Unlocking the Value of Canadian Oats: Evaluating Oat Starch By-Product Through Starch Noodle Production

By

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Abstract

The growing plant protein industry necessitates innovative approaches to valorize starch by-products, with Canada emerging as a key player due to its substantial production of pulses and cereal grains. Oats are garnering attention for protein extraction, raising concerns about the sustainability of the industry as the majority of the oat kernel's starch remains underutilized. While oat starch demonstrates desirable properties for various applications, genetic and environmental variations pose challenges to its consistent utilization. Chemically modifying starch offers a potential solution to tailor its properties for specific end uses.

The first study aimed to screen thirty oat cultivars and identify suitable genotypes for starch extraction. The objective of the second study was to analyze native oat starch and produce modified oat starch using citric acid cross-linking, elucidating physiochemical, pasting, and morphological differences. The objective of the last study was to optimize a method for oat starch noodle preparation and compare the quality characteristics of noodles made with native oat starch (NOS) to those substituted with varying levels of citrate-modified oat starch (COS). Subsequently, the digestibility implications of COS addition would be studied.

The first study revealed significant effects of genotype, environment, and their interaction on protein, total starch, and amylose content. Cultivar CDC Morrison was selected for further analysis based on these findings. Study two identified significant differences in NOS and COS, particularly in resistant starch content and pasting properties. Notably, COS exhibited an inability to form a paste, while NOS displayed higher pasting temperature and final viscosity. Chemical modification altered crystalline peaks and morphology, providing insights into their potential application in noodle production. Study three optimized a method for oat starch noodle production and observed that the addition of COS altered cooking time, texture, and colour compared to NOS noodles. NOS noodles exhibited similarities to commercial rice noodles. *In vitro* digestibility results revealed that substituting 25% of NOS with COS reduced glucose release, indicating that it was beneficial in slowing starch digestibility.

The studies collectively offer valuable insights into the valorization of oat starch by-products in the plant protein industry. Representative oat starch with unique properties was obtained through cultivar screening and starch extraction following industrial methods. Chemical modification further enhanced these properties, demonstrating potential applications in noodle production. The successful production of oat starch noodles, including those with modified starch, underscores the potential of oat starch by-products for human consumption. This research provides a novel approach for maximizing the value of oat starch by-products, contributing to the sustainability and efficiency of the plant protein industry.

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Dedication

This thesis is dedicated to my parents Tracey and Brad Alexander.

For their endless support and belief in me. And for their love, that has empowered me to overcome so many challenges and become the person I am today.

Thank you for inspiring me to reach for the stars and for being the pillars of strength in my life.

Contribution of Authors

Experimental Chapters

Chapter 3: Effects of Genotype and Environment on the Physiochemical Properties of Canadian Oat Varieties

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- My role for this experimental chapter included helping to adapt the original project design, performing the experiments, processing the experimental data, and writing of the original manuscript with input from all authors.
- Dr. Kirby T. Nilsen: Providing samples for analysis and contributing to the final version of the manuscript.
- Dr. Sijo Joseph: Planning the work and contributing to the final version of the manuscript.
- Dr. Trust Beta: Planning and supervising the work and writing of the manuscript.
- Dr. Lovemore Malunga: Devising the project, supervising, project funding, aiding in interpreting the results and writing of the manuscript.

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- Dr. Babak Sobhi: Contributing to the design and implementation of the research and editing and reviewing the manuscript.
- Dr. Sijo Joseph: Planning the work and editing and reviewing the manuscript.
- Dr. Trust Beta: Planning and supervising the work and writing of the manuscript.
- Dr. Lovemore Malunga: Developing the original project design, supervising, project funding, aiding in interpreting the results and editing and reviewing the manuscript.

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List of abbreviations

NOS- Native oat starch

COS- Citrate-modified oat starch

SEM- Scanning electron microscopy

XRD- X-ray diffraction

TA- Texture analyzer

NIR- Near-Infrared

GRAS- Generally recognized as safe

RS- Resistant starch

SDS- Slowly digestible starch

RDS- Rapidly digestible starch

RVA- Rapid visco analyzer

CVD- Cardiovascular disease

GxE- Genotype by Environment

DP- Degree of polymerization

Tgel- Gelatinization temperature

HTST- High temperature short time

ANOVA- Analysis of variance

PCA- Principal component analysis

RO- Reverse osmosis

Chapter 1: Introduction

Starch is a major component of cereal grains and an important energy source in the human diet. However, interest in using starch for food applications is minimal due to the negative associations a high starch diet has on human health (Xie & Liu, 2004). Protein is another important component found in cereal grains and has been explored for various food and non-food ingredients. The demand for protein from plants such as from oilseeds, legumes, and cereal grains continually expands. This is causing an increase in the extraction of the protein portion of the plant material to meet growing demands. In cereal grains protein makes up approximately 7-18%, while starch makes up approximately 50-80% (Garutti et al., 2022; Poutanen et al., 2022). With emphasis placed on cereal protein, rather than starch, this creates excess starch by-product that is typically used for animal feed rather than being reincorporated into human foodstuff (Cottee et al., 2022). While the growth of the plant protein industry is a welcome development, it also has the potential to create excess by-product in the form of starch, which if not prioritized for human consumption, can have negative implications on the sustainability of the plant protein industry.

This issue has brought about the opportunity to develop new markets and products to use the cereal grain starch by-product in human foods. Oat (*Avena sativa* L.) is an important crop grown and processed in Canada. In the past, most oat was grown for use in animal feed and only a small portion for human consumption. Interest in oats for human consumption has steadily increased over recent years. The vast nutritional properties of oats are one of the main reasons they are peaking in interest for consumption in the human diet (Rasane et al., 2015). Oats are consumed as a whole grain more often than other common cereal grains, which is why oats can provide many valuable nutrients. More research continues to be done on oat due to the increased awareness of their nutritional properties. As a result, the utilization of oat in the food industry is increasing. In particular, oat protein is gaining popularity as it can be used as a non-animal protein source. Oat contains higher amount of protein (9-20%) than other common cereals such as wheat (8-13%) and corn (8-10%) and the proteins are distributed heterogeneously throughout the grain. Along with this, oat protein is accepted by many consumers due to lack of off flavour produced, which is present in other plant alternatives (Boukid, 2021). However, protein is not the major component of oat, as it only makes up 9-20%. During protein extraction, an alkaline pH (9.2) is commonly used to solubilize the protein which subsequently separates starch from the protein and starch is recovered as the sediment (Boukid, 2021). Starch content in oat can exceed 60%, therefore it is the major component found in oat (Zhu, 2017). This starch is considered a by-product of protein extraction for the plant protein industry and is typically underutilized or repurposed for animal feed (Zhu, 2017). Studies have analyzed the rheological and physicochemical properties of oat starch to understand how it can be applied for food and non-food uses. Oat starch has unique properties such as a small granule size, high lipid content, and amylose and amylopectin ratio variations. The genotype and growing environment heavily influence the variations of these properties and directly impact the rheological and physiochemical properties of oat starch (Rhymer et al., 2005). For instance, oat starch requires less time and lower temperature to reach peak viscosity, which increases efficiency of gelatinization and would be beneficial for industrial applications (Punia et al., 2020). There is limited research on oat starch for food application, as most studies focuse on the utilization of oat flour for products such as baked goods, noodles, and snacks (Aydin & Gocmen, 2011; Galdeano et al., 2009; Punia et al., 2020). Therefore, there is an opportunity to explore novel ways to reintegrate the oat starch by-product coming from the plant protein industry.

Starch noodles, which are commonly consumed in Asia, use starch as the major raw material to produce the noodles. Starch properties heavily influence the quality of noodles and traditionally mung bean starch is used to achieve optimal characteristics. More research is focusing on the application of alternative starches for noodles such as from pea, sweet potato, and sorghum to observe their effect on eating quality (Bangar et al., 2023). Results from these studies agree on the fact that good quality starch noodles are produced from starch with high amylose content, restricted swelling, and small to intermediate sized granules (Bangar et al., 2023). For these reasons, among others, oat starch can be considered for the production of starch noodles. Although oat starch has beneficial properties for starch noodle production there is opportunity to enhance these qualities through the chemical modification of starch.

The chemical modification of starches can be done using different techniques with the most common being cross-linking. Cross-linked starches are used in the food industry to stabilize the granular structure, restrict swelling, and provide nutritional advantages. Citric acid is a common cross-linking agent as it is considered as the least toxic and is inexpensive (Kim et al., 2017). Cross-linking native oat starch using citric acid (resistant citrate starch) has the potential to improve properties such as thickening power, gelatinization properties, textural attributes, and increase resistant starch (RS) content (Hong et al., 2020; Kim et al., 2017; Obadi & Xu, 2021). These can aid in increasing the overall quality of starch noodles and improve the nutritional properties through creation of RS (Kim et al., 2017). Few studies have explored the influence of resistant citrate starch on noodle quality; however, the potential for its application in oat starch noodles has not been studied.

We hypothesize that blending native oat starch with different ratios of modified oat starch will positively influence the quality and digestibility of the starch noodles, that native oat starch alone would not achieve. The objective of the overall thesis is to find a novel application for the oat starch by-product, which is being achieved through the production of starch noodles using blends of native oat starch and modified oat starch (cross-linked using citric acid) to enhance rheological and nutritional properties. The results from this thesis will benefit the plant protein industry by creating a market that can utilize the starch by-product, stabilize a new grain market for Canadian farmers and create new products for consumers.

Chapter 2: Literature Review

2.1. Oat

The oat kernel comprises two main anatomical features, the hull and groat. The hull or husk is the outermost cover that surrounds the oat groat and makes up 20 to 36% of the oat kernel. It contains cellulose, hemicellulose, and lignin making it high in crude fibre (Ashogbon, 2023). Hulls are removed during milling, making it a by-product of oat milling that is typically transformed into fibre-rich livestock feed (Hareland & Manthey, 2003; Menon et al., 2016). The oat groat makes up 65-85% of the whole kernel with a greater proportion of oat groat being the most desirable in terms of milling efficiency and economic value. Within the oat groat there are three main layers, the bran, starchy endosperm, and germ. The bran is the outer layer of the groat which is made up of the pericarp, seed coat, and aleurone cells. The bran contributes a high amount of nutritional value to the oat as it contains a greater concentration of protein, lipid, fibre, vitamins, and minerals (Hareland & Manthey, 2003). This is the portion of the oat kernel that is rich in beta-glucan. The aleurone layers are removed as bran even though they are part of the endosperm which makes up 56-68% of the kernel fractions. The endosperm contains high amounts of digestible carbohydrates mainly in the form of compound and single starch granules. Within the starch compounds there exists two primary polymers which are amylose (linear polysaccharide) and amylopectin (branched polysaccharide) that can vary in amount depending on the oat variety and growing environment (Grundy et al., 2018; Hareland & Manthey, 2003). The germ represents the smallest portion of the oat groat (~4%) and is rich in lipids and hydrolytic enzymes along with some fibre, vitamins, and minerals (Gates, 2004).

2.1.1. History of the Canadian oat industry

Oat (*Avena sativa* L.) was first introduced to Canada by European settlers in the early 17th century. This crop was mainly cultivated for livestock feed, while only a small portion was used for bread baking (Derick, 1953; Prairie Oat Growers Association (POGA), 2023). Canada is now one of the largest producers and exporters of oat worldwide with an expected production and export of 4.3 and 2.65 kilotons, respectively in 2022-2023 (Agriculture and Agri-Food Canada, 2023a). Saskatchewan, Manitoba, and Alberta produce the largest volume of oat in Canada, with Saskatchewan contributing approximately 50% of oat grown each year. The majority of oat produced globally is used for livestock feed; however, as consumers become more aware of the nutritional benefits whole grain oat offers, more oat, especially those grown in Canada, are being used for human consumption (POGA, 2023). However, the current use of oat is still limited in terms of end-products produced for human consumption. However, with more research focused on oat, there is potential to develop novel value-added products intended for human consumption.

