaging usually depends upon three factors: type of polymer composition, film fabrication procedure, and film uniformity. Puncture resistance is defined as how resistant a material is to a stressful force over a small area (Anderson and Simsek, 2019b). It is an important parameter for food packaging due to the tension placed upon food packaging throughout its transportation from industries to consumers. Moreover, both high AX extraction and high AX purity may lead to higher AX film puncture resistance (Anderson and Simsek, 2019b). Unlike tear resistance assessment, puncture resistance determination does not require similar thicknesses when comparing two films from the same AX polymer.

The puncture resistances in DCB AX and WCB AX films were not significantly (P>0.05) different, both approximately estimated at 6.75 N (see above Table 3.4). However, the puncture resistance in the DDGS AX films was significantly (P<0.05) lower at 4.21 N (see above Table 3.4). It seems that the high extraction of AX from DCB and WCB made these films more

puncture resistant, while low AX yield from the DDGS led to lower puncture resistance for the DDGS AX films (see above Table 3.2 and Table 3.4). It has been reported that the high purity of AX from corn bran resulted in a higher AX film puncture resistance (Anderson and Simsek, 2019b). Also, less AX extract from DDGS results in a lower puncture resistance for the DDGS AX films (Anderson and Simsek, 2019b). In this study, the puncture extensibility in DCB AX films was 34.43 mm, significantly (P<0.05) higher than the 33.17 mm puncture extensibility for DDGS AX film samples (see above Table 3.4). In addition, the puncture extensibility in the WCB AX films was 33.78 mm, insignificantly (P>0.05) different from the two other samples (see above Table 3.4). It is likely that the puncture extensibility increased as the puncture resistance increased, mirroring the same trend seen in previous work (Anderson and Simsek, 2019b; Cazón et al., 2020).

An increase in both puncture resistance and puncture extensibility was observed when the AX film surfaces were suspended in lipase-acetate solution. Puncture resistance was increased, although insignificantly (P>0.05), for the modified AX films to 6.54 N when compared to 5.29 N for the unmodified DCB AX, WCB AX, and DDGS AX films (see above Table 3.4). The puncture extensibility of modified AX films was significantly (P<0.05) increased at 34.29 mm compared to 33.29 mm for the unmodified AX film samples (see above Table 3.4). Furthermore, film compositions and formation can have impact on puncture resistance and puncture extensibility of the film sheeting (Anderson and Simsek, 2019b; Cazón et al., 2020). Thus, the modification of the AX film surfaces tends to enhance puncture resistance, resulting in ductile or flexible AX films that can be used for food packaging materials.

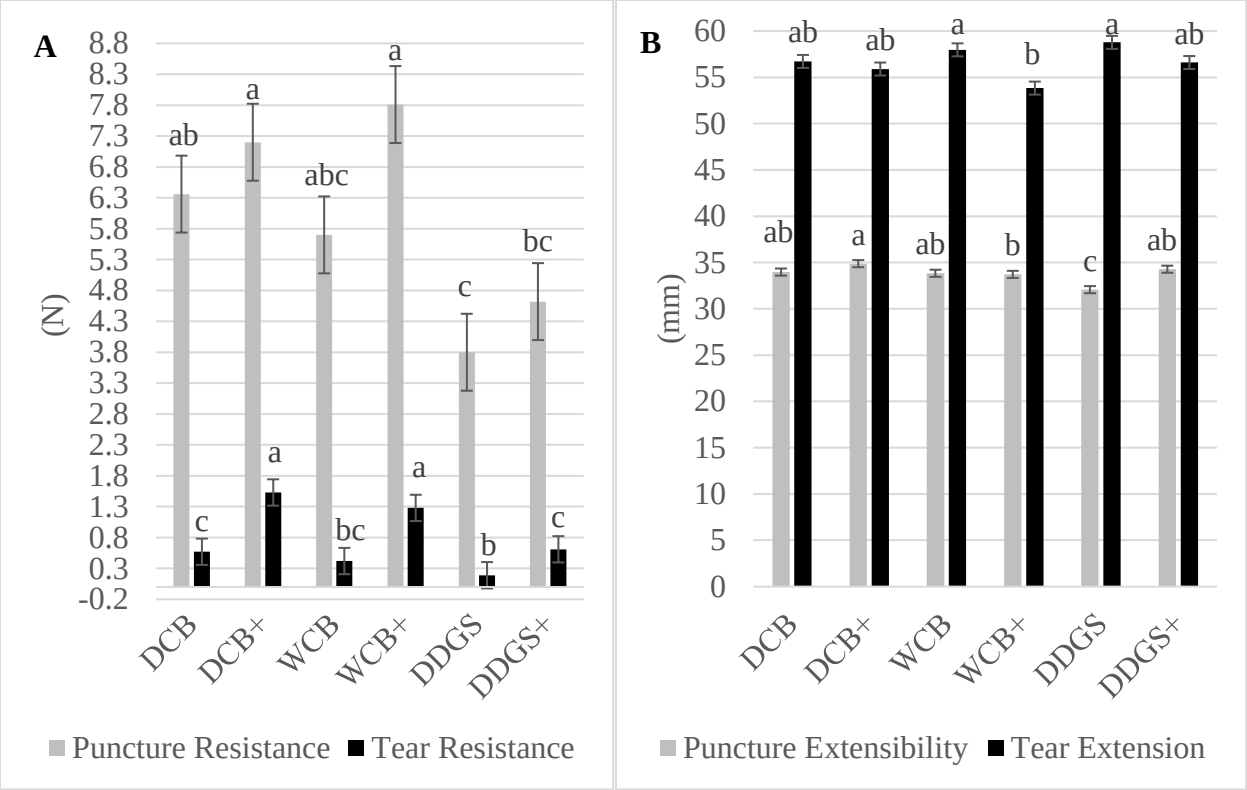

Figure 3.6. Interactions of tear and puncture resistances of unmodified and modified AX films. A: tear and puncture resistances. B: Tear and puncture extensibilities. Means with the same letter in the same box are not significantly different (P>0.05). The data are the means of three independent replicate experiments (n = 3). +: AX film that suspended lipase-acetate.

There was no significant (P>0.05) interaction for the puncture resistance of either the modified or unmodified AX films from one source (see above Figure 3.6. A). However, all modified AX films increased in puncture resistance compared to their unmodified AX films. Interestingly, there is a relationship between the surface film cracks and puncture resistance. SEM detected high surface cracks, which can decrease the film's puncture resistance. The unmodified DCB AX and WCB AX films had more surface cracks and lower puncture resistance, leading to a failure of ductile, elastomeric films. In contrast, modified DCB AX and WCB AX films had fewer surface cracks and thus demonstrated higher puncture resistance (see above Figure 3.4 and Figure 3.6. A). Low surface cracks and high puncture resistance provide more flexible and pliable films used in food packaging. There was only one significant (P<0.05)

interaction for the puncture extensibility of the modified and unmodified DDGS AX films (see above Figure 3.6. B). When the AX films were prepared with lipase-acetate, they had higher puncture resistance and puncture extensibility.

3.4.8. Structural Analysis

An FTIR analysis was performed for chemical compounds and functional groups contained in the AX films utilized in food packaging materials. FTIR spectra of modified and unmodified AX films from DCB, WCB, or DDGS showed a broad spectrum from 600 cm^{-1} to 3700 cm^{-1} (Figure 3.7 A, B, and C). A signal from 3265 cm^{-1} to 3277 cm^{-1} was related to O−H stretching present in the AX films and indicated hydroxyl groups (Figure 3.7 A, B, and C). The spectra also exhibited the uptake of C=O stretching at the wavenumbers from 1637 cm^{-1} to 1642 cm^{-1}, resulting in identification of a carbonyl group (Figure 3.7 A, B, and C). The characteristic band in the spectra appeared from 2917 cm^{-1} to 2935 cm^{-1} showing the existence of C−H stretching present in AX films, while the band from 1407 cm^{-1} to 1413 cm^{-1} demonstrated C−H bending in the AX films (Figure 3.7 A, B, and C). These bands were similar to those in a study conducted by Zhang et al., 2011.

In addition to hydroxyl group (OH), two functional groups were found in the AX films: carbonyl (CO) and ester compounds. Thus, the presences of OH, CO, and ester compounds in a plant-based film suggested that the films included good biodegradability properties (Behera et al., 2022; Rusianto et al., 2019). The absorption of OH, CO, and ester compounds in the modified DCB AX films was higher than the unmodified DCB AX films (Figure 3.7. A). However, the absorption of these functional groups, OH, CO and ester in the modified WCB AX films were not different from unmodified WCB AX films (Figure 3.7. B). There was a decrease in OH and increase in CO and ester compounds in modified DDGS AX films compared to

unmodified DDGS AX films (Figure 3.7. C). Overall, these results indicated that the DCB AX and DDGS AX films modified by suspension in lipase-acetate had excellent biodegradability properties.

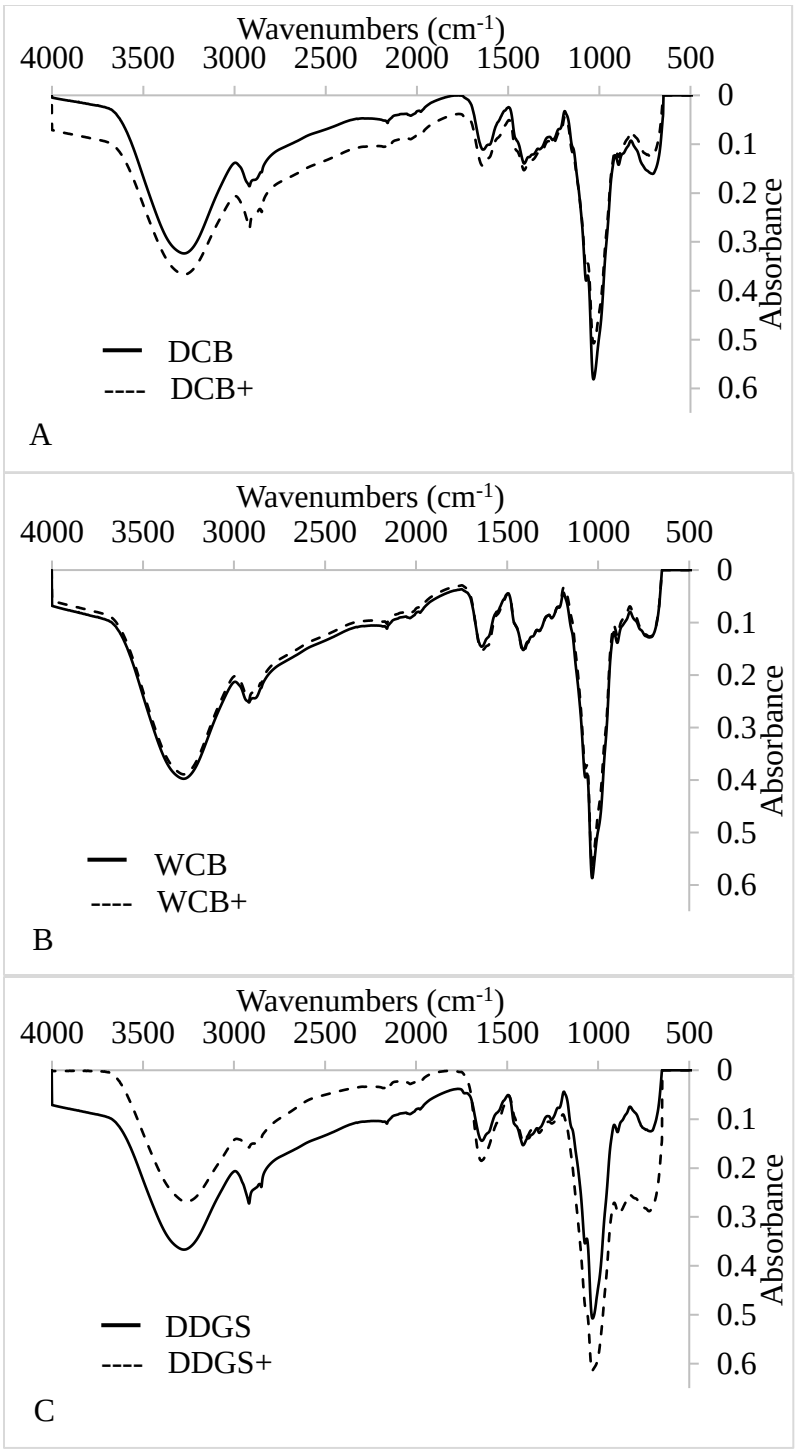

Figure 3.7. FTIR spectroscopy profiles for unmodified and modified AX films made with: corn bran from dry-milling (A), corn bran from wet-milling (B), dried distiller's grains with solubles (C).