2.1.2. Nutritional advantages of oat

Oat contains a wide variety of nutrients, making it an excellent raw ingredient to use in the food and beverage industry. In particular, oat has a good source of carbohydrates, dietary fibres, quality proteins, and high amounts of lipids, phytochemicals, vitamins, and minerals (Rasane et al., 2015). Table 1 shows the approximate availability of nutritional components found in whole grain oat.

Table 1. Nutritional composition of whole grain oat

Nutritional component	Availability (%)	Reference
Starch	51-65	Zhu (2017)
Protein	Total: 12-20	Boukid (2021)
	Globulins: 70-80	Nieto-Nieto et al. (2015)
	Prolamins: 4-15	
	Glutenins: <10	
	Albumin: 1-12	
Lipid	3-11	Zhu (2017)
Dietary Fibre	Beta-glucan: 3-8	
Phytochemicals	Tocotrienols and tocopherols: 0.002-0.003	Peterson (1995)
	Phenolic compounds: 5.7	
Vitamins	Thiamine: 0.002	Rasane et al. (2015)
	Riboflavin: 0.001	
	Niacin: 0.032	
Minerals	Calcium: 0.36	USDA (2019)
	Iron: 0.04	

The nutritional components outlined in Table 1 can contribute various functional and nutritional properties for those who consume products containing whole grain oat. Beta-glucan is a soluble fibre found in oat that consists of linear branched chains of D-glucose monosaccharides units that are joined by a combination of β (1$\rightarrow$ 3) and β (1$\rightarrow$ 4) linkages. Oat beta-glucan is found in the cell wall of the endosperm and is a major active component that provides different health promoting functions such as cholesterol lowering and antidiabetic (Paudel et al., 2021). The protein content in oats is divided among four main classifications: globulin, albumin, glutelin, and prolamin, as seen in Table 1. In oat, protein is mainly located in the germ and the bran and has attracted research and commercial interest since it is a low-cost dietary protein among other cereal proteins (Boukid, 2021; Runyon et al., 2015). Other minor components that promote health include antioxidants like tocols and phenolic compounds. The majority of phenolic compounds are located in the bran layer of the oat kernel and function as a defence mechanism against some pathogens. Consumption of these phenolic compounds has been associated with preventing diseases such as cancers and stroke (Paudel et al., 2021).

Type II diabetes is a common metabolic disorder that affects many people around the world. In 2021, 10.5% of the world population aged 20-79 were living with type II diabetes (Paudel et al., 2021; Sun et al., 2022). This disorder involves the loss of proper insulin function in the body, which results in failure to maintain blood sugar levels. Type II diabetes can be managed, prevented, or delayed with lifestyle changes such as eating nutritious foods (*What Is Diabetes?*, 2023). Various studies have shown that consumption of oat has the potential to reduce postprandial glucose level due to the presence of oat beta-glucan (Paudel et al., 2021). Starch digestibility is reduced by oat beta-glucan, because of its ability to alter the microstructure in food products, reducing starch's gelatinization. Oat beta-glucan is a high molecular weight polysaccharide, which even in low concentrations exhibits a high viscosity and this aids in decreasing the absorption and diffusion of glucose (Paudel et al., 2021). However, the benefits of oat beta-glucan can only be obtained when the whole oat kernel is consumed, which is commonly how oat is consumed, but this may not always be the case.

Foods rich in starch such as cakes, cookies, and pasta typically contain high amounts of rapidly digestible starch (RDS) and low amounts of slowly digestible starch (SDS) and resistant starch (RS), which causes a spike in postprandial glucose levels. Therefore, it is ideal for starchy foods to contain higher amounts of SDS and most importantly RS, as this will reduce the glycaemia level in the body (Zhong et al., 2018). Resistant starch can be found naturally in some food products; however, research is focused on finding ways to increase the RS content in food products naturally or by modification. High amylose starch contains a greater amount of dietary fibre, mainly in the form of RS and therefore can aid in decreasing glycemic response (Lim et al., 1992). Starches can naturally have higher amounts of amylose, while some are genetically altered to grow high amylose varieties. Oat is an example of a type of starch that has an amylose content that can vary from 2 to 38%, depending on the genotype and growing environment (Stevenson et al., 2007). Thus, the utilization of oat starch may be able to provide health benefits similar to oat beta-glucan due to the potentially high amylose content.

Cardiovascular disease (CVD) is common among many Canadians and evidence has shown that diet is a leading cause of CVD (Paudel et al., 2021). In 2010, Health Canada accepted the claim that relates the consumption of beta-glucan oat fibre to the reduction of blood cholesterol, which in turn can reduce the risk of coronary heart disease (Health Canada, 2010). The risk of developing CVD comes from high levels of serum cholesterol and low-density lipoprotein; however, the consumption of oat can help reduce these levels and benefit cardiovascular health. Literature has shown that a diet containing oat products, especially those containing unrefined and whole oat (rich in beta-glucan), can aid in reducing blood pressure and blood lipid profile levels (Paudel et al., 2021). It has also been reported that a diet rich in RS can reduce total cholesterol, total lipids, and plasma triglyceride, among others, which have been proven through various animal studies but few human studies (Ashwar et al., 2016). Oat starch naturally contains a higher amount of RS, which may contribute to the reduction of blood cholesterol as seen with oat beta-glucan. Furthermore, chemical modification of starch can increase resistant starch content in oat and could be considered for the reduction of cholesterol levels. However, more human studies would need to be conducted to better understand the influence of RS in oat on cardiovascular health (Ashwar et al., 2016).

2.1.3. Current novel applications for oat

In the past oats were mainly used in baked goods such as breads, cookies and cakes. The food and beverage industry has been dedicating more research toward using plant materials and with that we are seeing an evolution in the way plant materials are being used. Oats are being utilized in various novel ways to expand the food and beverage industry and to meet consumer demand, which is to have more plant-based alternatives. Some of those advancements include oat-based dairy alternatives and using oat protein for plant-based meat alternatives.

Dairy alternatives were originally produced and marketed towards consumers with cow's milk allergies and lactose intolerance. However, the popularity of plant-based dairy alternatives is continually rising due to its claimed sustainability and health benefits. Cow's are a major contributor to greenhouse gases compared to other animal farming, which has put the dairy industry in a negative view (Martínez-Padilla et al., 2020). The processing of plant-based dairy alternatives involves extracting the plant source with water and removing the sediments from the liquid. Other ingredients such as vitamins, minerals, stabilizers, sugar, and are added to the fluid extraction, and then it is homogenized and pasteurized. Although plant-based beverages are highly processed and have low protein content, they are considered healthier options due to the low saturated fats and cholesterol (Martínez-Padilla et al., 2020). This poses a debate about whether these plant-based beverages are a nutritional substitute for cow's milk, as cow's milk has many nutritional qualities of its own.

For production of plant-based beverages it is important to consider the protein quality, which is determined by the amino acid composition, bioavailability and protein digestibility. Cereals such as oats and rice contain low amounts of indispensable amino acids, which is a predictor of the quality of dietary protein sources. Martínez-Padilla et al. (2020) conducted an analysis on commercial plant-based alternatives to cow's milk to investigate the *in vitro* protein digestibility and fatty acid profile. Their findings showed that the most easily digested samples were cow's milk, almond drink, and oat drink with no significant difference in total digestibility compared to cow's milk. Moreover, soy drinks have the lowest digestibility, which is likely due to their antinutrient content. It is also important to understand the differences in protein intake between plant-based beverages and cow's milk to ensure these alternatives provide an adequate protein source. Some studies have pointed out the fact that plant-based beverages provide protein levels below the recommended intake for growth in childhood (Martínez-Padilla et al., 2020). Martínez-Padilla et al. (2020) suggests that a way to combat this issue and increase protein quality would be to combine plant sources such as legumes with grains or nuts and seeds, which would allow for a complete amino acid profile. Overall, oat beverages have the potential to be considered as a good milk alternative; however, further nutritional research would need to be done.

Trends have shown that the plant-based protein industry will be a leader in helping us meet the growing demand for food (Sesotec, 2020). Plants such as cereals, legumes, and oilseeds contain an abundant source of protein for human consumption and optimal protein composition can be achieved by mixing various plant foods together. Consumption trends are moving toward incorporating plant substitutes into the diet for many reasons such as health, animal welfare and the environment (Iqbal, 2022). These meat alternatives have traditionally been made from soybeans but have expanded into the use of peas, beans, and oilseeds. Cereal grains, mainly wheat, are also used in plant-based foods and currently oats are attracting a significant amount of interest for protein extraction. These meat alternatives are commonly referred to as meat analogs, which are produced with the intention to mimic the texture, flavour, colour, and appearance of animal meat by use of plant proteins (Li et al., 2022). Many benefits exist from the consumption of meat analogs since these are cholesterol free and have low amounts of saturated fats. Development of meat analogs involves the use of texturized vegetable proteins, which are typically produced using high moisture extrusion cooking using a twin-screw extruder. Raw materials used for texturization include protein isolates typically from soy and pea, but recent developments have expanded into the use of lupin, mung bean, hemp, and wheat gluten (de Angelis et al., 2020). Opportunity exists to use a pea-oat protein blend to produce meat analogs with enhanced nutritional value. de Angelis et al. (2020) has explored the use of dry-fractionated pea and oat protein to produce meat analogs using low-moisture extrusion. Their study showed that use of dry-fractionated protein can support the efforts towards efficiently producing sustainable plant-based meat analogues. Dry-fractionated protein had a lower protein content than protein isolates; however, de Angelis et al. (2020) was able to show that the dry-fractionated protein could still produce meat analogues even with a lower protein content. They found that by mixing dry-fractionated oat and pea protein, 55 g of protein was achieved in an 100 g serving size, which is acceptable for meat analogue production.

2.2. Generation of starch by-product from oat protein extraction

Various novel methods are being developed for use of oat in the plant-based food and beverage industry, as previously discussed. Many of these methods focus on using oat protein, which leaves a large portion of the oat unused. Starch is the major component in oat, making up approximately 60% of the kernels dry weight, while protein is approximately 20% (Sowa & White, 1992; Zhu, 2017). Increased research on the topic of oat proteins has attracted the plant-based protein industry to develop ways to utilize said protein. This poses a concern because demand for extraction of oat protein will increase and as a result there will be higher amounts of starch by-product. In the plant protein industry, the starch by-product is not being reintegrated into the mainstream food system, rather it is used in animal feed or discarded (Mäkinen et al., 2016). This contradicts the sustainability efforts set out by the plant-based food industry, as large amounts of waste are still being produced. Oat starch can easily be reintegrated into the mainstream food system to help feed the growing population, meet sustainability efforts, and provide novel and nutritional options to consumers.

2.2.1. Oat protein extraction

Extraction of protein from oat starts from oat raw material which is commonly oat flour or oat bran. These materials are subjected to dry or wet processing to extract protein. Wet extraction is the most popular method to extract plant proteins such as from oat. The method of wet extraction, illustrated in Figure 1, involves the use of aqueous solution to extract protein and centrifugation or decantation to fractionate the protein.