3.5. Conclusion

The proximate compositions of DCB, WCB, and DDGS showed high discrepancies among the samples because of how they were processed: dry-milling, wet-milling, or ethanol production. The higher yield of AX from WCB as opposed to the DCB AX and DDGS AX was likely due to the difference in the processing level. The moisture, ash, protein, starch, AX polymer, A/X, and M_W of the AX extracts resulted in significant variances based on the three sample sources. Corn dry-milling separates the main physical fractions of the grain: germ, bran, and endosperm. However, corn wet-milling separates the kernel's main chemical compositions of starch, protein, fiber, and oil. Ethanol production requires more steps, such as fermenting and distilling, which make the procedure difficult to extract pure AX from DDGS.

The AX films made from DCB and DDGS were modified by suspending these AX film surfaces in lipase-acetate mixture, which increased the thick sheeting of both AX films. The modified AX films of WCB exhibited a similar thickness to the unmodified film samples. In addition, the tensile properties of modified AX films created from DCB and DDGS were significantly increased compared to their unmodified AX films. The modified AX films of WCB improved in tensile properties compared to unmodified AX films. The AX film surfaces were affected by suspending them in lipase-acetate mixture, making the modified films ductile, bendable, and more resistant to deformation when stretched compared to unmodified AX films. Surface modifications of AX films made from all three sample types (DCB, WCB, and DDGS) significantly enhanced their tear resistance, indicating an increase in AX film material flexibility and pliability. Puncture resistance was also increased, although insignificantly, for the modified AX films compared to unmodified AX films made from DCB, WCB, and DDGS. Overall, modified AX films with high mechanical properties (tensile strength, tear tolerance, and puncture

resistance) resulted in stronger packaging materials. Finally, the modified AX films made from DCB showed better physical and mechanical properties compared to AX films made from WCB and DDGS.

4. BIODEGRADABILITY AND WATER INTERACTIONS OF ENZYMATIC-CHEMICAL MODIFIED BIODEGRADABLE FILMS FROM CORN BYPRODUCTS

4.1. Abstract

Non-biodegradable plastic materials are hazardous to the environment and cause pollution. Therefore, arabinoxylan (AX) films were created for potential use in food packaging due to the need to replace non-biodegradable plastics. The water interaction characteristics of the biodegradable AX films were evaluated after extracting AX from corn bran byproducts of dry-milling (DCB), wet-milling (WCB), and dried distiller's grains with solubles (DDGS) using acid-alkali procedure. Packaging materials were then created using the three AX extracts combined with laccase and sorbitol as the basis for each film. These three films were modified by immersing their surfaces in a lipase-acetate solution. Moisture content of modified AX films made from DCB, WCB, and DDGS was significantly decreased ($P<0.05$) compared to the unmodified AX films. Water solubility of modified DCB AX films was significantly reduced ($P<0.05$); however, water solubility of modified WCB AX films was insignificantly decreased ($P>0.05$) compared to their unmodified AX films counterparts. Water vapor permeability of modified AX films created from WCB and DDGS were significantly reduced ($P<0.05$), compared to their unmodified AX film counterparts. Conversely, modified DCB AX films decreased insignificantly in water vapor permeability ($P>0.05$), as opposed to unmodified DCB AX films. The contact angle of unmodified and modified AX films was not significantly ($P>0.05$) different from one film to another. Since a contact angle with > 90° assumes a low wettability of a film's surface, the contact angles of all AX films accounted for > 100°, making AX films hydrophobic materials. The biodegradation rates of modified WCB AX and modified DDGS AX films increased after 63 and 99 days, respectively, compared to their unmodified AX

films counterparts. Thus, the hydrophilic nature of AX polymers of WCB and DDGS enhances biodegradation rate of the surface films when incubated in the lipase-acetate mixture. This study found that modified WCB AX film was more hydrophobic and modified DDGS AX film was more biodegradable than modified DCB AX film.

4.2. Introduction

Corn is one of the three most commonly produced grain crops in the world, including wheat and rice (Delcour and Hoseney, 2010d; García-Lara and Serna-Saldivar, 2019). As a primarily agricultural crop, corn has a significant economic value and global impact on food supply, for both human nutrition and animal feed. One byproduct of the corn industry is corn bran (CB), which comes from either dry-milling (DCB) or wet-milling (WCB) procedure. Additionally, a coproduct of ethanol production using corns is dried distiller's grains with solubles (DDGS). While DCB, WCB, and DDGS are low-cost ingredients and are considered as wastes of some industries, these materials can be utilized for innovative food packaging materials, which could be replaced by the non-biodegradable plastics.

The CB is a byproduct made via dry-milling or we-milling operations that CB is often used for animal feed because it is undesirable for patent flour (Apprich et al., 2014; Rose et al., 2010a). The dry-milling and we-milling operations generate DCB and WCB, respectively, which are not the same in terms of color and chemical compositions. The corn dry milling procedure contains tempering, conditioning in warm water, heating, drying, grinding, and sifting, which rubs the corn particles to remove the germ and bran, resulting in endosperm-flour (Anderson and Almeida, 2019; Anderson and Lamsal, 2011). On the other hand, the corn wet milling procedure separates the corn kernel into the grain's main chemical compounds of starch, protein, oil, and fiber-bran. This process is comprised of steeping in 0.2% SO_2, drying, grinding into coarse

grounds, separating germ, reducing into finer particle sizes, separating bran, removing gluten, washing, and screening into starch flour (Rausch et al., 2019; Zhang et al., 2021). CB's chemical composition is mainly made up of dietary fibers: higher in arabinoxylan and lower in cellulose (Agger et al., 2010; Carvajal-Millan et al., 2007). AX has a higher proportion of insoluble fiber and a much smaller proportion of soluble fiber (Dikeman et al., 2006).

DDGS are a coproduct of processing corn but are obtained from the manufacturing for ethanol production instead of corn milling industry (Zarrinbakhsh et al., 2013). Ethanol production technologies have recently improved the conversion of polysaccharides to alcohol. DDGS are mostly used as an animal feed that produce from corn grains by the following steps: grinding in-between 3 to 5 mm, slurrying by adding water, liquefying with amylolytic enzymes, fermenting, distilling, centrifuging, and drying (Chen et al., 1999; Lin and Tanaka, 2006; Torija et al., 2003; U.S. Grains Council, 2018). The fermentation is a key step using the *Saccharomyces cerevisiae* yeast, which converts sugars to ethanol at 32 ^{o}C for 12 h (Pretorius, 2000; Torija et al., 2003). Then, ethanol is collected by distillation columns and the residual fluids and solids are called whole stillage, which is centrifuged to remove coarse solids from the liquid (U.S. Grains Council, 2018). The coarse solids are called wet cake and are subjected to a drying process that yield DDGS, which after drying includes 30 g kg^{-1} protein, 15 g kg^{-1} cellulose, and 20 g kg^{-1} hemicellulose; half of the hemicellulose is AX (Xiang et al., 2014; Zarrinbakhsh et al., 2013).

AX is a complex molecule consisting of a linear xylose backbone of D-xylanopyranosyl (Xyl*p*) residues attached by β-1,4-glycosidic bonds that include arabinose (Ara*f*) substituents (Correia et al., 2011; Rumpagaporn et al., 2015; Zhang et al., 2014). AX structure in corn is that of a β-(1,4) linked Xyl*p* backbone at C (O)-2 with 4-O- methyl-D-glucuronic acid and at C (O)-2 and/or C (O)-3 positions of Ara*f* residues (Agger et al., 2010; Correia et al., 2011; Saeed et al.,

2011). Most of the phenolic acid found in CB is ferulic acid (FA), which is esterified to AX, but FA is presented at the C (O)-5 position of Ara*f*. In addition, although FA is found in small amounts, FA has important impacts on the molecular weight, solubility, and gel capacity of AX in the CB and after separation (Kale et al., 2010; Kale et al., 2013; Rumpagaporn et al., 2015).

The FA has crosslink AX molecules: dimers and trimers, which are commonly presented in CB. Because of this, AX is subdivided into water extractable (WEAX) and water unextractable (WUAX) fractions. WUAX is naturally crosslinked within the cell wall. However, WEAX is not crosslinked because its ester bonds can be broken with an alkaline treatment, leading it to be soluble in water and resulting in a viscous solution (Rumpagaporn et al., 2015). Thus, in turn, WEAX can also exhibit strong gelatinous characteristics because of the oxidative crosslinking of FA moieties (Kale et al., 2013). AX can be extracted from DCB, WCB, or DDGS using several methods including acid-alkali extraction, which is a good method for estimating AX and evaluating AX characteristics (Anderson and Simsek, 2019b; Xiang et al., 2014).

A film made by extracted AX is usually brittle, causing the film to be fragmented when handled, leading to low extensibility and flexibility of the film. Water interaction characteristics of AX films must be low, namely moisture content, water solubility, and water vapor transmission rate, for elongating the shelf life of the packaged foods (Anderson and Simsek, 2019a; Hansen and Plackett, 2008). In contrast, contact angle and biodegradability have to be high in order to increase hydrophobicity of AX films (Anderson and Simsek, 2019a; Antoniou et al., 2014). These water barrier properties of the films generated from DCB, WCB and DDGS for use in the food packaging manufacturing to produce desirable and biodegradable packaging materials.

Plasticizers, often sorbitol or glycerol, are utilized to decrease brittleness and improve the AX film's flexibility, and both are recognized as safe compounds, humectants, and non-carcinogenic (Antoniou et al., 2014; Robertson, 2013b). Sorbitol or glycerol can significantly affect the characteristics of packaging materials; while films made with glycerol include good mechanical properties such as an extension and resistance, films containing sorbitol have a high hydrophobicity for food packaging materials, making them suitable for a packaged product (Anderson and Simsek, 2019a; Antoniou et al., 2014).

The AX casting film might be modified with a lipase to increase film's hydrophobicity, with the lipase suspended in vinyl acetate (Stepan et al., 2013). Thus, the use of lipase tends to reduce the acetate amount needed for the esterification reaction (Bengtsson et al., 2003; Stepan et al., 2013). Lipase has a high level of reaction specificity in hydrolyzing ester bonds, leading to the esterification of AX (Kontkanen et al., 2004; Sakai et al., 2008; Utsugi et al., 2008). This lipase activity can show an esterified monosaccharide or oligosaccharide of AX, resulting in the developing of biomaterial film (Stepan et al., 2013).

The aims of this research were to use AX extraction to create biodegradable film materials, to determine how the AX films interact with water and soil (in aerobic biodegradation), and to evaluate if these interactions are associated with the properties of AX polymer. Biodegradable food packaging material has been established through the mixture of AX extraction, laccase, and sorbitol (Anderson and Simsek, 2019b); in this research the surface of casting AX film was immersed in a reagent of acetate with lipase.

4.3. Materials and Methods

4.3.1. Collecting and Milling

Milled DCB was bought from Archer Daniels Midland (ADM) Company, Decatur, IL. The WCB specimen was also purchased from ADM, but this sample needed to be ground in the laboratory. The DDGS specimen was bought from Elk Mound Seed Company, Elk Mound, WI. Each DCB, WCB, or DDGS sample had three replications. The color of DCB flour was brown, but the WCB specimen was ground to yellow flour using a UDY miller, cyclone sample mill from UDY corporation. The sample of DDGS was milled to sticky flour using the UDY miller. Then, the milled DCB, WCB, and DDGS specimens were added in plastic bags and stored in a freezer until use for polymer extraction.

4.3.2. Proximate Composition

The proximate analysis of DCB, WCB, or DDGS flour and their extracted AX were the same as those utilized in Chapter Three on page 43, but listed briefly here for reference. Table 4.1 demonstrates the official procedures for analysis of proximate compounds including non-official method for lipid analysis. The analyses performed contained: moisture content (AACC International, 1999d), total ash content (AACC International, 1999c), total protein content (AACC International, 1999b), total starch content (AACC International, 1999a), and TDF, SDF, and IDF (AOAC, 2022.01). The lipid content of DCB, WCB, and DDGS flours was determined according to the non-official method using a hexane.

Table 4.1. Official methods of proximate compositions excepted lipid analysis.

Parameter	Method name	Method number
Moisture	Moisture Air-Oven	AACCa Method 44-15.02
Ash	Ash-Basic	AACCa Method 08-01.01
Protein	Crude Protein-Combustion	AACCa Method 46-30.01
Total starch	Megazyme Enzyme Assay Kit K-TSTA	AACCa Method 76-13.01
Total DF, SDF, IDF	IDF/SDF ANKOM Dietary Fiber Analyzer	AOACb 2022.01*
Total lipid	Use solvent organic: hexane	Non-official*

[a] American Association of Cereal Chemists. [b] Association of Official Agricultural Chemists.
*Only for DCB, WCB, and DDGS specimens.

4.3.3. Arabinoxylan Extraction

The extraction of the AX from the milled DCB, WCB, and DDGS specimens is the same as that described in Chapter Three on pages 44 and 45. There were three main steps of AX extraction: defatting, acid-alkali extraction, and ethanol fractionation that procedure is explained concisely here for reference. To begin the extraction, the three samples were defatted by hexane that DCB, WCB, or DDGS flour was mixed with hexane 1:10 w/v (Anderson and Simsek, 2019b). The hexane-flour solution was twice stirred at 900 rpm, each stirring for 1 h at 25 °C using a LED Digital Stirrer (see above in Chapter Three Figure 3.1). Arabinoxylan was extracted from the defatted DCB, WCB, and DDGS flour using 0.25M HCl and 3.0M NaOH before centrifuging to obtain solubilized arabinoxylan. (Figure 3.1). The 50 g of defatted DCB, WCB, or DDGS were placed to 500 mL of 0.25M HCl and stirred at 450 rpm on a hot plate set to 75 °C, holding the slurry temperature at 45 °C for 2 h. Then, 100 mL of 3.0M NaOH was poured to the slurry, while maintaining stirring and heating for another 2 h. The solution of DCB, WCB, or DDGS specimen was neutralized to pH=7 using concentrated HCl. The solution was centrifuged at 4945 rpm for 10 min, and the supernatant was collected. The AX fraction was precipitated with 95% ethanol for 1 h at 25 °C with 900 rpm, and it was then collected by centrifugation (6600 rpm, 10 min) before drying in an oven at 50 °C (see above in Chapter Three Figure 3.1).

Recording a weight of the pellet AX sample (from DCB, WCB, or DDGS) after each extraction and storing it in the freezer were addressed.

4.3.4. Arabinoxylan Characteristics

The Ara*f* and Xyl*p* ratio of DCB, WCB, DDGS, or AX samples were determined by High Performance Anion Exchange Chromatography-Pulsed Amperometric Detection (HPAEC-PAD). 1 mL aliquot of 1M HCl was separately added to 5-6 mg of each sample to certify an adequate time for removing the acidic solvents by evaporation. (Nagel et al., 2014). The samples were incubated in the heating blocks at 100 ^{o}C for 1 h. The samples were cooled and neutralized using 1 mL of 1M NaOH to simplify the separation of Ara*f* and Xyl*p*. After that, the solutions were filtered through a 0.2 μm nylon syringe filter, and the sugar compounds of filtered samples were determined using HPAEC-PAD with a CarboPac PA20 column according to the method of Nagel et al. (2014). Sugar compositions of DCB, WCB, DDGS, or AX samples were calculated according to the formula 1 mentioned above in page 45.

Weight average molecular weights (M_W) and polydispersity index (PI) were determined by HPSEC-MALS-RI (Nishitsuji et al., 2022). The system was Shimadzu HPLC coupled to a Wyatt Opti lab RI detector and Wyatt MALS Dawn Heleos II. Separation was done using two columns (PL Aquagel-OH 40 and 60) connected in series at a flow rate of 0.5 mL/min. The mobile phase used was 0.05% sodium azide in double distilled water. The extracted AX of DCB, WCB, or DDGS was dissolved in 50mM $NaNO_3$ (2 mg/mL) for 16 h, and the solution was filtered through a 0.45-μm filter. The 100 μm of sample was injected to the HPSEC-MALS-RI system. The dn/dc value was 0.146 that M_W and PI were calculated using Astra 6.1.6 software. The light scattering model was Zimm with a fit degree of 1.

4.3.5. Casting and Modifying of AX Film

The procedure used for film solution and casting are mentioned in detail in the Chapter Three on pages 46 and 47; however, a brief description is given here as well. To start the modification of the film solution, film casting was done in deionized water including D-sorbitol (Anderson and Simsek, 2019b) with a slight modification (see above in Chapter Three Figure 3.2). The film solution was produced by making a 2.7% (w/v) of AX extraction from DCB, WCB, and DDGS specimens in deionized water with laccase from *Aspergillus sp.* Laccase (130 µL/2.7% AX film solution) was added to create cross-linked gel formations (Kala et al., 2013). The AX solution was stirred for 24 h at 25 ^{o}C using a Cimarec i Poly 15 Stirrer. Then, the solution was incubated for 15 min at 90 °C in a water-shaking bath. D-Sorbitol (98% powder hygroscopic) was placed as a plasticizer to AX solution at 25% (w/v). After adding D-sorbitol, the solution was heated for 15 min at 90 °C in the water-shaking bath. Then, the AX film solutions from DCB, WCB, and DDGS were casted onto a Nunc Square BioAssay Dish and dried at 60 °C overnight in an air oven (VWR® Forced Air Ovens) (Figure 3.2). Once dried, all AX films were maintained at Boveda 50-60% relative humidity (RH) and at 25 ^{o}C in a dry keeper desiccator cabinet. Each unmodified AX film from per sample included three replications. Each modified AX film also had three replications.

In order to increase hydrophobicity of the AX films, the surfaces of the DCB, WCB, and DDGS samples were constantly incubated into lipase-acetate mixture (Stepan et al., 2013). The lipase was suspended in vinyl acetate and the modified AX films were suspended in the reaction mixture at 40 ^{o}C for 24 h in a closed system (Stepan et al., 2013). After 24 h, the surfaces of the modified films were washed multiple times using methanol and hexane and dried in the fume hood for 10 min (see above in Chapter Three Figure 3.2). Finally, both the modified and

unmodified AX films were stored at 50-60% RH and 25 ^{o}C; finally, their water interactions and biodegradability were first evaluated and then compared.

4.3.6. AX Film Moisture Content

The moisture content of each modified and unmodified AX film (from DCB, WCB, or, DDGS) was measured by heating these packaging materials within a short time and high temperature condition (Garcia et al., 2004). To start the moisture procedure, the initial weight of each AX film was recorded. Next, the film was heated at 130 °C for 1 h in an air oven (VWR® Forced Air Ovens). After 1 h, the film was cooled in the desiccator for 30 min, and then the final weight of each film was recorded. Thus, the moisture content of the AX film was determined by the equation 2:

$$Film\ moisture\ content\ (\%) = \frac{\text{Initial film mass} - Final\ film\ mass}{\text{Initial film mass}a} \times 100 \qquad (2)$$

4.3.7. AX Film Water Solubility

The water solubility of AX film was determined according to previous studies (Garcia et al., 2004; Anderson and Simsek, 2019a). Firstly, each film was cut into a 2 cm × 3 cm piece, and the initial weight of AX film was recorded. Next, the piece of film was placed into Fisher brand plastic centrifuge tube, and 40 mL of distilled water was poured in the capped centrifuge tube. The tubes were shaken at 25 °C, 70 rpm for 1 h using a Lab Shaker Rocker. Then, each film-water solution was filtered through filter paper using a vacuum. The remaining solid material was dried at 25 ^{o}C overnight in the fume hood. A day later, the filter papers were folded and dried at 60 ^{o}C for 6 h in an air oven (VWR® Forced Air Ovens). The final weight of the film was determined, and the percentage of water-soluble film was calculated using the equation 3:

$$Film\ water\ soluble\ (\%) = \frac{\text{Initial film mass} - \text{Final film mass}}{\text{Initial film mass}a} \times 100 \qquad (3)$$

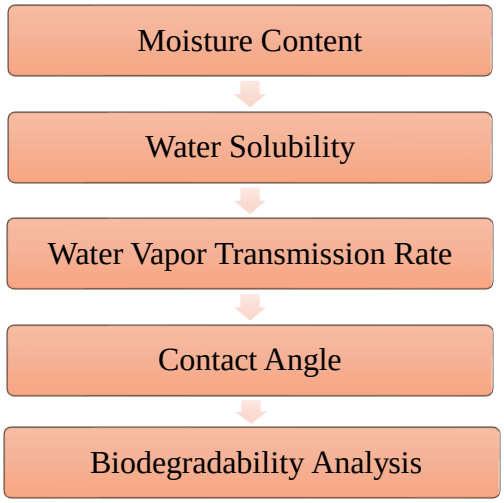

Figure 4.1. Determination of the water interaction and biodegradability properties of AX films.