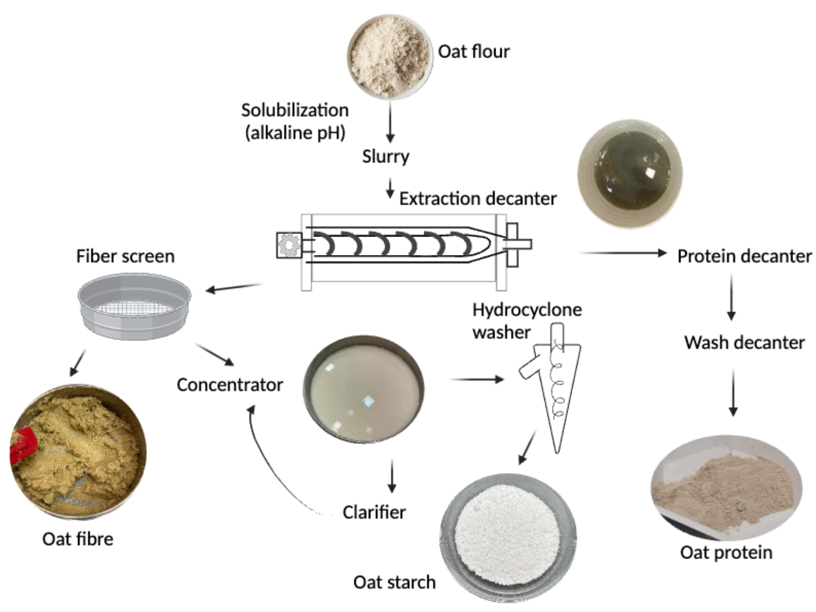

Figure 1. Oat protein extraction with starch and fibre recovery. Created with BioRender.com

The first step involves blending the raw material into water at alkaline pH to make a slurry. Through actions such as centrifugation, decanters, hydrocyclones and separators, the protein is separated from the other components, which are mainly starch and fibre (Alfa Laval, 2023). Protein is purified following extraction such as by precipitation and ultrafiltration. This is possible based on isoelectric precipitation, which depends on the dissolution of proteins in the alkaline solution (Chandran et al., 2023). The precipitated proteins will be separated from solution by use of decanters and separators. From here, the separated protein is heat treated to lower the risk of microbial contamination, while maintaining the nutritional and functional properties of the protein (Alfa Laval, 2023). During the extraction process the starch/fibre is recovered by separation and drying, making starch and fibre a by-product of the protein industry. This by-product is utilized by some industries such as for animal feed, while for others it becomes a wasted material.

The use of alkaline pH (pH > 9.0) for protein extraction results in an increased yield and purity of oat protein (Chandran et al., 2023; Cluskey et al., 1976; Kriger et al., 2018; Spaen & Silva, 2021). The recovery of oat protein can also be dependent on the starting raw material. For instance, Liu (2014) found that by use of wet extraction for oat protein at acidic pH, a protein recovery of 60.4-72.1% was obtained. This range was mainly due to the oat starting material, which had either been defatted or not. However, other studies have shown that extraction of oat protein at alkaline pH yields a higher protein content compared to acidic pH extraction. This is evidenced by studies that have obtained a protein recovery between 54.6-13.0% using acid pH (pH< 6) (Cluskey et al., 1976; Kriger et al., 2018).

In the process of oat protein extraction, the biggest by-products are fibre and starch, as shown in Figure 1. These constituents are effectively separated from protein at alkaline pH, which can be further processed by passing the fibre and starch blend through a sieve to get isolated fibre and starch can be further clarified. Lim et al. (1992) found that soaking oat flour in an alkaline pH solution made using sodium hydroxide, yielded starch with a low protein content (0.3-0.4%) and high recovery (90-95%). Thus, protein extraction at alkaline pH is also ideal for recovery of oat starch and fibre, which can make it easier for these by-products to be utilized by other industries.

2.3. Influences of genotype and environment on oat properties

The increasing popularity of oat for human consumption can be partially attributed to the nutritional qualities it expresses. With increasing knowledge about the dietary benefits associated with consumption of the oat grain, demand for the raw material increases. There is emphasis for breeders to develop specific oat cultivars that meet nutritional requirements set out by the food industry. Along with this, it is also important to choose oat cultivars that have the milling quality desired by the milling industry (Howarth et al., 2021). Various factors can contribute to the variation seen in the yield and physical and chemical quality of cereal crops; however, growing environment and genotype are typically the most influential.

2.3.1. Oat grain yield and quality

The quality and yield of an oat crop are a major determinant of value to the producer, which can be affected by climate fluctuations, genotype and/or their interactions. Yield and quality are typically quantified using various measurements of grain characteristics that define milling quality, which primarily include groat content and ease of hull removal, also known as hullability. These characteristics are closely related to the yield extracted at the mill and milling efficiency (Howarth et al., 2021). For instance, an oat cultivar with poor hullability may require a greater impact force to separate groat from hull during the dehulling phase and this can lead to more groat breakage and lower yield extracted. Therefore, it is important to improve the physical properties of the oat grain to ensure higher yields and quality; however, this only comes from understanding how genotype and environment impact these characteristics.

Some difficulties that arise when developing new oat varieties to meet consumer and producer demand is that while one trait may be optimized, this typically comes with the cost of other traits being compromised. Howarth et al. (2021) explained that this is the case for plant breeders when trying to improve beta-glucan content, high groat content, and high yield in oat. Another important aspect to consider when developing oat varieties is their adaptability in different environments. For a variety to be deemed successful in terms of quality and quantity, plant breeders must also test the degree of interaction of said variety in various growing environments. Therefore, analysis of the genotype (G), environment (E) and genotype by environment interaction (GxE) is crucial to evaluate quality of oat varieties. Many sources of variance need to be considered when analyzing quality of cereal grains using GxE. These can include but are not limited to growing year, soil type, latitude, longitude, genotype, weather conditions, and crop management. Environmental factors usually have a big influence on yield and the different quality parameters of oat and other cereal grains. Studnicki et al. (2016), observed how the yield and quality traits of spring wheat is affected by genotype, environment, and crop management. They defined quality traits as test weight, protein content, sedimentation value, wet gluten content, and starch content. Their results showed that depending on the soil conditions, yield and quality traits were affected differently. Climate can be highly correlated with grain yields and quality factors. In North America, warm spring weather is favourable to produce high yields of oat. This allows for early planting and the seeds develop at an accelerated pace. On the other hand, high temperatures in the night during the summer months (July and August), when the plants are in final stages of grain development, negatively impact oat yields, as mentioned by

Doehlert et al. (2001). These results show that studying GxE interactions is crucial when selecting an oat genotype that will maximize the genetic potential of the genotypes for breeders and farmers (Studnicki et al., 2016).

2.3.2. Oat groat chemical composition

The evaluation of oat composition is crucial for understanding groat quality. The major indicators of quality include protein, oil, and beta-glucan. Variation in composition can be seen in oat groats, which is usually attributed to genotype and environment (Doehlert et al., 2001; Howarth et al., 2021). Protein composition is typically ranked as the most important indicator of quality. Compared to other grains, oat protein is more nutritionally significant, making it a crucial determinant of quality. Studies have shown that both genotype and environment can impact protein concentration. For instance, nitrogen supply has been shown to affect the protein concentration that is found in oat (Doehlert et al., 2001).

Oil content in oat is higher than in most other grains. The amount of oil desired in the oat depends on the intended industry, which makes it an important factor to consider. In the animal feed industry, oat with higher oil content is desired as this gives a higher caloric content for the animal feed. For food applications, a high oil content can be detrimental, as this increases chances for rancidity and production of off flavours (Doehlert et al., 2001). It has been reported that environmental conditions such as temperature have an impact on oil content. In particular, cooler temperatures have shown to increase accumulation of oil in oat groats (Doehlert et al., 2001; Howarth et al., 2021). Oil and protein content have been debated to be negatively correlated among different genotypes. However, cultivars with both high oil and protein content have been developed (Doehlert et al., 2001).

In recent years, various studies have looked at the application of beta-glucan coming from oat due to the associated health benefits. Cereal beta-glucans are a dietary fibre that make up 2-8% of dietary fibre in oat and can aid in lowering cholesterol and providing anti-cancer properties (Zhu, 2017). A high beta-glucan content is therefore, important for selection of an ideal oat cultivar for producers and consumers (Yan et al., 2016). It is known that a strong interaction exists between beta-glucan content and genotype; however, the same cannot always be said for environmental effects. One study has shown that there was a significant interaction between nitrogen and location, and nitrogen and year, that affected beta-glucan content in oat groats. In contradiction to this, another study showed nitrogen having no significant effect on beta-glucan content (Brunner & Freed, 1994; Humphreys et al., 1994). In a more recent study, it was found that beta-glucan content was significantly affected by environment, genotype, and their interaction for eight oat cultivars grown in 20 locations across western Canada (Herrera et al., 2016).

The major storage component found in oat is starch. The greatest value in oat is said to come from the non-starch components, as previously discussed, which means starch concentration is not always considered when evaluating the quality of oat (Doehlert et al., 2001). However, as industries become more interested in using all parts of the grain to reduce waste, then starch content in oat becomes an important parameter for measuring quality. According to a study done by Doehlert et al. (2001), the amount of starch found in an oat groat was positively correlated with warm growing temperatures as well as precipitation. This signifies that environmental conditions have a strong influence on the amount of starch that can be produced in an oat groat. Starch accumulation may also impact protein concentration, as noted by Doehlert et al. (2001). Their results showed that high precipitation later in the growing season stimulated starch accumulation in the oat groat which in turn, diluted the protein concentration. This inverse relationship may be due to the washing away of remaining nitrogen from the root zone and is supported by other research (Rhymer et al., 2005; Silveira et al., 2016). The effects of genotype on total starch vary between different studies. However, most results show that genotype has a significant effect on total starch; however, the dominance over the environmental effect differs (Doehlert et al., 2001; Herrera et al., 2016; Rhymer et al., 2005).

2.3.3. Final product quality

Selection of genotype and growing environment thus far has shown to be crucial for the growers in terms of ease of production and yield and for obtaining a desirable physical quality and chemical composition for processing and further applications. With that said, for these selected components to be optimized, the final products made need to be attractive for consumers (Hüttner et al., 2011). This means that selection of oat genotype and growing environment requires careful consideration not only for the needs of breeders and producers but also how they affect final product quality to meet consumer's needs. Thus, allowing the production and consumption of oats to increase in a sustainable way.

Selection of the optimal oat variety and growing location depends on the end use of oat. For instance, the use of oat for bread making requires a selection of different properties compared to oat used for flakes. It was determined by Hüttner et al. (2011) that oat varieties with lower protein content resulted in a better bread product likely due to less disruptions in the starch gel during baking. They also found that oat varieties with a higher fat content improved bread quality, as it acts to increase the resistance to deformation of the batter. In comparison, selection of oat for production of oat flakes have different considerations. Hydration capacity of oat flakes is one property that needs to be considered, as rapid hydration is more desirable for final product quality. Rhymer et al. (2005) found that genotype, environment, and their interaction significantly affected oat flake hydration capacity, and oat varieties that produced smaller flakes had an increased rate of hydration.

Oat based products have received considerable attention over the years mainly due to their well-known health benefits. Thus, selection of oat varieties that contain higher nutritional components such as beta-glucan, polyphenols, and vitamins is very important, considering their nutritional quality is one of the main attractions for producers and consumers irrespective of the end-product (van den Broeck et al., 2015). Therefore, the GxE effects need careful consideration for meeting producer and consumer needs in terms of quantity and quality.

2.4. Oat starch

Starch is the main component found in oat kernels and exhibits many unique properties, which gives it the potential to be used in various food and non-food applications. Compared to starch from other cereal grains, oat starch has unique chemical, physical, and structural properties. These include smaller granule size, high lipid content, higher crystallinity values, and an increased content of short-chain amylose (Punia et al., 2020). Amylopectin and amylose are two polymers of glucose that are the main carbohydrate components of oat starch granules. Amylose is the linear polymer that is bonded by α (1,4) glycosidic bonds, while amylopectin has dense branching and shorter (1,4) straight chains, branched by α-D- (1,6) linkages. The properties associated with the amylose and amylopectin molecules along with the proportions contribute to the diversity of oat starch composition (Kasturi & Bordenave, 2013).