4.3.8. Water Vapor Transmission Rate

The water vapor permeability experiment had two parts: the water vapor transmission rate (WVTR) and water vapor permeance (WVP), which were measured by following the ASTM E96/E96M-22a$^{\varepsilon 1}$ (ASTM International, 2022b). The water method was utilized, and the testing condition was at 25 ^{o}C and 50-60% RH for each modified and unmodified AX film. During testing, the specimen weights were determined by an analytical balance scale, recording the weight at 0, 30, 60, 90, 120, 150, 180, 210, and 240 min, including 24 and 48 h (Anderson and Simsek, 2019a). The tools of the test contained: 10 mL distilled water, 15 × 60 mm polystyrene petri dish, and two steel washers (with inner diameters of 3.2 cm and outer diameters of 5.7 cm) to hold the 3 cm x 4 cm AX film piece. The parafilm wax was used to secure the sample's steel washers and seal the entire apparatus. The equations 4 and 5 below were used to evaluate WVTR and WVP, respectively.

$$\text{WVTR (g h}^{-1}\text{ m}^{-2}) = \frac{G}{tA} = \frac{(G/t)}{A} \quad (4)$$

Where: G = weight change in grams, t = time which G happened in hours, G/t = slope of G per h, A = test area (inner diameter mouth area). In this study A = 8.19 ft^2.

$$\text{WVP (g/s m}^2\text{ Pa)} = \frac{WVTR}{\triangle p} = \frac{WVTR}{S(R^1 - R^2)} \quad (5)$$

Where: WVTR = water vapor transmission rate (g h^{-1} m^{-2}), $\triangle$p = vapor pressure difference in (mm Hg), S = saturation vapor pressure at test temperature in (mm Hg), R_1 = relative humidity at the source, R_2 = relative humidity at the vapor sink. In this study S = 19.84, R_1 = 1 and R_2 = 0.619702.

4.3.9. Contact Angle

The contact angle was determined by distilled water dispensing one drop of the water on each AX film by following ASTM D7490-13 (ASTM International, 2022a). The testing condition was at 25 ^oC and 50-55% relative humidity for each modified and unmodified AX film. The measurement of contact angles was performed by a Dynamic Contact Angle Analyzer including a charge coupled device camera (Anderson and Simsek, 2019a). One drop of distilled water was applied on the surface of cleaned AX film by a syringe, and the camera rapidly took a picture of the drop's angles. The contact angles were measured by the ImageJ software program in the range from 0° to 180° based on the angles of the drop.

4.3.10. Biodegradability Analysis

The biodegradability analysis for each modified and unmodified AX film was performed in two replicates using soil under alkaline condition (Anderson and Simsek, 2019a). This testing was conducted by the following official method ASTM D5988-18 (ASTM International, 2018a). Briefly, 100 g of sandy soil was added into a 12 oz. Clear Round Wide-Mouth Plastic Jar. 300 mg of the AX film was placed in the middle of the soil in the jar, and the film was covered with the remaining soil. An uncapped Clear Round Wide-Mouth Plastic Jar 4 oz. with 20 mL of 1N NaOH was placed on the top of the soil, and the 12 oz. jar was tightly sealed. A control specimen of soil was used as a blank, and the capped jar and blank were stored out of light at 23-25 °C.

Then, carbon and carbon dioxide balance (C-CO_2) production was determined over 168 days. The jar containing NaOH was removed from the soil-film jar on days 6, 12, 19, 26, 33, 41, 63, 99, and 168 to replace with new jar filling 20 mL of fresh NaOH solution. The 3 drops of diluted phenolphthalein were added to used NaOH, which was titrated using 1N HCI solution until 7.75 pH reached. The carbon content of each modified and unmodified AX films needed to be addressed for determining the amount of biodegradability. The equations 6 and 7 below were applied to determine the C-CO_2, leading to calculate percentage of biodegradability of each modified and unmodified AX films.

$$Release\ of\ C\ per\ 100\ g\ CO^2 = (A - B\ )\ (Acid\ Molarity)(Eq.g\ C\text{-}CO_2) \quad (6)$$

Where: A = the initial amount of NaOH utilized in the attached-film in mL, B = the amount of HCI used in titration sample in mL, AM = the molarity of HCI in M, and Eq.g. C-CO_2 = the equivalent gram of hemicellulose. In this study A = 20 mL, Acid Molarity = 1, Eq.g C-Co_2 = 6.

$$Film\ biodegradability\ (\%) = \frac{CO_2\ \text{soil with film} - CO_2\ \text{soil without film (blank)}}{\text{mg C in film}} \times 100 \quad (7)$$

4.3.11. Statistical Analysis

Statistical analysis was completed with SAS 9.4 for Windows, SAS Institute, Cary, NC. The first factor was the source (DCB, WCB, and DDGS), and the second factor was the modification of AX films. For each dependent variable, we used two-way effects Model ANOVA to evaluate the main effects of source and modification of AX films as well as their interaction. In all cases, the interaction was either not significant or if significant, it was of a diverging nature such that the main effects were still interpretable. Fisher's least significant difference (LSD) was utilized for mean separation of the levels of the main effects. A Cell Means model was utilized to evaluate differences among the interaction means, and LSD was utilized for mean separation. All tests were evaluated using the significant level of 0.05.

4.4. Results and Discussion

4.4.1. Proximate Composition of CB

The proximate composition results of DCB, WCB, or DDGS flour were the same as those data in Chapter Three on pages 51, 52, 53, and 54, but mentioned here for reference.

4.4.1.1. Moisture and Ash

The flour samples from DCB, WCB, and DDGS were analyzed for proximate compositions, which demonstrated large variations among the three samples due to processing as dry-milling, wet-milling, or ethanol production (see above in Chapter Three Table 3.1). Moisture was the first analysis completed upon receiving the pre-milled DCB and after the WCB and DDGS were milled by the researcher. The moisture content is usually used as a baseline for determining other compositions such as ash, protein, oil, starch, and fibers (Haynes et al., 2009; McCleary et al., 2012). The DCB had the largest percentage of moisture content at 9.96%, followed by DDGS at 7.96% and WCB at 1.33% (see above in Chapter Three Table 3.1). Hence, the moisture content of the three samples was significantly (P<0.05) different from one sample to another. These DCB, WCB, and DDGS samples varied in moisture content due to processing methods and storage conditions.

Ash is mostly found in the CB, the location of the inorganic, mineral compounds present in the grain (Anderson and Lamsal, 2011). However, ash content is a good indicator of bran contamination in white flour or flour extraction rate (Anderson and Almeida, 2019). The DDGS contained the largest amount of ash content with 4.29% compared to DCB and WCB samples with 0.49 and 0.31%, respectively (see above in Chapter Three Table 3.1). Therefore, the ash content of all specimens was significantly (P<0.05) different from one sample to another. DDGS had a greater ash content because DDGS processing does not include a bran separation step (U.S.

Grains Council, 2018; Zarrinbakhsh et al., 2013). As the rate of flour extraction is increased, the amount of contamination with non-endosperm increases and the ash content increases (Anderson and Almeida, 2019).

4.4.1.2. Protein, Oil, and Starch

Protein facilitates in building structure and support for the corn kernel cell walls (Anderson and Almeida, 2019; Larkins, 2019). Processing affects the protein content of DCB, WCB, and DDGS, as observed in the significant differences of the protein contents in this experiment. The amounts of protein in the DCB and WCB were 4.89% and 9.93%, respectively, much lower than the percentage of protein in the DDGS with 34.45% (see above in Chapter Three Table 3.1). These protein percents were all significantly ($P<0.05$) different amongst the three samples. It is most likely that DCB and WCB included lower proteins because of separating germ in the dry-milling and removing gluten in the wet-milling (Anderson and Lamsal, 2011; Zhang et al., 2021).

Oils are present in small amounts in the CB when the CB flour is contaminated by germ particles (Anderson and Almeida, 2019). Although the second centrifugation step in the ethanol production process extracts oil, the DDGS has a much greater oil content compared to DCB and WCB (U.S. Grains Council, 2018). The results of lipid analysis in this research bears this out. Thus, the oil contents of these byproduct materials followed this trend: 1.32% oil in DCB, 4.66% oil in WCB, 8.82% lipid in DDGS (see above in Chapter Three Table 3.1). These percentages of lipid contents had significant ($P<0.05$) differences from one specimen to another. In the milling process, the heating step might be applied to deactivate the lipid-enzymes, thus maintaining the oil content (Anderson and Almeida, 2019; Rausch et al., 2019).

The endosperm is where the kernel stores almost all the kernel's starch, which is relatively low amounts in the CB byproducts (Anderson and Almeida, 2019). The total amounts of starch in corn grains can differ based on harvesting conditions and milling procedures (Zhang et al., 2021). In this study, the contents of total starch in WCB and DDGS were 5.42% and 6.05%, respectively, both significantly (P<0.05) lower than the 7.03% starch for DCB sample (see above in Chapter Three Table 3.1). It is possible that the CB made from wet-milling contained the lower percentage of starch because of the removing the prolamin-starch matrix (Larkins, 2019). Similarly, the DDGS process requires enzymatic and yeast treatments, leading to more free sugars and less starch. Even though the starch content of DCB was higher compared to WCB and DDGS, it should be in the range of 9-20% as reported in the previous research (Anderson and Almeida, 2019; Zhang et al., 2021).

4.4.1.3. Dietary Fibers

The CB is generally low in moisture, lipid, protein, and starch but rich in TDF, with a greater proportion of IDF and a much fewer SDF (Dikeman et al., 2006). In this study, the results of TDF, IDF, and SDF matched these DF trends in the three samples. The DCB included the greatest TDF content, accounting for 91.88%, followed by WCB with 72.64% and DDGS with 44.51% (see above in Chapter Three Table 3.1). Similarly, DCB had the highest amount of IDF content at 88.81% compared to WCB and DDGS samples with 69.66% and 39.55%, respectively (see above in Chapter Three Table 3.1). Thus, the TDF and IDF percentages of the three samples were significantly (P<0.05) different from one specimen to another. In contrast, SDF amounts in the DCB and WCB were not significantly (P>0.05) different, both estimated at 3% (see above in Chapter Three Table 3.1). However, the SDF in the DDGS was significantly (P<0.05) higher at approximately 5% (see above in Chapter Three Table 3.1). It seems that CB as a by-product of

dry-milling has more DF than WCB or DDGS procedure, leading to the highest DF in DCB. The tempering step of dry-milling process toughens the bran and facilitates the easy separation of the CB from the corn kernel (Anderson and Lamsal, 2011). In addition, CB is a co-product of ethanol production process, which has converted the compositions (protein, sugars and non-starch bound lipids) to ethanol, resulting in the lowest DF in DDGS material.

One of the main DF in CB is AX, determined using HPAEC-PAD. Then, the Ara*f*/Xyl*p* ratio (A/X) was measured. AX is a non-cellulose polysaccharide chiefly found in the cell walls and is mainly made up of two monosaccharides: Ara*f* and Xyl*p* in the fiber (García-Lara et al., 2019; Rose et al., 2010a; Zhang et al., 2014). The AX concentration with its A/X are different and dependent upon the growing condition and corn processing. The total AX contents of the DCB, WCB, and DDGS samples were as follows: 62.26%, 54.25%, and 35.49%, respectively (see above in Chapter Three Table 3.1). In addition, the A/X for the samples were varied at 0.80 for DCB, 0.85 for WCB, and 1.06 for DDGS (see above in Chapter Three Table 3.1). These AX concentrations and A/X values were all significantly different ($P<0.05$) amongst three samples. The estimated percentages of AX content in the DCB, WCB, and DDGS samples were in accordance with the values of TDF (see above in Chapter Three Table 3.1). Thus, TDF increased in the CB as AX increased, in line with previously published study (Agger et al., 2010; Carvajal-Millan et al., 2007). It is likely that the low A/X in the AX of the DCB and WCB samples was due to their low SDF (Zannini et al., 2022).