Starches have the ability to form pastes and gels when water and heat are added. This process involves the swelling and thus disruption of starch granules, which leads to the formation of three-dimensional networks of amylose and amylopectin. When the formed starch gel or paste begins to cool, amylose and amylopectin undergo retrogradation, which is the process of reorganization. The gelatinization, retrogradation, and pasting properties of starch vary depending on the source and the molecular characteristics of starch. The mentioned properties are what make starch a great option for use as food ingredients such as to stabilize emulsions and modify texture (Kasturi & Bordenave, 2013).

2.4.1. Molecular analysis and characterization

On a molecular level, as previously mentioned, starch is comprised of two polymers of glucose: amylose and amylopectin. Amylose is a straight, non-branched chain composed of D-glucopyranosyl residues linked through α-(1,4)-bonds. However, it has been demonstrated that amylose may occasionally feature extended branches composed of α-D-(1,4)-glucopyranosyl chains connected to the main chain through α-(1,6) linkages (Kasturi & Bordenave, 2013). Amylopectin is an extensively branched glucan, comprising short chains of α-D-(1,4)-glucopyranosyl linked by α-(1,6) connections. The configurations of branching and the lengths of chains differ across various starch sources and growing conditions. It has been found that the ratio of amylose to amylopectin as well as their chain length have a major influence on the digestion of starch. Iqbal et al. (2021) found that high amylose maize starch had a slower hydrolysis behaviour, which led to formation of a dense gel network causing a decrease in the digestibility of starch. This is thought to be due to increasing amounts of SDS and RS, which is likely a result of high amylose content. The decreased digestibility of starch can provide potential health benefits such as decreasing the chances of some chronic diseases like obesity, insulin resistance, and type 2 diabetes. Although other factors such as degree of branching and fine molecular structures may also influence rate of digestion (Kaur et al., 2022; Xu et al., 2017).

Chain length is a way to differentiate between amylose and amylopectin molecules. Components that have a degree of polymerization (DP) > 100 are classified as amylose chains, while those with a DP < 100 are classified as amylopectin chains. Gel permeation chromatography can demonstrate the distribution of amylose and amylopectin chains in a starch sample. Xu et al. (2017) analyzed oat starch and found that there were two peaks for amylopectin, one sharp peak for short chain amylopectin (DP up to 37) and another small peak for long chain amylopectin (DP 37 to 100). They also obtained a small wide peak that represents amylose chain-length distribution (DP 100-30,000). Their results showed that there were more short chain amylopectin molecules than long chain, based on the smaller peak at DP 37-100. Based on *in vitro* digestion models, oat starch with increased short chain amylopectin molecules can aid in decreasing digestion rate of starches (Syahariza et al., 2013; Xu et al., 2017).

Molecular weight plays a crucial role in determining various properties of starch, such as viscosity in pastes or suspensions for processing considerations, the rate of digestibility for nutritional concerns, and film-forming characteristics for packaging applications (Kasturi & Bordenave, 2013; Xu et al., 2017). The weight-average molecular weight of amylose and amylopectin (small and large fractions) in oat starch are $1.68\text{x}10^5$ and $3.19x10^6$ and $1.36\text{x}10^7$, respectively (Zhu, 2017). It was reported by Xu et al. (2017) that oat starch had a lower digestibility compared to rice starch, which is thought to be due to various characteristics, one of those being the larger molecular weight of amylose. As mentioned, viscosity can be influenced by starch granule molecular weight. The viscosity of maize starch was investigated by Iida et al. (2008) and they found that decreasing the molecular weight of starch by sonification resulted in a decreased viscosity of starch after gelatinization. Thus, it is predicted that starch granules with higher molecular weight such as those from oats may result in higher viscosity pastes and/or gels.

2.4.2. Granule shape and crystallinity

Oat stands out among other cereal grains for producing distinctive grains characterized by small starch granules. The size and morphology of these granules play a crucial role in shaping the physiochemical properties of the associated starch (Zheng et al., 2015). Notably, starch granules display growth rings, alternating between highly ordered and amylopectin-rich regions, and amorphous sections that are richer in amylose. The ordered growth rings reveal the association of adjacent side chains of amylopectin in double helices, organized within two crystalline structures, denoted as A-type and/or B-type. Cereal starches, in general, can be categorized into two granule types based on size: A-type (>10 µm in diameter) and B-type (<10 µm in diameter) (Kasturi & Bordenave, 2013; Zheng et al., 2015).

Oat starch granules exhibit unique clustering during growth, resulting in irregular or polygonal shapes, while some forming on the outer layer of clusters showcase a combination of polygonal and ovoid characteristics (Kasturi & Bordenave, 2013; Punia et al., 2020). Oat starch clusters typically measure between 20 and 150 µm in diameter, while individual clusters range from 2 to 12 µm in diameter following starch extraction, which is similar in size to rice starch (Table 2). These granules exhibit weak birefringence, appearing smooth without any discernible fissures (Punia et al., 2020). Due to their natural tendency to cluster, accurately classifying the shape of oat starch granules as type A or B becomes challenging. The variations in the physicochemical properties of oat starch can be attributed to distinct granule structures and compositions. Starches characterized by fewer short chains and an increased presence of long B chains tend to demonstrate higher gelatinization temperatures and a greater percentage of retrogradation (Zheng et al., 2015). This highlights the impact of granule structure on the functional characteristics of oat starch.

Table 2. Starch granule morphology for common cereal grains

Starch Type	Granule Shape	Granule Diameter (μm)	Reference
Oat	Polygonal and Irregular	2-12	Kasturi & Bordenave (2013); Punia et al. (2020)
Rice	Polygonal, lenticular, irregular	2-7	Wani et al. (2012)
Wheat	Disc shaped, spherical, irregular	2-36	Guo et al. (2023); Perez & Bertoft (2010); Zheng et al. (2015)
Maize	Spherical and polygonal	5-20	Guo et al. (2023); Perez & Bertoft (2010)
Potato	Lenticular and irregular	2-70	Guo et al. (2023)

Starch granules exhibit a high density of semi-crystalline structures, and the characterization and identification of their crystalline structure is commonly accomplished through the application of X-ray diffraction (XRD). This fundamental technique serves as a crucial tool in assessing the level of organization within starch granules, which is important for understanding the mechanical properties of starch granules. Based on the most prominent diffraction peaks detected by XRD, starch can be classified into three types: A, B, or C. Zhu et al. (2023) reported that the X-ray pattern for oat starch showed peaks at the Bragg angles of 15°, 17°, 20°, and 23° 2θ. These patterns are indicative of the crystal structures commonly associated with A-type; a distinctive characteristic found in cereal starches. It is also important to note that the 20° peak represents the high amounts of amylose-lipid complexes commonly found in oat starch. These results are in agreement with other literature (Kasturi & Bordenave, 2013; Punia et al., 2020).

2.4.3. Gelatinization and pasting properties

The gelatinization process of starch is characterized by the irreversible breakdown of molecular order within a starch granule, occurring upon heating beyond a designated temperature known as the gelatinization temperature (Tgel), in the presence of an abundance of water. During this procedure, there is a significantly higher absorption of water due to application of heat as opposed to cold water, and starch polymers, beginning primarily with amylose, are leached out of the granule. Molecular characteristics of amylose and amylopectin influence the Tgel (Kasturi & Bordenave, 2013). For instance, a longer chain length of amylopectin requires a higher temperature for gelatinization to occur. This is likely due to the fact that amylopectin may form longer double helices, which can increase their thermal stability. In contrast, amylose content typically follows a negative correlation with gelatinization enthalpy. This is a result of decreasing amount of amylopectin double helices, as amount of amylose increases (Kasturi & Bordenave, 2013; Li, 2022). Differential scanning calorimetry is a common method used to measure the gelatinization properties of many cereal starches. One noticeable difference between oat starch and other common cereal starches (wheat, barley, and rye) is their location of swelling. Oat starch granules have the tendency to expand uniformly and concurrently in all three dimensions, in a similar manner to maize starch, while other cereal starches first start swelling along the radial axis and then to other axes. Swelling power is a key indicator of organization of starch granules. It is measured as the ratio of the volume of the swollen starch granules to dry starch. In oat starch, swelling power and amylose leaching increases steadily between the temperature range of 60 to 85°C, then decreases rapidly between 90 and 95°C (Kasturi & Bordenave, 2013). Some common factors that impact the gelatinization of oat starch is the amount of amylose and the small amounts of endogenous lipids present in the starch (Punia et al., 2020; Zhu, 2017). Zhu et al. (2023) found that oat starch with higher percent amylose and fat influence resistance to swelling and resistance during the formation of starch gel, primarily due to the complexing of amylose with lipids (Kasturi & Bordenave, 2013; Zhu et al., 2023).

The pasting properties of starch can be analyzed using methods such as Rapid Visco Analyzer (RVA), Brabender Amylograph, and an Ottawa Starch Viscometer; however, the results can be sensitive to the selected method (Kasturi & Bordenave, 2013; Zhu, 2017). These instruments can measure the variation in viscosity of starch pastes or suspensions as temperature changes during shear. When water is present and the temperature is above gelatinization, starch granules swell, losing molecular order. Amylose and amylopectin then leach out. Mechanical stress, such as shear, disrupts granules, dispersing amylose and amylopectin, forming a paste (Kasturi & Bordenave, 2013). Various properties are known to impact the pasting properties of oat starch, as observed in previous studies (Hüttner et al., 2011; Rhymer et al., 2005; Zhu et al., 2023). These include the size and shape of starch granules, amylose and amylopectin structure and composition, and the minor components that may be present such as lipids and proteins (Zhu, 2017). These differences can be influenced by oat genotype and/or the growing environment. Rhymer et al. (2005) found that pasting properties of oat starch were more heavily influenced by the growing environment, particularly the growing year, which suggests the climate has a greater influence on the pasting properties of oat starch. The pasting characteristics need careful consideration for the selection of an optimal oat variety for industrial use. The time it takes for starch to reach peak viscosity is of particular interest to food manufactures. Oat genotypes with a shorter time and lower temperature to reach peak viscosity are thought to be more commercially acceptable (Kasturi & Bordenave, 2013; Rhymer et al., 2005). This is beneficial from the point of view of the manufacturer, as it can aid in decreasing processing times, making oat starch a strong contender for its application in food.

2.4.4. Current applications and options for modifications

Oat starch has been relatively overlooked in industrial applications, likely because of its availability and cost compared to other cereal starches. However, it possesses distinct properties that render it beneficial for both food and non-food purposes. Its notable characteristics, such as high lipid content and small granule size, make it applicable to the pulp and paper industry, particularly in the coating and sizing of papers. There has also been research on the use of plasticizers on oat starch to enhance its film-making properties. Galdeano et al. (2009) found that the higher lipid content in oat starch can act as a water vapour barrier, which can aid in film formulations, allowing a variety of plasticizers to be used with little impact on the oat starch film properties. While exploration of the potential of oat starch as a food ingredient has been limited, preliminary tests have been conducted, including its use as a fat replacer in mayonnaise (Werlang et al., 2021), for improving texture in various food products and as an ingredient in cake batter (Kumar et al., 2018; Mirmoghtadaie et al., 2009). The unique properties exhibited in oat starch such as its pasting properties, provide the opportunity for more research to be conducted for its use as a food ingredient. However, limitations still exist, as oat starch is an unfamiliar ingredient and has some characteristics that may be less applicable to the food industry. This could be overcome with chemical or physical modification.

The inherent characteristics of native starch granules, including low solubility, retrogradation, thermal decomposition, and elevated viscosity post-gelatinization, impose limitations on their industrial application. To broaden the range of uses for starch, it is crucial to investigate and apply various methods of modification (Compart et al., 2023). Starch is naturally comprised of

RSD, SDS and RS, in which RS is typically found in low amounts. RS typically decreases the energy and accessible carbohydrate content in food. Through fermentation in the large intestine, RS generates short-chain fatty acids, offering health benefits comparable to the functional properties of dietary fibre. Consequently, enhancing the quantity of RS through modification holds the potential to enhance the nutritional advantages of native starch (Kim et al., 2017; Remya et al., 2018). Chemical modification can be used to obtain RS, which is referred to as resistant starch type 4 (RS4) and is among five different RS subtypes. Various methods of chemical modification have been investigated to produce RS4 and are outlined in Table 3.

Table 3. Types of chemical modification methods for production of RS4

Type of chemical modification	**Findings**	**Reference**
Cross-linked	Rice starch was crossed linked with citric acid at varying concentrations (1, 10, and 30%). The modifications resulted in an increase in RS content of rice starch. As degree of solubility and the concentration of cross-linked citric acid increased, the swelling factor, light transmittance, and thermal and pasting properties decreased. Limited access for digestive enzymes, decreased digestibility.	Kim et al. (2017)
Hydroxypropylated	Native sweet potato starch reacted with propylene oxide had lower gelatinization temperatures and an increased swelling power and paste clarity.	Lee & Yoo (2011)
Acetylated	Acetic anhydride added to corn starch slurry resulted in an increase in the amount of RS by decreasing amounts of SDS in ungelatinized starch and decreased RDS in gelatinized starch. Giving lower glycemic index values.	Chung et al. (2008)
Oxidized	Oxidation of oat starch with hypochlorite resulted in increased water binding and solubility. Oxidation of oat starch decreased viscosity and increased pasting temperature.	Berski et al. (2011)

Within the food industry, cross-linked starch is commonly utilized to provide a stable granular structure, restrict swelling, and offer nutritionally beneficial effects (Kim et al., 2017; Remya et al., 2018). It was found by Menzel et al. (2013) that when citric acid is added to starch films, the water and water vapour properties of the films are improved. This makes citric acid a good option for its use as a food contact material, since it has also been approved as a food additive under Good Manufacturing Practice (Health Canada, 2022). It is in general agreement due to the various literature reports on chemical modification of starch, specifically cross-linking with citric acid, that RS content can be increased, thus resulting in changes to gel properties and decreased digestion. Investigation of the chemical modification of oat starch would need to be explored further to confirm the same results.

2.5. Starch noodles

2.5.1. Processing methods for starch noodles

Starch noodles are prepared from starch as the major raw material opposed to flour. Noodles made from starch can be classified based on their shape, most commonly vermicelli (thin noodles) and broad strip (flat, thick noodles) (Bangar et al., 2023; Wang et al., 2012). Traditional production of starch noodles uses mung bean starch and involves gelatinizing a small portion of the starch, which is then mixed with the remaining starch (ungelatinized starch) and water to create a dough with 50% moisture. This dough is then extruded into noodles of the desired shape at room temperature. Following this, the noodles are briefly boiled, cooled in water, then partially dried at 4°C and frozen overnight to finally dry completely at 40°C (Tan et al., 2009). Mixing the dough plays an important role in determining the noodle quality. In dough that uses flour as the main raw ingredient, protein and starch determine the mixing properties; however, in dough that only uses starch, there is negligible influence from protein. Bangar et al. (2023)

suggests that the lack of protein in a dough formulation lowers the water absorption. It is crucial to produce a starch dough that has optimal moisture content and amount of starch paste. Increased moisture content and decreased amount of starch paste will lead to decreased viscosity, while low moisture content increases viscosity and the energy needed to mix the dough (Tan et al., 2009). Mixing can also influence cooking characteristics such as cooking loss, colour, and noodle firmness. Molding is the next step that shapes the noodles into the desired shape. Some starch noodles are prepared by cutting thinly rolled dough sheets into strips. However, most processes utilize an extruder for molding the dough (Bangar et al., 2023). The use of high temperature short time extrusion (HTST) for starch noodle making was investigated by Wang et al. (2012). They found that use of HTST successfully produced starch noodles from pea starch that were comparable to commercial starch noodles. This method can eliminate the need for addition of pre-gelatinized starch and post extrusion cooking of noodles, which decreases processing time. The cooked starch noodles are immediately transferred to cool water and then stored at cold temperatures (-18 to 5°C), which is part of the aging process. Theoretically this process is similar to retrogradation of starch because as starch noodles age in the cold temperatures, the gel matrix is stabilized (Tan et al., 2009). Lee et al. (2005) found that as the aging time of starch noodles increased, the elasticity of the noodles decreased while firmness of starch gel increased. Lastly, noodles are dried at 40°C in a convection dryer before packaging. The temperature of drying has been seen to have little effect on the textural properties of cooked noodles (Lee et al., 2005).

Another method for starch noodle production is one that originally utilized arrowroot. This traditional method is termed kuzukiri, which is a Japanese dessert. This method is known for production of noodles that are translucent, thin, and gelatinous. In general, noodles are made by cooking a starch slurry at 50 to 60°C, pouring the slurry into a thin plate, steaming the starch paste and then the completely gelatinized starch is aged at refrigeration temperatures. The aged starch gel can then be passed through an extruder or cut into strips for drying in an air oven (30-40°C) (Lee et al., 2005). Compared to traditional ways of preparing mung bean starch noodles, this method does not involve freezing the cooked noodles. This may be beneficial to noodle processors as it decreases processing time and production costs. However, the freezing step produces starch noodles with a chewy and elastic texture, which is desired by most consumers. Lee et al. (2005) found that sweet potato starch cooked using the kuzukiri method resulted in a stickier and softer texture than commercial noodles. Thus, there are some limitations to the preparation of starch noodles using the kuzukiri method, mainly in the form of texture. These drawbacks could be addressed using modified starch for noodle production (Bangar et al., 2023).

2.5.2. Properties of starch noodles

The properties of starch noodles are directly related to the source of starch. Mung bean starch is most preferred for the preparation of starch noodles, mainly due to their appealing appearance and desirable texture. In contrast, noodles made from rice starch, potato starch, and corn starch exhibit moderate elasticity, a dull or opaque appearance, and may experience high cooking loss and swelling during cooking, making the noodles excessively soft. Enhancing the quality of starch noodles requires a foundational understanding of their structure. Traditionally, variations in starch noodle quality have been ascribed to factors such as amylose content, the ratio of amylose to amylopectin, fat and protein content in the starch, and the size of starch granules (Tan et al., 2009). Most starch noodles typically contain substantial quantities of rapidly digestible starch, leading to the rapid release of glucose, which is associated with adverse health effects such as elevated blood sugar, diabetes, and hypertension. To address the limitations of native starches, modified starches have been proposed for use in noodle production. These modifications aim to enhance digestibility and improve overall quality, addressing issues like low viscosity and thickening power (Bangar et al., 2023). Various literature has investigated the application of different starches for noodle preparation in which the common parameters to consider are the morphological properties, texture, and cooking quality.

2.5.2.1. Morphological characteristics

During the processing of starch noodles, starch undergoes gelatinization and retrogradation, which allows the structure of the noodle to be formed. The granular size of starches will have an impact on the noodle structure. Chen et al. (2003) found that the larger sized granules from sweet potato and potato starch (> 53µm) resulted in poor noodle quality and in some cases could not form a noodle strand. They also found that noodles made from sweet potato starch with small granule size (< 20µm) were more transparent and had a more acceptable texture in terms of firmness and stretchability. This could imply that noodles made from starch with smaller granule sizes, like oat starch, have a better chance of producing good quality noodles. Another possibility is by the addition of modified starch, which is likely to change the starch granule shape and surface appearance. Remya et al. (2018) found that chemical modification of tuber starches with citric acid resulted in cracks, a rough surface, and some agglomerations of the starch granules. These alterations to the starch granule structure can be seen in the structure of starch noodles, such as that modified starch can increase porosity of a noodle strand. Zhou et al. (2015) found that the small pores created by addition of modified starch to noodles can improve water absorption from the granules and enhance peak viscosity.

2.5.2.2. Texture

The texture of noodles are commonly assessed using a texture analyzer (TA), providing quantitative measurements that indicate the textural characteristics. Some common measurements made using a TA include cohesiveness, hardness, chewiness, firmness, elasticity, and stickiness among others. In general, sought-after attributes in starch noodles involve minimizing stickiness and cohesion while enhancing firmness and elasticity. These qualities can be verified through sensory analysis (Bangar et al., 2023; Punia et al., 2019). The textural properties of mung bean starch noodles are considered to be the standard, thus achieving similar texture characteristics with alternative starches is desired (Tan et al., 2009). The higher amylose content of mung bean starch contributes to some of the ideal properties exhibited in the noodles. Tan et al. (2006) found that when comparing mung bean and sweet potato starch noodles, the mung bean starch had higher amylose content (~33%), which led to higher quality noodles due to good cohesiveness. The assessment of cohesion reflects the way noodles engage with the texture probe during measurement and the degree to which they undergo disruption upon compression. Starch noodles exhibiting favorable compression characteristics have been observed to possess improved cooking properties and mouthfeel (Tan et al., 2009). Considering these results, it could be suggested that pure starches with higher amylose content, such as oat starch, could be a probable contender for starch noodle application as they may be able to provide similar textural properties to mung bean starch noodles.

2.5.2.3. Cooking quality

Evaluation of the cooking quality of starch noodles is useful to understand as it affects consumer acceptance of the noodles. The parameters measured include cooking time, cooking loss and cooked weight. For starch noodles it is more favourable to have noodles with low cook loss, cooked weight, and cook time (Tan et al., 2009). Baek & Lee (2014) found that cooking loss of white and brown rice starch noodles increased with increased cooking time, which can be explained by the fact that keeping the noodles longer in boiling water decreases their resistance to disintegration. They also observed that brown rice starch noodles had a significantly higher cooking loss than white rice starch noodles. The reason for this is thought to be due to the degree of starch gelatinization. Brown rice starch has a lower degree of starch gelatinization, which may contribute to a weakened structure and consequently an increased cooking loss (Baek & Lee, 2014). Choosing starches that can form a stronger gel network may help strengthen the noodle structure and reduce cooking loss (Chansri et al., 2005). Chemical modification of starches may also help combat this issue. Ačkar et al. (2022) found that chemical modification was able to increase the gel strength of maize, wheat, and tapioca starches. Gel firmness can be used as a predictor for the cooked weight of noodles, such that as gel firmness increases the cooked weight of noodles would decrease. The process of starch recrystallization influences gel characteristics, particularly gel firmness. This phenomenon occurs as noodles age and is expedited by the presence of amylose. Consequently, starches with elevated amylose levels may contribute to enhancing gel firmness, ultimately leading to a lower cooked weight of the noodles (Bangar et al., 2023). As discussed, studies have reported on the properties of oat starch (Hoover et al., 2003; Rhymer et al., 2005; Zhu et al., 2023) and majority conclude that oat starch has the potential to be used as a food ingredient. However, studies have not reported on the use of oat starch for starch noodles and there is great potential for their application due to the functional properties of oat starch.