4.4.2. Composition and Characterization of AX

The proximate composition results of AX extracts from DCB, WCB, or DDGS flour were the same as those data in Chapter Three on pages 54, 55, 56, 57, and 58, but mentioned here for reference.

4.4.2.1. Extracts

Acid-Alkali extraction method was utilized to extract AX from each sample (DCB, WCB, and DDGS) that chemical extraction of AX is shown in the Figure 3.1. Extracted AX was analyzed for proximate composition, AX polymer, A/X, and M_W which resulted in significant variances based on the three samples (see above in Chapter Three Table 3.2). These compositions and characterizations of AX obtained in this research has provided the information about the effectiveness of the acid-alkali extraction method used. The AX yields of DCB and DDGS were around 23.30% and 23.01%, respectively, while the WCB AX accounted for 25.39% (see above in Chapter Three Table 3.2). The yields of AX were not significantly (P>0.05) different from DCB to DDGS, but significantly (P<0.05) different from the AX yield of WCB. AX is extracted from CB where the greatest concentration of AX is in the ranging 25-35% of the total bran (Saeed et al., 2011; Zannini et al., 2022). The higher yield in the WCB AX as compared to the DCB AX and DDGS AX was likely because of the difference in the processing level. Corn dry milling separates the main physical portions of the kernel: germ, bran, and endosperm (Anderson and Almeida, 2019). In contrast, the corn wet milling process separates the grain's main chemical compositions of starch, protein, fiber, and oil (Zhang et al., 2021). Ethanol production procedure needs more steps, such as fermenting, distilling, and centrifuging, which make the process difficult to extract AX from DDGS (Xiang et al., 2014).

4.4.2.2. Moisture and Ash

Moisture of AX extracted from DCB, WCB, and DDGS samples was also analyzed. The moisture content is frequently used as a baseline for measuring other proximate compositions (Haynes et al., 2009; McCleary et al., 2012). The DCB AX contained the greatest moisture percentage of 14.14%, followed by the DDGS AX with 13.85% and the WCB AX with 13.15%

(see above in Chapter Three Table 3.2). Thus, the moisture contents of the AX yields were significantly (P<0.05) different amongst three AX extracts. The moisture contents of these AX extracts increased from the starting flour materials (see above in Chapter Three Table 3.1 and Table 3.2). Therefore, these AX yields from DCB, WCB, and DDGS samples possibly changed in moisture content due to material sources and storage conditions.

The AX concentration is influenced by the mill stream, and a high relationship between ash and AX contents has been found that a sample had low ash content with low AX content (Ramseyer et al., 2011). The DDGS AX had the highest percentage of ash content, 18.36%, compared to DCB AX and WCB AX samples with 5.36% and 5.86%, respectively (see above in Chapter Three Table 3.2). Hence, the ash contents of all AX extracts were significantly (P<0.05) different from one AX to another. The ash remaining in the AX fractions increased from the starting flour materials (see above in Chapter Three Table 3.1 and Table 3.2). Therefore, ash remaining in the AX yields from DCB, WCB, and DDGS followed the relationship that high ash content led to high AX content (Ramseyer et al., 2011). After AX extraction, DDGS AX increased in ash content as it increased in AX polymer.

4.4.2.3. Protein and Starch

Protein supports in building structure of the cell walls in the corn kernel (Anderson and Almeida, 2019; Larkins, 2019). However, acid-alkali extraction procedure of AX consists of disrupting covalent and hydrogen bonds and reducing the CB matrixes, making it low in protein and starch (Cyran et al., 2004). The protein contents of DCB AX and DDGS AX were 6.67% and 6.51%, respectively, which both were significantly (P<0.05) lower than 9.33% protein for the WCB AX extract (see above in Chapter Three Table 3.2). The protein percentages of the extracted AX reduced from both WCB and DDGS flours except for the AX yield of DCB, which

increased (see above in Chapter Three Table 3.1 and Table 3.2). It seems that the use of acid-alkali extraction of AX decreased in protein contents of WCB AX and DDGS AX because of the collapsing protein matrixes in the chemical extraction.

Both the protein and the starch percentages found in the WCB AX and DDGS AX were decreased. However, these two compositions in the AX yield of DCB increased compared to the starting flour materials (see above in Chapter Three Table 3.1 and Table 3.2). The starch content of WCB AX and DDGS AX was about 2.5% each, which was significantly (P<0.05) smaller than 8.83% starch for AX yield of DCB (see above in Chapter Three Table 3.2). As the total starch contents of WCB and DDGS were relatively low in percentage, their AX extracts contained a lower percentage of starch. It is possible that chemical extraction method of AX decreased in starch contents of WCB AX and DDGS AX because of hydrolyzing effect on the starch and releasing more non-polysaccharides using the acid-alkali extraction (Cyran et al., 2004; Zhang et al., 2014).

4.4.2.4. Arabinoxylan Polymers and Molecular Weight

After acid-alkali extraction of AX, the polymer and its A/X were also determined by HPAEC-PAD. The AX concentration with its A/X varies based on the byproduct or coproduct sources and extraction process of AX yields. The AX polymers for the extracted AX of the DCB, WCB, and DDGS samples were different as follows: 76.60%, 77.64%, and 66.68%, respectively (see above in Chapter Three Table 3.2). The A/X were also dissimilar from one to another at 1.02 for DCB AX, 1.20 for WCB AX, and 1.32 for DDGS AX (see above in Chapter Three Table 3.2). The polymer of AX and A/X were significantly different (P<0.05) amongst all three types of extracted AX. The AX polymer and its A/X in the AX fractions increased from the DCB, WCB, and DDGS flours (see above in Chapter Three Table 3.1 and Table 3.2). This

growth of AX polymers is due to the higher yields after acid-alkali extraction, indicating a successful chemical method to extract AX from the bran byproducts (Cyran et al., 2004).

The M_W and PI of the AX yields from all samples were measured by HPSEC-MALS-RI. From the highest to lowest, the M_W of the three AX extracts were 4063667 for DCB AX, 1876333 for WCB AX, and 879133 for DDGS AX (see above in Chapter Three Table 3.2). These AX M_W were significantly different (P<0.05) amongst all three yields of AX. This is most likely due to the high concentration of AX, resulting in higher M_W compared with AX's low content (Kale et al., 2010). The M_W obtained in this research matched this concept when compared to AX contents and their M_W of the AX yields from WCB and DDGS (see above in Chapter Three Table 3.2). Additionally, the low soluble DF of DCB and WCB had low A/X of AX yields (see above in Chapter Three Table 3.1 and Table 3.2). This is probably because of the higher M_W of DCB AX and WCB AX (Acosta-Estrada et al., 2019).

The PI is a measure of the width of the variation in the M_W polymer distribution, which is a ratio between weight average molecular weight and number average molecular weight. Also, PI is a measure of the heterogeneity of M_W polymer as its size functions (Chang, 2005). Same as the M_W, the PI for all AX extracts was significantly (P<0.05) different from one AX yield to another. Thus, the PI of these AX extracts followed this trend from the greatest to the smallest: DCB AX (3.05), WCB AX (2.37), and DDGS AX (1.80) (see above in Chapter Three Table 3.2). As all PI of AX extracts were higher than 1, their M_W were greater than their number average molecular weights (Chang, 2005). These trends indicated that there was more heterogeneity in the three AX extracts from DCB, WCB, and DDGS.

4.4.3. AX Film Moisture Content

The moisture content of a film influences the mechanical properties and the water interactions of a solid film. When the moisture content of AX films increases, they become hydrophilic; however, when the moisture content of the film decreases, it indicates that the AX film has become more hydrophobic (Anderson and Simsek, 2019a). In this research, for the AX sources utilized in the casting films, the moisture content of the DDGS AX films was 17.03%, significantly (P<0.05) higher than that of both WCB AX and DCB AX films, at 15.13% and 14.77%, respectively (Table 4.2). The films made with the DCB AX were the most hydrophobic, while the AX films made from the DDGS were the least hydrophobic. The percentages of moisture amount between the WCB AX films and the DCB AX films were not significantly (P>0.05) different. The presence of water indicates the moisture or water content of the AX films, which can vary depending upon the AX source.

There was a significant (P<0.05) correlation between the incorporation of lipase-acetate in the AX films' formulation and their moisture contents. The moisture content of modified AX films of the three samples was 14.79%, significantly (P<0.05) lower than the 16.50% for all unmodified AX films (Table 4.2). The AX films suspended in the lipase-acetate solution resulted in a lower moisture content and thus an increase in AX film hydrophobicity. The modification of AX films may cause a barrier between water and the hydrophilic nature of the AX chains, resulting in a reduction in the availability of the OH group and, in turn, a decrease in water interactions in the AX chains through hydrogen bonds. This outcome can lead to a decrease in the film moisture content, as has been shown in previous research (Ghasemlou et al., 2011; Salvada et al., 2022).

Interactions between a source and its modification affected the AX film moisture content. A significant (P<0.05) decrease in moisture content was observed when the AX film surfaces were immersed in the lipase-acetate mixture. The moisture contents of all modified DCB AX, WCB AX, and DDGS AX films were significantly (P<0.05) reduced compared to their unmodified AX film samples (Figure 4.2. A). The AX films suspended in lipase-acetate solution were the most hydrophobic, while the unmodified AX films were the least hydrophobic materials. The tendency for increasing moisture content was due to the lack of lipase-acetate incorporation of the unmodified AX films. This is probably because the free OH group is available to interact with water molecules, leading to an AX film structural affinity for water uptake (Ghasemlou et al., 2011; Salvada et al., 2022).

4.4.4. AX Film Water Solubility

Food already has a high moisture content, so packaging it in water-soluble materials is detrimental to both food safety and food quality. The DDGS AX films included the highest solubility in water, accounting for 96.94%, followed by WCB AX films with 87.08% and DCB AX films with 79.92% (Table 4.2). Furthermore, the water solubility percentages of three AX films were significantly (P<0.05) different from one specimen to another. As can be seen in Table 4.2, low moisture contents of AX films produced low water solubility, leading to hydrophobic materials. In this study, the AX component had an impact on the AX film water solubility. This is because A/X decreases as the AX structure increases in crystallinity, which leads to a lower water-solubility in AX films (Heikkinen et al., 2013). Again, in this study, the A/X of CB flours and AX extracts both increased in the DDGS, thus the AX structure indicated small crystallinity, resulting in an increase in water solubility for the DDGS AX films (see above in Chapter Three: Table 3.1, Table 3.2, and below Table 4.2). In contrast, A/X of CB flours and

AX yields decreased in the DCB, therefore structure of AX tended to be more crystalline, leading to a decrease in solubility in water for the DCB AX films (Table 3.1, Table 3.2, and Table 4.2).

The water solubility was also evaluated for AX film surfaces which were submerged in a solution of lipase-acetate. A significant (P<0.05) decrease in water-soluble films was observed for the modified DCB AX, WCB AX, and DDGS AX films compared to unmodified DCB AX, WCB AX, and DDGS AX films. The water solubility of modified DCB AX, WCB AX, and DDGS AX films was 81.61%, significantly (P<0.05) lower than the 94.35% water solubility for unmodified DCB AX, WCB AX, and DDGS AX films (Table 4.2). Water solubility of these unmodified AX films increased without suspending their surfaces in the lipase-acetate mixture. A relationship between an increase in the value of the water solubility present in the AX films and an increase in the existence of disubstituted xylose could be because of more spaces between the polymers and water (Zannini et al., 2022). After arabinose units are removed, disubstituted xylose is left. Then water enters and dissolves the materials, leading to the high solubility of AX films (Anderson and Simsek, 2019a; Zannini et al., 2022). Submerging the AX films in lipase-acetate solution resulted in a low water solubility, which in turn leads to more hydrophobic films and increased structural strength of food packaging materials.

The statistical interaction between the water solubility of the modified DCB AX films was significantly (P<0.05) decreased compared to its unmodified AX films (Figure 4.2. B). The water solubility for modified WCB AX films were reduced, but not significantly (P>0.05) different from its unmodified AX films (Figure 4.2. B). This decrease in the water solubility for both modified AX films means that they may be appropriate for hydrophobic materials for food packaging. Film low solubility and high hydrophobicity are essential characteristics that indicate

more water-tolerance for food packaging materials (Salvada et al., 2022). There was a correlation between film morphology and film water solubility that indicated cracks may be an entryway for water into the polymer matrix, leading to the film dissolving. Research bears out these results of the AX film morphology and water solubility (Salvada et al., 2022). The modified DCB AX and WCB AX films had lower surface cracks, resulting in lower water solubility for both modified DCB AX and WCB AX films compared to their unmodified AX films (see above in Chapter Three Figure 3.4 and see below Figure 4.2. B). The water solubility of the modified DDGS AX film remained unchanged as opposed to the unmodified DDGS AX films (Figure 4.2. B). This is possibly because of the AX hydrophilic nature of the DDGS AX films. Higher water film solubility influences the commercial purpose of the packaging materials because aqueous food can ingest the film (Behera et al., 2022).

Table 4.2. Determination of water interactions of unmodified and modified AX films.

Factors	AX films	Moisture content	Water solubility	Water vapor transmission rate	Water vapor permeance
		(%)	(%)	(g h^{-1} m^{-2})	(g/s m^2 Pa)
Sources	DCB	14.77a	79.92a	52.24a	6.92a
	WCB	15.13a	87.08b	48.85b	6.47b
	DDGS	17.03b	96.94c	50.42b	6.68b
Modification	Unmodified	16.50a	94.35a	53.08a	7.03a
	Modified	14.79b	81.61b	47.93b	6.35b

Means with the same letter in the same column for the same sample type are not significantly different (P<0.05). The data are the means of three independent replicate experiments (n = 3).

4.4.5. Water Vapor Transmission Rate

The water method was used to measure the AX film water vapor transmission rate (WVTR) and water vapor permeance (WVP). WVTR is the steady water vapor flow in time through the area of flat material under specific temperature and humidity between two parallel

surfaces. WVP is the time rate of water vapor transmission via the area of flat material induced by vapor pressure difference between two specific surfaces. A timed water method (every 30 min for 4 h, then at 24 h and 48 h) measured the vapor movement rate through the AX film from the distilled water to 23-25 ^{o}C. Because the 48 h measurement is the most effective for confirming the evaluation of WVTR and WVP, the following results section focuses on the measurement of water vapor permeability at 48 h. From the lowest to highest, the AX films WVTR from WCB, DDGS, and DCB were 48.85, 50.42, and 52.24 g h^{-1} m^{-2}, respectively (see above Table 4.2). The difference between the WVTR of the WCB AX films and the DDGS AX films was not significant (P>0.05); however, both were significantly (P<0.05) lower from the WVTR of the DCB AX films (see above Table 4.2). Similarly, the WVP of the WCB AX, DDGS AX, and DCB AX films were 6.47, 6.68, and 6.92 g/s m^2 Pa, respectively (see above Table 4.2). The difference between the WVP of the WCB AX films and the DDGS AX films was insignificant (P>0.05); however, both were significantly (P<0.05) decreased from the WVP of the DCB AX films (see above Table 4.2).

Generally, the WCB AX films were the least permeable to water vapor; thus, these hydrophobic films have a good potential for future biodegradable materials. In fact, films low in WVTR and WVP are hydrophobic substances, thus minimalizing their interactions with water (Ciannamea et al., 2018). In contrast, the DCB AX films were the most permeable to water vapor, resulting in hydrophilic and hygroscopic films. The increase in WVTR and WVP can be attributed to hydrophilic nature of AX and the heterogeneity of the AX films made from several sources (Salvada et al., 2022; Weng et al., 2021). Water vapor permeability could also be increased due to the random irregularities, such as bobbles and voids, during the film casting and drying process. These irregularities allow water to enter through films, resulting in decreased AX

matrix molecular density—which in turn, facilitates more water vapor diffusion and more water interactions (Salvada et al., 2022; Weng et al., 2021).

The addition of the lipase-acetate solution onto the surfaces of the AX films was followed by decreases of both WVTR and WVP since the incorporation of a lipase-acetate mixture can hinder water molecules and decrease water vapor transport. Thus, the WVTR of the unmodified AX films was 53.08 g h^{-1} m^{-2}, significantly (P<0.05) decreased to 47.93 g h^{-1} m^{-2} for the modified AX films (see above Table 4.2). Correspondingly, the WVP of the unmodified AX films was 7.03 g/s m^2 Pa, significantly (P<0.05) decreased to 6.35 g/s m^2 Pa for the modified AX films (see above Table 4.2). These decreases of WVTR and WVP can be related to an increased hydrophobic character due to the presence of oil droplets within the film forming (Ciannamea et al., 2018). It is likely that the additional lipase-acetate mixture could enhance the moisture barrier characteristics of the modified AX films. Materials with low WVTR and WVP are more resistant to moisture transfer between packaged food and the surrounding atmosphere, reducing microbial spoilage and prolonging the food shelf life (Naidu and John, 2020; Salvada et al., 2022). There was a significant (P<0.05) correlation between thickness and water vapor permeability. An increase in thickness in the modified AX films led to a decrease in the both WVTR and WVP of the modified AX films.

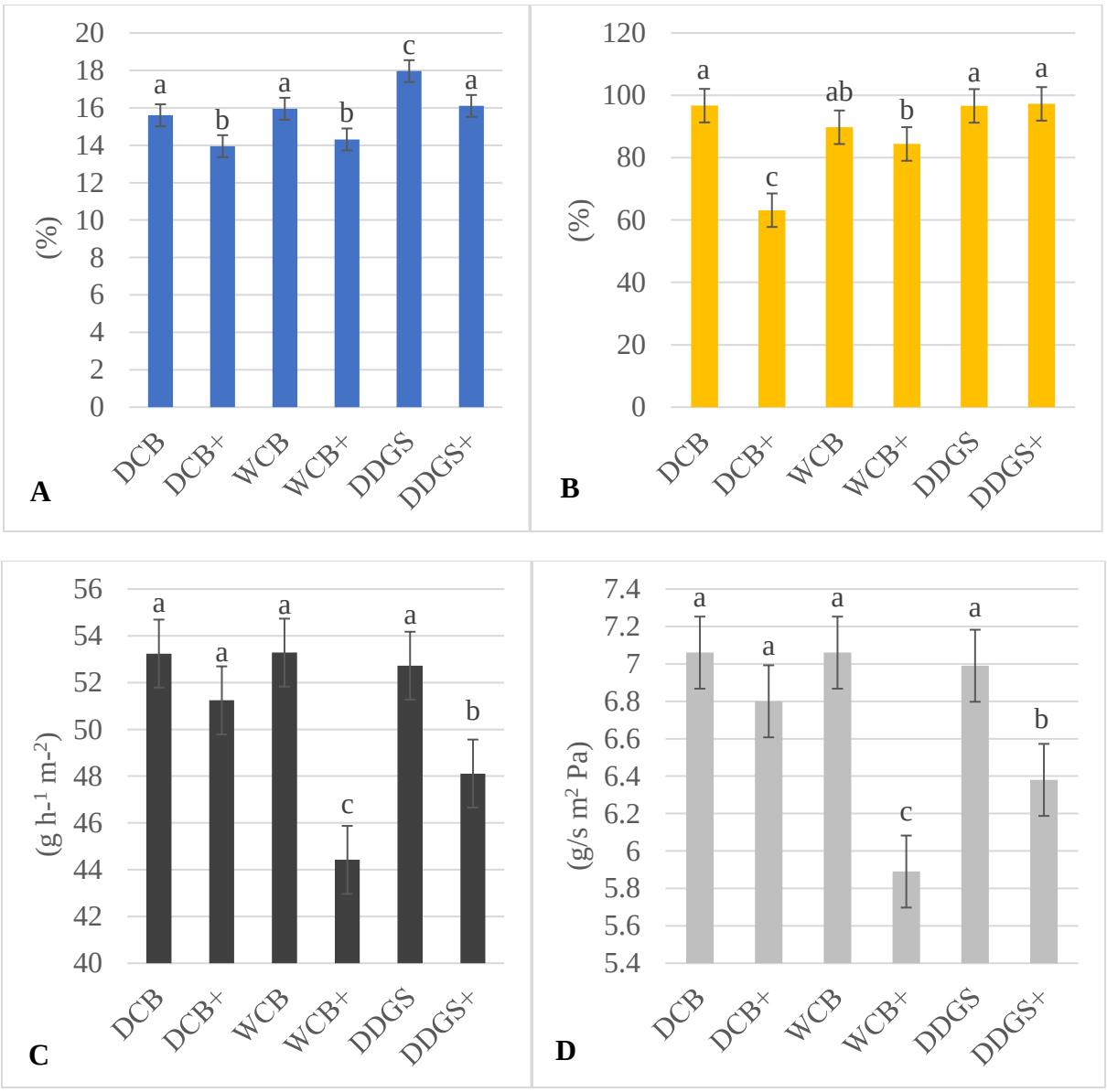

Figure 4.2. Water interaction evaluations of unmodified and modified AX films. A: Moisture content. B: Water solubility. D: Water vapor transmission rate. C: Water vapor permeance. Means with the same letter in the same box are not significantly different (P>0.05). +: AX film that suspended lipase-acetate.

The overall result of the interactions in WVTR and WVP was observed for all three types of AX films. The WVTR and WVP of modified WCB AX and DDGS AX films were significantly (P<0.05) reduced compared to their unmodified films (Figure 4.2. C and D). However, the WVTR and WVP of modified DCB AX films were also decreased, but (P>0.05) insignificantly, compared to the unmodified DCB AX films (Figure 4.2. C and D). It is most

probable that the increases in the thicknesses of both modified DCB AX and modified DDGS AX films resulted in decreases of their WVTR and WVP in this research. As film thickness increases, water vapor permeability decreases creating an equilibrium water pressure on the interior surface of the films (Behera et al., 2022). Hydrophobicity was significantly increased with immersing AX films in a lipase-acetate solution made from WCB and DDGS, while the modified DCB AX films seemed to be hygroscopic materials. The reduction of WVTR and WVP was assumed to be because the AX films suspended in lipase-acetate; AX polymer chains may become less mobile, resulting in less water vapor diffusion, leading to low water vapor permeability. This assumption was in agreement with the water vapor permeability measurement reported (Ghasemlou et al., 2011).