Chapter 3: Effects of Genotype and Environment on the Physiochemical Properties of Canadian Oat Varieties

3.1. Abstract

The relationship of oat grain composition and physical attributes as influenced by oat genotype and Canadian growing environments were investigated. Thirty Canadian oat (*Avena sativa* L.) genotypes that were grown in three Canadian growing locations (Brandon, Manitoba; Portage la Prairie, Manitoba; and Lacombe, Alberta) over two consecutive years (2020-2021) were analyzed. Analysis of variance showed that the protein, total starch and amylose content were significantly affected by genotype, environment, and their interaction. Principal component analysis biplot illustrated that protein and total starch have an inverse relationship and are more affected by growing year, while amylose content had a negligible influence. Majority of genotypes were stable across environments, while some genotypes like CDC Morrison, were more influenced by different environments. Correlation analysis suggested that drought-like conditions early in the growing season generated oat genotypes that favoured accumulation of protein, beta-glucan, and oil. The results obtained through this study provide detailed knowledge on the relationship between important oat chemical and physical traits and different growing environments, which can aid breeders in concurrently enhancing characteristics to obtain high quality oat grains and thus high-quality end products.

3.2. Introduction

Oat (*Avena sativa* L.) is a common cereal crop grown in Canada. In 2023, the total area seeded with oat in Canada was 1.023 million hectares (Agriculture and Agri-Food Canada, 2023; Canadian Grain Commission, 2023). This may be attributed to increasing interest in the consumption of oat, possibly due to decreased nutrient requirement for cultivation, recognized nutritional qualities of oat grain and renewed interest in research and development on novel oat products and ingredients for human consumption. (Howarth et al., 2021; Rasane et al., 2015). Thus, developing oat genotypes that can meet demands of producers, manufacturers, and consumers is important. Consequently, the relationship between oat genotype (G) and growing environment (E), as well as genotype by environment interaction (GxE), must be studied. Plant breeders will then gain an understanding of the factors that influence the chemical composition, physical characteristics, functionality, and end-use potential of oat (Howarth et al., 2021; Rhymer, 2002). Some studies have focused on the effects of GxE interactions on oat grain yield, quality, in terms of thousand kernel weight, groat percentage, and hullability, and a major focus on beta-glucan content (Howarth et al., 2021; Huang et al., 2016; Mut et al., 2016; Sapega & Sh Tursumbekova, 2022; Wodebo et al., 2023). The research is valuable for understanding the agricultural production capability and milling quality; however, the research is lacking in terms of a Canadian perspective. Most literature utilizes data from oat grown in other parts of the world, most commonly Europe and those from Africa, and West and South Asia (Alemayehu et al., 2021; Howarth et al., 2021; Mut et al., 2016; van den Broeck et al., 2015; Wodebo et al., 2023).

Another factor to consider is the small sample sizes used in majority of the literature. GxE variations observed may not be very representative when smaller sample sizes are used, making it crucial to study larger sample sets as achieved in the present study. Limited research has focused on starch, the major component in oat. In general, whole grain oat can contain 51-65%, 9-20% protein, 3-11% oil and 2-8% dietary fibre in the form of beta-glucans (Malunga et al., 2022; Rasane et al., 2015; Zhu, 2017). The characteristics of starch can have a major influence on the final product quality, making it crucial to investigate and enhance our understating of how oat can be applied to the production of products for human consumption with good eating and nutritional quality (Zhu, 2017).

Oat starch has considerable differences in terms of physiochemical properties compared to other cereal starches. Starch content in oat varies with genotype and growing location, and physiochemical differences have also been observed among oat genotypes (Rasane et al., 2015; Rhymer et al., 2005). Oat starch granules also contain amylose (linear polysaccharide) and amylopectin (branched polysaccharide), which are both polymers of α-D-glucose. Depending on the source of starch, the ratio of amylose to amylopectin can vary. It has been observed that amylose content in oat starch can range from 10-34% depending on genotype and environmental factors as well as analytical methods used (Hoover et al., 2003; Sowa & White, 1992; Wang & White, 1994). The higher content of amylose in oat can be beneficial for food applications, as it is known that starches with a higher amylose content have a lower rate of digestion (Li et al., 2023).

Though some research has been done on the influence of genotype and environment on oat, to the best of our knowledge it typically involves data from non-Canadian oat varieties and growing locations, smaller sample sets, and is mainly focused on yield and whole grain oat quality, rather the main chemical components of protein and starch with emphasis on amylose content. It is also important to study oat genotypes currently being grown, as will be done in the present study, to ensure findings are relevant to the industry. Therefore, the present research aims to fill the gaps in the existing knowledge on oat GxE effects. The objectives of this study were to a) determine the effects of genotype, environment, and GxE interactions on the core chemical traits, b) to investigate to what degree of significance are the analyzed traits affected by genotype and environmental interactions, c) and to analyze relationships among core traits and climatic factors as well as physical attributes and various chemical components to provide a representative analysis of Canadian oat genotypes for application in breeding trials and to better understand their end use potential.

3.3. Materials and methods

3.3.1. Sample description and experimental design

All experiments were carried out using thirty Canadian oat genotypes (Table 4) that were grown in three Canadian growing locations: Brandon, Manitoba (49.8437°N, 99.9515°W); Portage La Prairie, Manitoba (49.9731°N, 98.2925°W); and Lacombe, Alberta (52.4682°N, 1137305°W) over two growing years (2020 and 2021) (Table 5). The soil order in all locations was Chernozemic, differing only in colour; where Brandon and Portage La Prairie, MB it was black while in Lacombe, AB it was black and grey. All thirty oat genotypes were grown in a single replicate test at multiple locations and years after which samples from the field reps were composited.

Table 4. Pedigree, height and lodging resistance of Canadian oat genotypes

Genotype	Pedigree	Height	Lodging resistance	Reference[1]
AC Morgan	OT526/OT763	Tall	Very good	Kibite & Menzies (2001)
Camden CS	SW Betania/Dominik	Short	Very good	
Summit	AC Ronald/OT299	Short	Good	
Souris	ND90141/ND900118	Medium	Good	
Triactor	SW 9619019/Stork	Medium	Good	
CDC Dancer	OT344/OT269	Tall	Good	
Leggett	OT294/Pc94	Medium	Good	Mitchell Fetch et al. (2007)
CDC Minstrel	OT293/CDC Dancer	Medium	Very good	
CDC Morrison	CDC Sol-Fi/HiFi	Medium	Very good	
CDC Ruffian	OT399/OT599	Medium	Good	
AAC Justice	ND020965/SA050100	Tall	Good	Mitchell Fetch et al. (2022)
ORE3542M	Leggett/CDC Minstrel	Short	Very good	
ORE3541M	Leggett/CDC Minstrel	Short	Very good	
CDC Norseman	OT590/CDC Minstrel	Tall	Good	
CDC Arborg	SA050479/OT595	Tall	Very good	
Akina	SW Betania/SW 032009	Medium	Good	
Bolina	01RAT23/OT397	Short	Good	
Bullet	Souris/07774-3-9-1-1a-1	Tall	Very good	Yan, McElroy, Fregeau-reid, Xue, et al. (2017)
Dieter	Pc68/Donegal//Capital	Short	Fair	
Hidalgo	OT385/OT2006	Medium	Very good	
Kara	SW 01218/SW 02293	Short	Good	
Kolosse	Spurs/07786-1-10-1-1	Tall	Very good	Yan et al. (2016)
Nice	QO_617_16/DONEGALOgle// RL2897*2/Kent	Medium	Good	
Nicolas	09030-4/08979-3	Medium	Very good	
Noranda	09033-4/08987-1-3	Medium	Very good	
Orrin	OT349/J775-1	Short	Fair	
Richmond	OT3008/07836-2-9-3-1 //07836-2-9-3-1 /OA1048-6	Tall	Good	
Rigodon	/3/ Q.O.174.19 (RL2897=OT187)	Tall	Good	Yan, McElroy, Fregeau-reid, Martin, et al. (2017)
Roskens	Sel. from 08387;Souris/07836-2-5-2-la-2	Medium	Good	
Synextra	Q.O.187.11/Laurent	Tall	Fair	

[1]All other data (not listed as a reference) was obtained from the Canadian Food Inspection Agency (2024) or T3/Oat (2024).

Table 5. Seeding date and climate conditions recorded at the three growing locations

Location	Year	Seeding date	Climate Conditions[1]				
			Month	Mean daily high temperature (°C)	Mean daily low temperature (°C)	Rainfall (mm)	Growing degree days (°C)
Brandon	2020	May 29	May	17.1	3.7	8.2	322.1
			June	25.0	10.4	205.5	513.0
			July	26.3	14.1	54.0	606.4
			August	25.9	11.8	57.1	565.4
			September	19.0	4.2	11.2	346.9
Lacombe	2020	June 4	May	16.3	3.0	80.0	289.1
			June	19.8	8.0	91.2	416.3
			July	21.8	9.7	99.1	488.8
			August	23.0	8.7	51.2	490.3
			September	19.0	4.6	16.0	354.1
Portage	2020	June 3	May	17.0	4.9	21.3	339.5
			June	24.7	12.1	50.3	551.4
			July	26.8	15.3	60.0	652.2
			August	25.8	12.9	45.6	560.8
			September	18.9	6.4	22.7	380.2
Brandon	2021	May 18	May	17.7	2.1	25.8	307.7
			June	26.0	11.6	101.2	565.2
			July	28.2	12.9	0.2	472.0
			August	24.0	11.0	156.8	542.2
			September	23.2	6.9	21.6	452.1
Lacombe	2021	May 21	May	16.1	2.5	49.1	288.4
			June	23.4	9.5	75.1	493.5
			July	24.7	11.4	57.6	558.4
			August	23.2	8.6	62.2	492.7
			September	19.4	3.6	14.6	346.4
Portage	2021	June 1	May	18.0	3.6	49.5	334.2
			June	26.7	13.4	70.3	601.2
			July	29.2	15.3	12.7	690.5
			August	25.1	12.6	78.1	565.4
			September	22.7	9.9	22.8	488.4

[1]Data obtained from Government of Canada (2024).

3.3.2. Sample preparation

Oat samples were dehulled using the Codema Laboratory Oat Huller LH 5095 system (Codema, Inc., Minneapolis, MN, USA) to obtain groats. Prior to milling the groats, remaining hulls and broken groats were removed by hand. The cleaned groats were subsequently milled with a

Retsch Ultra Centrifugal Mill ZM200 (Retsch GmbH, Haan, Germany) using a 0.5mm sieve to obtain whole oat flour.

3.3.3. Proximate composition

Compositional analysis was conducted on whole oat flour for protein, total starch, and moisture according to the AACC approved methods 46-30.01, 76-13.01, and 44-15.02, respectively. Amylose content was determined using the Megazyme kit K-AMYL (Megazyme International, Wicklow, Ireland) from Megazyme International. Duplicate analysis was performed for each sample.

Oil and beta-glucan content for whole oat flour was quantified using Near-Infrared (NIR) Spectroscopy (Unity SpectraStar 2400, Unity Scientific, Columbia, MD, USA), where single analysis was done for each sample.

3.3.4. Colorimetry

Colour properties of oat groats and whole oat were measured using a Konica Minolta Chromo Meter (model CR-410, Minolta Co., Osaka, Japan) with a 50mm aperture size. The CIE $L^*a^*b^*$ colour space was used where L^* represents lightness, and a^* and b^* represent chromaticity and measure from green to red and blue to yellow respectively. Whole oat and oat groats were packed into the granular material attachment (CR-A50) to obtain CIE $L^*a^*b^*$ colour parameters. Duplicate analysis was performed for each sample for the 2021 growing season.

3.3.5. Statistical analysis

Two-way analysis of variance (ANOVA) was preformed using the R software package (R version 4.1.2, R foundation for Statistical Computing, Vienna, Austria) to study the effects of genotype, environment, and their interaction on the physiochemical properties of oat. Six environments were established based on growing location and year (Brandon 2020, Lacombe

2020, Portage la Prairie 2020, Brandon 2021, Lacombe 2021, Portage la Prairie 2021). Tukey-Kramer honest significant difference method was used to compare means at $P < 0.05$ using R statistical software. The relationship between core traits (protein, total starch, and amylose) and weather data along with all measured traits with each other were determined using Pearson correlation with the R software package.

Principal component analysis was performed with JMP software (SAS Institute Inc., Cary, NC, USA) to examine the genotypic and environmental relationships for protein, total starch, and amylose content. This analysis method models the obtained data in a graphical format known as a biplot and is based on principal components. The data set that is represented graphically is based on the means of their duplicate analyses. Results are displayed on the biplot as principal component 1 (PC1) and principal component 2 (PC2), where genotype and environment are represented as markers and traits are shown as vectors (Peterson et al., 2005).

3.4. Results and Discussion

3.4.1. Colour of whole oat and oat groats

Colour of oat groats and whole oats were measured on 30 oat genotypes grown in the three locations in 2021. The colour values ranged from 56.9-63.0, 3.8-4.8, and 18.4-21.9 for *L**, *a**, and *b**, respectively for oat groats. *L**, *a**, and *b** for whole oat were 54.7-64.9, 1.9-4.0, and 16.5-22.3 for *L**, *a**, and *b**, respectively (Figure 2).

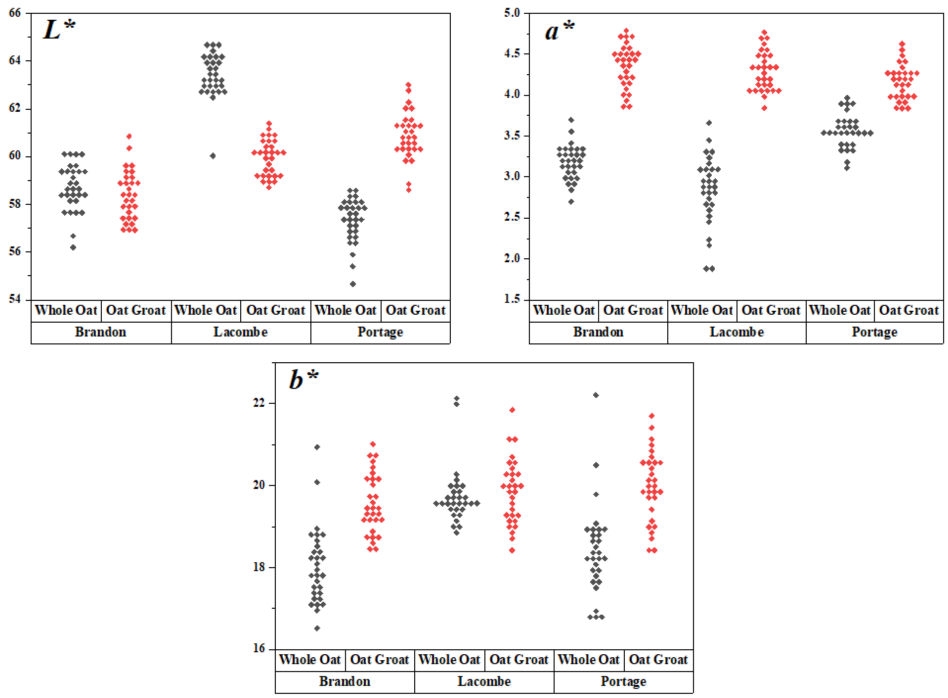

Figure 2. Distribution of colour values *L** (lightness), *a** (redness/greenness), *b** (yellowness/blueness), for whole oat and oat groats, based on three growing environments pooled from 30 genotypes.

3.4.2. Chemical composition of oat groats

The composition of 30 Canadian oat genotypes grown in six environments is shown in the distribution plot in Figure 3. Values ranged from 12 to 24% for protein (Table 6), 49 to 67% for total starch (Table 7), 21 to 33% for amylose content (Table 8), 3-7% for beta-glucan, and 5-10% for oil content (Figure 3).

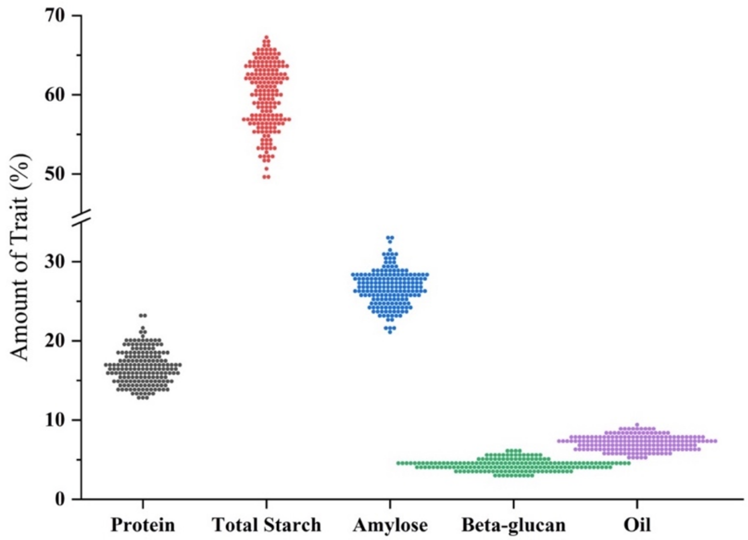

Figure 3. Distribution of oat chemical composition based on 30 genotypes pooled from six growing environments.

3.4.3. Means and ANOVA

Genotypic and environmental means for the 30 oat genotypes grown in six growing environments are given for protein (Table 6), total starch (Table 7), and amylose (Table 8). The results found are similar to those reported in previous literature with some deviations (Malunga et al., 2022; Rhymer et al., 2005; Tester & Karkalas, 1996). The variations seen in the data are attributed to environmental and genotype effects, agronomic management, and their interactions. Genotypic means for protein content showed cultivar CDC Morrison stood out amongst the rest as it had consistently high mean protein content (Table 6), low mean total starch (Table 7), and low mean amylose content (Table 8). Genotypes Bolina, CDC Ruffian, and CDC Minstrel behaved similarly in terms of protein and total starch content, where each genotype comparably had the highest mean total starch and lowest mean protein content.

The environmental means for protein, total starch and amylose content showed noticeable differences among locations across the 2020 and 2021 growing years. This is explained by the fact that the 2021 growing year displayed higher mean values for protein and amylose and lower mean values for total starch content across all locations than the 2020 growing year means (Table 6-8). No location displayed a consistent trend for the parameters investigated.

Table 6. Mean protein content of oat genotypes (%, db), measured on milled oat groats from Canadian growing locations[1]

Genotypes	Environments						Mean
	Brandon 2020	Lacombe 2020	Portage 2020	Brandon 2021	Lacombe 2021	Portage 2021	
AC Morgan	15.1 ± 0.0	16.7 ± 0.5	12.8 ± 0.2	19.1 ± 0.2	14.2 ± 0.1	16.6 ± 0.3	15.8^{bc}
Camden CS	17.2 ± 0.0	16.8 ± 0.2	16.1 ± 0.3	21.7 ± 0.3	16.1 ± 0.0	19.1 ± 0.3	17.8^{abc}
Summit	14.1 ± 0.2	16.3 ± 0.4	13.4 ± 0.2	18.4 ± 0.3	16.1 ± 0.3	16.9 ± 0.1	15.9^{bc}
Souris	14.2 ± 0.2	16.3 ± 0.4	16.4 ± 0.6	19.0 ± 0.2	17.9 ± 0.3	18.9 ± 0.2	17.1^{abc}
Triactor	16.8 ± 0.1	16.8 ± 0.4	15.0 ± 0.4	19.9 ± 0.2	17.2 ± 0.4	17.8 ± 0.2	17.3^{abc}
CDC Dancer	15.4 ± 0.3	15.6 ± 0.2	13.8 ± 0.3	17.1 ± 0.1	16.8 ± 0.4	16.9 ± 0.0	15.9^{bc}
Leggett	16.5 ± 0.1	16.3 ± 0.4	15.8 ± 0.3	21.5 ± 0.0	17.2 ± 0.3	18.8 ± 0.8	17.7^{abc}
CDC Minstrel	13.1 ± 0.5	13.6 ± 0.2	15.8 ± 0.5	17.8 ± 0.2	16.1 ± 0.0	15.3 ± 0.2	15.3^{c}
CDC Morrison	17.5 ± 0.0	17.6 ± 0.4	17.1 ± 0.6	23.6 ± 0.2	20.5 ± 0.1	23.4 ± 0.0	19.9^{a}
CDC Ruffian	14.0 ± 0.0	15.2 ± 0.3	13.5 ± 0.3	18.4 ± 0.8	14.0 ± 0.0	16.3 ± 0.1	15.2^{c}
AAC Justice	13.4 ± 0.1	14.7 ± 0.2	15.5 ± 0.1	17.1 ± 0.3	16.5 ± 0.3	18.2 ± 0.2	15.9^{bc}
ORE3542M	14.5 ± 0.2	15.1 ± 0.2	14.5 ± 0.4	19.6 ± 0.3	17.6 ± 0.2	18.0 ± 0.1	16.6^{bc}
ORE3541M	16.7 ± 0.2	17.7 ± 0.6	14.0 ± 0.6	18.9 ± 0.0	18.5 ± 0.3	17.6 ± 0.2	17.2^{abc}
CDC Norseman	14.9 ± 0.1	16.2 ± 0.2	13.5 ± 0.0	21.2 ± 0.2	18.5 ± 0.2	20.2 ± 0.3	17.4^{abc}
CDC Arborg	15.2 ± 0.2	14.1 ± 0.2	16.3 ± 0.1	20.4 ± 0.9	14.2 ± 0.3	15.7 ± 0.1	16.0^{bc}
Akina	15.9 ± 0.2	16.4 ± 0.2	13.9 ± 0.2	19.4 ± 0.0	15.6 ± 0.0	18.0 ± 0.1	16.5^{bc}
Bolina	14.2 ± 0.1	14.9 ± 0.3	13.9 ± 0.2	18.2 ± 0.1	14.6 ± 0.1	15.7 ± 0.3	15.2^{c}
Bullet	15.4 ± 0.2	17.2 ± 0.3	16.2 ± 0.0	18.3 ± 0.8	17.2 ± 0.1	19.8 ± 0.0	17.3^{abc}
Dieter	16.0 ± 0.3	17.1 ± 0.2	14.9 ± 0.0	20.2 ± 0.0	16.6 ± 0.5	19.9 ± 0.4	17.4^{abc}
Hidalgo	16.9 ± 0.3	15.8 ± 0.2	14.5 ± 0.1	18.7 ± 0.5	16.8 ± 0.4	17.3 ± 0.2	16.7^{bc}
Kara	15.3 ± 0.2	17.7 ± 0.6	15.9 ± 0.5	20.2 ± 0.9	17.6 ± 0.1	18.6 ± 0.5	17.6^{abc}
Kolosse	15.2 ± 0.3	17.2 ± 0.5	17.4 ± 0.4	19.9 ± 0.4	18.6 ± 0.0	17.2 ± 0.2	17.6^{abc}
Nice	13.9 ± 0.1	15.8 ± 0.2	15.4 ± 0.6	20.4 ± 0.1	19.6 ± 0.2	18.5 ± 0.5	17.2^{abc}
Nicolas	14.8 ± 0.1	17.0 ± 0.3	16.9 ± 0.1	20.4 ± 0.1	18.0 ± 0.1	17.4 ± 0.3	17.4^{abc}
Noranda	17.4 ± 0.2	14.0 ± 0.0	14.4 ± 0.6	20.4 ± 0.2	16.1 ± 0.0	19.7 ± 0.2	17.0^{abc}
Orrin	15.2 ± 0.3	16.1 ± 0.4	14.8 ± 0.3	18.7 ± 0.3	17.0 ± 0.2	18.5 ± 0.0	16.7^{bc}
Richmond	16.1 ± 0.0	15.1 ± 0.1	14.5 ± 0.4	18.8 ± 0.1	14.5 ± 0.3	16.6 ± 0.0	15.9^{bc}
Rigodon	16.1 ± 0.1	18.7 ± 0.2	18.7 ± 0.7	20.5 ± 0.4	18.9 ± 0.8	19.5 ± 0.0	18.7^{ab}
Roskens	16.6 ± 0.2	15.0 ± 0.1	13.0 ± 0.3	20.0 ± 0.2	20.0 ± 0.1	19.5 ± 0.1	17.4^{abc}
Synextra	17.1 ± 0.2	19.5 ± 0.1	15.5 ± 0.1	20.7 ± 0.3	17.6 ± 0.3	19.8 ± 0.0	18.4^{ab}
Mean	15.5^{DE}	16.2^{D}	15.1^{E}	19.6^{A}	17.0^{C}	18.2^{B}	

[1]Mean values for locations shown with the same uppercase superscripts are not significantly different (P < 0.05). Mean values for genotypes with the same lowercase superscripts are not significantly different (P < 0.05). Data is expressed as mean ± standard deviation.