4.4.6. Contact Angle

The contact angles inform us about the hydrophobicity of the solid surface being analyzed. Due to the polarity of water, films have a high degree of content angles, resulting in higher hydrophobic and lower hydrophilic materials appropriate for food packaging (Salvada et al., 2022). The WCB AX films were the most hydrophobic with average contact angles of 109.46°; the DDGS AX films were the next hydrophobic with an average contact angle of 108.87°; the DCB AX films were the least hydrophobic with an average contact angle of 106.11° (Table 4.3). Therefore, the contact angle of all films was not significantly (P>0.05) different from one sample to another. There was an important correlation between the A/X ratio of AX extract and the contact angle of the AX films with water (Anderson and Simsek, 2019a). As A/X ratio of AX extract decreased in the DCB, the disubstituted xylose or branching on the AX polymer increased and the DCB AX films became more hydrophilic (see above in Chapter Three

Table 3.2; see above Table 4.2; and see below Table 4.3). A decrease in the AX substitution can lead to an increase in hydrophobicity of the AX films (Anderson and Simsek, 2019a).

For both unmodified and modified AX films, the contact angle changed minimally when the surfaces of the AX films were immersed with a lipase-acetate solution. The contact angle of unmodified AX films was 109.86°, not significantly (P>0.05) higher than the contact angle of 106.43° for the modified AX films (Table 4.3). Water was used to determine contact angle because the contact angle is very sensitive to surface contamination (ASTM International, 2022a). Thus, like other studies, the contact angle precision in this study was not recorded by laboratory testing; however, for this study, the measurement of the contact angle on a solid surface was based on general disciplinary practice (Briassoulis and Giannoulis, 2018). Figure 4.3 provides the pictures of contact angles of unmodified and modified AX films; it was difficult to measure precisely the contact angles and wettability of these AX films. When the hydrophilicity of plant films improves, the capacity of the water absorption for the film seems to be increased (Behera et al., 2022). Depending upon the contact angle results, the hydrophobicity of the AX based-films suspended in the lipase-acetate solution reduced, which tended to increase the water absorption capacity of the modified AX films.

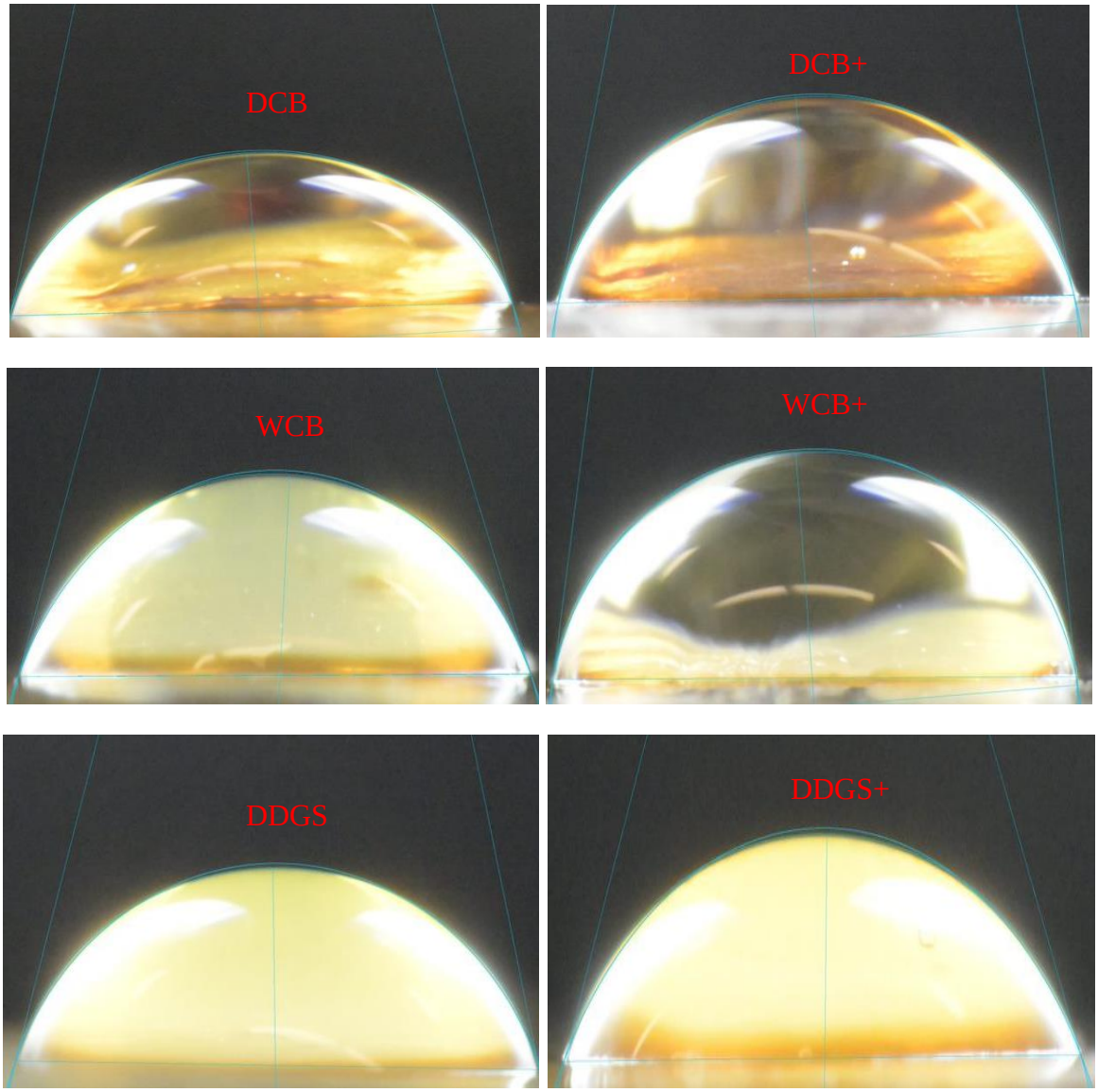

Figure 4.3. Contact angle pictures of unmodified and modified AX films. +: AX film that immersed lipase-acetate.

The contact angle value of modified DCB AX films was 105.15°, but was not significantly (P>0.05) reduced compared to the contact angle of 107.08° for the unmodified DCB AX films (Table 4.3). Similarly, the contact angle value of modified WCB AX films was 103.23°, but it insignificantly (P>0.05) decreased compared to the contact angle of 115.70° for the unmodified WCB AX films (Table 4.3). This suggests that the suspended lipase-acetate AX film surfaces were more hydrophilic with a higher affinity for water than their unmodified film

surfaces. It is most likely that both modified DCB AX and WCB AX films had smooth surfaces, which was an indicator for their hydrophilic films (see above in Chapter Three Figure 3.4 and see below Table 4.3). The film roughness or smoothness plays an essential role in the precision of contact angle measurement and water interaction between particles (Salvada et al., 2022). In contrast, the contact angle value of modified DDGS AX films was 110.92° but increased with no (P>0.05) significant difference compared to the contact angle of 106.81° for the unmodified DDGS AX films (Table 4.3). Thus, the surface of DDGS AX films was affected by its suspension in the lipase-acetate mixture; thus, the modified DDGS AX films were hydrophobic with a lower affinity for water. Since a contact angle with > 90° assumes a low wettability of a film's surface (Salvada et al., 2022), the AX films in this study were clearly hydrophobic materials with low wetting tendencies.

4.4.7. Biodegradability

Soil is a species-rich source of inoculum for the determination of biodegradability of the packaging materials. Also, the biological activity of the soil is considerable under alkaline condition when maintained the oxygen availability. In this study, the biodegradability of AX films was evaluated by determining the amount of CO_2 produced over period of 6 months that AX films were incubated in sandy soil. C-CO_2 content of all AX films is presented in the Table 4.3, showing no significant (P>0.05) differences regarding the three factors: sources, modification, and interaction. The WCB AX films were found to be the most biodegradable material with average of 36.09%, followed by DCB AX films accounting for 31.82%; however, the DDGS AX films were the least biodegradable material with amount of 28.91% (Table 4.3). Consequently, the biodegradability of WCB AX films was significantly (P<0.05) different from the biodegradability of DDGS AX films. There was a critical correlation between the

hydrophobicity and the biodegradability of the AX based-films. As the WCB AX films had the highest hydrophobicity based on the contact angle results, the WCB AX films showed the greatest biodegradability amongst all samples (Table 4.3). An increase in the heterogeneity of the AX polymers can provide a decrease in the biodegradability of the AX films due to the lack of the microbial breakdown of the films (Anderson and Simsek, 2019a). Similarly, the DDGS AX films included the lowest biodegradability as the AX polymers from DDGS had more ingredients (proteins, lipids, free sugars) which are more heterogenous in the films.

No improvement was found in the biodegradability when the surfaces of AX films were suspended in the lipase-acetate solution. The percentage of biodegradability of the modified AX films was 32.24%, but not significantly (P>0.05) different form the amount of biodegradable material at 32.31% in the unmodified AX films (Table 4.3). This outcome demonstrates that both unmodified or modified AX films were biodegradable and ecological amicable, this can facilitate low toxicity in the materials used for food packaging. The film biodegradation is associated with the film water solubility as higher solubility leads to greater biodegradation of films by the microbial organisms in the soil (Leon-Bejarano et al., 2020). Although, based on the AX film sources in this research, the water solubility and biodegradability of the AX films results did not support this correlation. This study also found that higher the biodegradability, greater is the water solubility of the WCB AX and DDGS AX films when their surfaces were suspended in the lipase-acetate solution (see above Figure 4.2 B and Table 4.3).

Table 4.3. Hydrophobicity and biodegradability of unmodified and modified AX films.

Factors	AX films	Contact angle	C-CO_2	Biodegradability
		(°)	(mg)	(%)
Sources	DCB	106.11a	9.84a	31.82ab
	WCB	109.46a	12.43a	36.09a
	DDGS	108.87a	11.73a	28.91b
Modification	Unmodified	109.86a	11.45a	32.31a
	Modified	106.43a	11.22a	32.24a
Interaction	DCB	107.08a	12.27a	34.35ab
	DCB+	105.15a	7.41a	29.30bc
	WCB	115.70a	14.25a	37.82a
	WCB+	103.23a	10.62a	34.36ab
	DDGS	106.81a	7.83a	24.75c
	DDGS+	110.92a	15.63a	33.06ab

Means with the same letter in the same column for the same sample type are not significantly different (P<0.05). The data of contact angle are the means of three independent replicate experiments (n = 3). The data of biodegradability are the means of two independent replicate experiments (n = 2). +: AX film that suspended lipase-acetate.

The modified DDGS AX films had initially a slower biodegradation rate; however, it increased quickly after 26 days and had a rapid increase after 99 days compared to the unmodified DDGS AX films (Figure 4.4). The biodegradation of modified WCB AX films increased slowly after 41 days, and it gradually improved after 63 days compared to the unmodified WCB AX films (Figure 4.4). It is most likely that the modified WCB AX and modified DDGS AX films increased in the rate of biodegradability because their water solubilities were not significantly (P>0.05) changed when compared to the water solubility of their unmodified AX films counterparts. (see above Figure 4.2 B and see below Figure 4.4). It was observed that the hydrophilic nature of AX polymers of WCB and DDGS improved the rate of biodegradation of their surface films when incubated in lipase-acetate mixture. By contrast, the biodegradation of modified DCB AX films did not increase within 168 days compared to the

unmodified DCB AX films (Figure 4.4). Interestingly, the water solubility of modified DCB AX films significantly decreased (P<0.05) from its unmodified AX films, leading to lower biodegradability (see above Figure 4.2 B and see below Figure 4.4). The rate of film biodegradation is related to the film solubility in water, which means that lower solubility reduces the biodegradability of films (Leon-Bejarano et al., 2020). The AX films in this research were generally biodegradable with modification of the suspending their surfaces in the lipase-acetate solution.

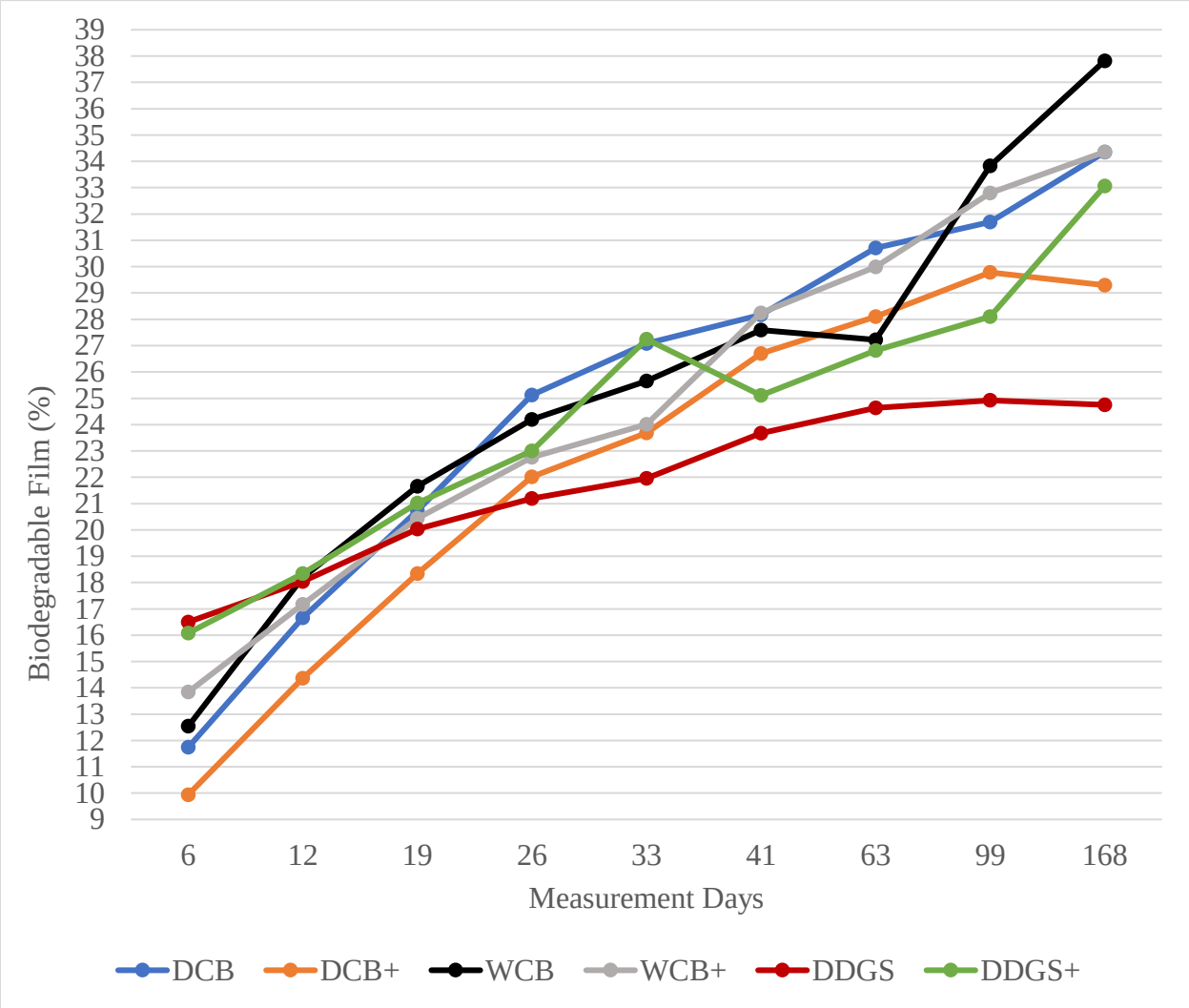

Figure 4.4. Biodegradability profiles for unmodified and modified AX films made with: corn bran from dry-milling (DCB), corn bran from wet-milling (WCB), dried distiller's grains with solubles (DDGS). +: AX film that suspended lipase-acetate.

4.5. Conclusion

The proximate compositions of DCB, WCB, and DDGS exhibited large discrepancies among these samples due to processing by dry-milling, wet-milling, or ethanol production. The greater extract of AX from WCB as compared to the DCB AX and DDGS AX was expected because of the difference in the processing level. The moisture, ash, protein, starch, AX polymer, A/X, and M_W of the AX yields resulted in significant differences based on the three sample sources. Corn dry-milling separates the main physical portions of the kernel: germ, bran, and endosperm. However, corn wet-milling separates the grain's main chemical compositions of starch, protein, fiber, and oil. Ethanol production needs more steps, such as fermenting and distilling, which make the process difficult to extract pure AX from DDGS.

Hydrophilic and non-biodegradable materials can shorten shelf-life foods and are not ecologically amicable. The moisture content of AX films casted from DCB, WCB, and DDGS was reduced by suspending the film surfaces in the lipase-acetate solution, making the modified AX films hydrophobic materials based on the results of moisture contents. Water solubility of modified DCB AX films was significantly reduced and the solubility of modified WCB AX films was diminished, albeit insignificantly, compared to their unmodified AX films. Water vapor permeability of modified AX films made from WCB and DDGS was significantly decreased compared to their AX film counterparts. These decreases in water solubility and water vapor permeability for the modified AX films can help to produce hydrophobic materials for safer food packaging with a longer shelf-life. The biodegradation rates of modified DDGS AX and modified WCB AX films increased after 63 days compared to their unmodified AX films counterparts. Hence, the hydrophilic nature of AX polymers of WCB and DDGS improves the biodegradation rate of surface films when incubated in the lipase-acetate mixture. Overall, the

WCB AX and DDGS AX films were more hydrophobic and biodegradable than DCB AX films with modification of immersing the surfaces in the lipase-acetate solution. In summary, Hydrophobicity and biodegradability of modified WCB AX films provide competent material which can be used for food packaging.

5. SUMMARY AND CONCLUSIONS

5.1. Summary of Outcomes

The proximate compositions of DCB, WCB, and DDGS showed great discrepancies among the samples due to how they were processed: dry-milling, wet-milling, or ethanol production. The moisture, ash, protein, starch, AX polymer, A/X, and M_W of the AX extracts resulted in significant variances based on the three sample sources.

The AX films made from DCB and DDGS were modified by suspending these AX film surfaces in lipase-acetate mixture, which increased the thick sheeting of both AX films. The tensile properties of modified AX films made from DCB and DDGS were significantly increased as opposed to their unmodified AX films. The modified AX films made from WCB had improved tensile properties, albeit insignificantly, as compared to unmodified WCB AX films. Surface modifications of AX films made from all three sample types (DCB, WCB, and DDGS) significantly enhanced their tear resistance, indicating an increase in AX film material flexibility and pliability. Puncture resistance was also increased, but again insignificantly, for the modified AX films as compared to the unmodified AX films made by DCB, WCB, and DDGS.

The moisture content of AX films made from DCB, WCB, and DDGS was reduced by suspending their film surfaces in the lipase-acetate solution, making the modified AX films hydrophobic based on their moisture content. Water solubility of the modified DCB AX films was significantly reduced; the water solubility of modified WCB AX films was also diminished, but insignificantly, as opposed to the unmodified AX films. The water vapor permeability of modified AX films made from WCB and DDGS was significantly decreased compared to the unmodified AX films. The modified AX films made from DCB showed an insignificant reduction in water vapor permeability as opposed to the unmodified DCB AX films. Only

modified DDGS AX films showed an increase in the contact angle value compared to its unmodified films, making the modified DCB AX and WCB AX films less hydrophobic based on the results of contact angles. The biodegradation rates of modified DDGS AX and modified WCB AX films increased after 63 days compared to their unmodified AX films.

5.2. Overall Conclusion

The higher yield of AX from WCB as compared to the DCB AX and DDGS AX was likely due to the difference in the processing level. Corn dry-milling separates the main physical fractions of the grain: germ, bran, and endosperm. However, corn wet-milling separates the kernel's main chemical compositions of starch, protein, fiber, and oil. Ethanol production requires more steps, such as fermenting and distilling, which makes it more difficult to extract pure AX from DDGS.

The surfaces of the AX films were affected by suspending them in lipase-acetate mixture, making the modified AX films ductile, bendable, and more resistant to deformation when stretched compared to unmodified AX films. Modified AX films with high mechanical properties (tensile strength, tear tolerance, and puncture resistance) produced a stronger packaging material. The modified AX films made from DCB showed better physical and mechanical properties, as opposed to modified AX films created from WCB and DDGS.

The decrease in moisture content, water solubility, and water vapor permeability for the modified AX films could lead to hydrophobic materials for safer food packaging with longer shelf lives for food. Contact angle is very sensitive to film surface contamination, and the precision of contact angle has not been documented by laboratory testing. The WCB AX and DDGS AX films were more hydrophobic and biodegradable than DCB AX films with modification of immersing the surfaces in the lipase-acetate solution. The modified AX films

made from WCB could also be used for food packaging materials because of hydrophobicity and biodegradability.

5.3. Future Work

Oxygen gas permeability is very essential to food packaging materials because oxygen can interact with food ingredients leading to oxidation reactions. Food oxidation and spoilage will develop more slowly when oxygen is not present in the food system. To this end, oxygen gas permeability analysis should be performed on AX films suspended in lipase-acetate solution; this kind of work would furnish information about the possibility of using these biodegradable films with an oxygen barrier to package foods. Because AX includes the ability to create a crosslinked network that is extremely dense and has a low molecular mobility (Kale et al., 2013; Vansteenkiste et al., 2004), these AX films can have excellent barrier properties containing low oxygen gas permeability. The standard test method for determining the oxygen permeability of AX films is the American Society for Testing and Materials (ASTM) Method D3985-17 (ASTM International, 2017).

Another additional analysis that should be done is the evaluation of the modified AX film color. Considering aesthetic appeal is essential for food marketing; thus, the color of the food packaging material can be purposefully modified for consumer appeal. There was color variation in the modified AX films in this research. For example, the DCB AX films were darker with brown shades while the WCB AX films were lighter and more yellow. AX film color can be determined by a MacBeth Color Eye test from the CIELab and the Hunter Lab (Salvada et al., 2022). No report was found regarding the yellow and white properties of biodegradable films created from any of the corn bran AX films incubated in a lipase-acetate solution.

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