Table 7. Mean total starch content of oat genotypes (%, db), measured on milled oat groats from Canadian growing locations[1]

Genotypes	Environments						Mean
	Brandon 2020	Lacombe 2020	Portage 2020	Brandon 2021	Lacombe 2021	Portage 2021	
AC Morgan	65.2 ±1.6	63.8 ± 0.8	67.4 ± 1.0	56.4 ± 0.4	59.5 ± 0.9	58.9 ± 1.7	61.3[a]
Camden CS	61.8 ± 0.9	61.9 ± 0.9	62.2 ± 0.8	57.2 ± 2.6	54.0 ± 3.0	53.5 ± 1.1	58.4[ab]
Summit	63.8 ± 0.2	62.1 ± 0.9	64.8 ± 0.9	58.4 ± 0.8	56.4 ± 1.7	53.0 ± 1.5	59.3[ab]
Souris	63.5 ± 1.7	62.0 ± 0.9	63.1 ± 0.5	60.9 ± 2.4	55.1 ± 0.9	55.6 ± 0.8	59.8[ab]
Triactor	60.9 ± 0.7	60.4 ± 0.4	61.6 ± 1.1	56.5 ± 1.5	54.4 ± 1.9	56.9 ± 1.4	58.2[ab]
CDC Dancer	65.9 ± 0.4	64.1 ± 0.2	66.9 ± 0.1	61.3 ± 0.7	58.9 ± 0.2	60.3 ± 0.8	62.4[a]
Leggett	62.7 ± 0.7	61.7 ± 0.9	62.1 ± 1.0	52.4 ± 2.0	56.8 ± 2.0	57.4 ± 0.8	58.8[a]
CDC Minstrel	67.1 ± 1.2	65.4 ± 0.7	65.1 ± 1.2	60.7 ± 3.0	57.2 ± 0.9	60.8 ± 3.1	62.8[a]
CDC Morrison	61.3 ± 0.5	59.5 ± 0.6	61.0 ± 0.3	49.6 ± 1.0	50.9 ± 1.0	51.7 ± 0.3	55.4[b]
CDC Ruffian	64.6 ± 0.5	63.9 ± 0.6	66.2 ± 0.2	59.6 ± 2.3	59.1 ± 1.3	58.7 ± 2.8	61.6[a]
AAC Justice	64.2 ± 0.1	62.6 ± 0.6	62.5 ± 0.9	59.9 ± 3.3	56.5 ± 0.1	56.3 ± 0.3	60.4[ab]
ORE3542M	65.5 ± 2.3	64.3 ± 0.4	64.5 ±1.3	60.3 ± 1.1	57.0 ± 1.9	57.9 ± 0.3	61.6[ab]
ORE3541M	64.6 ± 0.3	62.5 ± 0.6	65.6 ± 1.1	57.4 ± 0.2	55.9 ± 0.7	57.0 ± 1.8	60.0[ab]
CDC Norseman	65.1 ± 1.2	63.3 ± 0.5	64.7 ± 0.3	56.1 ± 2.0	55.8 ± 1.0	52.5 ± 0.4	59.4[ab]
CDC Arborg	65.4 ± 0.6	65.1 ± 0.4	64.0 ± 1.2	58.5 ± 1.7	58.2 ± 0.8	60.2 ± 0.8	62.1[ab]
Akina	63.7 ± 1.1	62.5 ± 0.1	63.7 ± 0.4	55.5 ± 1.2	57.9 ± 0.5	55.5 ± 0.9	59.6[ab]
Bolina	63.9 ± 0.0	64.2 ± 0.4	66.4 ± 1.4	57.3 ± 0.8	60.4 ± 2.4	60.6 ± 2.3	61.8[a]
Bullet	65.7 ± 1.4	63.1 ± 0.2	63.2 ± 1.0	57.8 ± 0.1	58.6 ± 1.2	57.0 ± 0.5	60.9[ab]
Dieter	65.6 ± 0.1	63.3 ± 0.6	65.7 ± 0.8	59.1 ± 1.2	59.2 ± 0.5	57.0 ± 0.0	61.2[ab]
Hidalgo	61.2 ± 0.5	62.3 ± 0.3	63.0 ± 0.6	49.9 ± 0.8	57.6 ± 0.5	57.1 ± 1.5	58.4[ab]
Kara	62.9 ± 2.5	60.2 ± 0.2	61.8 ± 0.6	51.9 ± 0.4	53.8 ± 1.2	52.4 ± 2.0	56.9[ab]
Kolosse	66.2 ± 0.6	63.1 ± 0.7	62.3 ± 0.6	56.4 ± 0.7	53.7 ± 0.9	57.8 ± 1.8	60.0[ab]
Nice	63.9 ± 0.1	62.5 ± 0.8	62.3 ± 2.4	53.5 ± 2.8	54.7 ± 1.7	56.7 ± 0.3	59.0[ab]
Nicolas	64.6 ± 1.7	63.6 ± 1.4	64.1 ± 0.7	57.7 ± 0.6	55.8 ± 2.5	56.9 ± 0.7	60.4[ab]
Noranda	61.5 ± 0.3	62.8 ± 0.4	62.7 ± 1.2	55.4 ± 0.7	57.0 ± 0.8	54.1 ± 0.6	58.9[ab]
Orrin	63.6 ± 0.4	62.7 ± 0.1	64.2 ± 0.1	58.1 ± 1.3	57.5 ± 1.3	55.6 ± 0.9	60.0[ab]
Richmond	65.5 ± 1.0	66.6 ± 0.7	65.8 ± 0.4	60.2 ± 0.4	59.8 ± 3.1	59.4 ± 1.7	63.0[a]
Rigodon	62.4 ± 0.3	60.4 ± 0.0	59.3 ± 1.2	55.5 ± 0.5	55.3 ± 0.0	52.6 ± 1.2	57.8[ab]
Roskens	61.6 ± 1.0	61.3 ± 0.3	63.2 ± 0.2	56.5 ± 0.8	53.8 ± 0.8	54.4 ± 1.0	58.2[ab]
Synextra	61.8 ± 0.1	59.8 ± 0.9	64.4 ± 0.6	56.4 ± 1.4	55.9 ± 2.7	54.2 ± 1.5	58.0[ab]
Mean	63.9[A]	62.7[A]	63.8[A]	56.9[B]	56.6[B]	56.4[B]	

[1]Mean values for locations shown with the same uppercase superscripts are not significantly different (P < 0.05). Mean values for genotypes shown with the same lowercase superscripts are not significantly different (P < 0.05). Data is expressed as mean ± standard deviation.

Table 8. Mean amylose content of oat genotypes (%), measured on milled oat groats from Canadian growing locations[1]

Genotypes	Environments						Mean
	Brandon 2020	Lacombe 2020	Portage 2020	Brandon 2021	Lacombe 2021	Portage 2021	
AC Morgan	26.5 ± 0.6	26.2 ± 0.8	25.4 ± 1.2	27.3 ± 0.0	29.2 ±1.3	31.3 ± 0.8	27.6^{abcdef}
Camden CS	26.3 ± 1.3	28.2 ± 0.3	26.6 ± 0.7	27.6 ± 0.6	28.4 ± 0.8	27.5 ± 1.3	27.4^{abcdefg}
Summit	23.2 ± 0.8	23.9 ± 1.3	23.9 ± 0.6	28.7 ± 0.8	27.7 ± 0.6	26.3 ± 0.7	25.6^{hijk}
Souris	26.6 ± 0.4	28.7 ± 1.6	30.5 ± 1.3	28.1 ± 1.1	28.7 ± 1.0	26.9 ± 0.5	28.2^{abc}
Triactor	24.2 ± 1.1	26.0 ± 0.0	25.8 ± 0.5	26.8 ± 1.2	27.7 ± 0.1	27.8 ± 1.2	26.4^{fghi}
CDC Dancer	27.7 ± 0.1	23.5 ± 0.9	31.6 ± 0.6	29.4 ± 0.6	29.5 ± 1.2	29.0 ± 1.3	28.5^{ab}
Leggett	23.7 ± 0.4	21.9 ± 0.3	24.1 ± 0.6	27.2 ± 0.8	29.3 ± 1.0	25.8 ± 0.6	25.3^{ijk}
CDC Minstrel	25.6 ± 1.2	28.7 ± 0.7	27.1 ± 1.4	28.3 ± 0.3	28.8 ± 0.6	28.3 ± 0.1	27.8^{abcde}
CDC Morrison	21.6 ± 0.0	25.5 ± 0.6	24.2 ± 0.8	26.0 ± 0.7	24.5 ± 1.1	24.2 ± 0.9	24.4^{k}
CDC Ruffian	27.6 ± 0.5	23.2 ± 0.3	27.3 ± 0.1	28.3 ± 1.0	32.6 ± 1.3	28.9 ± 1.4	28.0^{abcd}
AAC Justice	22.8 ± 0.3	24.5 ± 0.3	24.7 ± 0.6	26.6 ± 1.3	25.2 ± 1.3	26.0 ± 1.4	25.0^{jk}
ORE3542M	23.9 ± 1.1	22.0 ± 1.1	24.8 ± 0.7	27.3 ± 1.1	30.1 ± 1.0	28.7 ± 1.1	26.1^{ghij}
ORE3541M	25.2 ± 1.3	23.6 ± 0.2	28.3 ± 0.2	28.1 ± 1.2	26.6 ± 1.2	30.6 ± 0.9	27.1^{bcdefg}
CDC Norseman	25.7 ± 0.8	24.1 ± 0.8	26.4 ± 1.0	27.2 ± 1.3	27.7 ± 1.2	29.0 ± 1.5	26.7^{defghi}
CDC Arborg	26.9 ± 0.4	21.1 ± 0.4	26.3 ± 0.0	27.6 ± 1.7	33.4 ± 1.8	28.4 ± 0.1	27.3^{abcdefg}
Akina	25.2 ± 0.1	27.1 ± 2.0	23.4 ± 1.2	27.7 ± 0.0	31.3 ± 0.6	28.6 ± 0.3	27.2^{bcdefg}
Bolina	25.9 ± 0.8	25.7 ± 0.5	25.0 ± 0.4	27.6 ± 1.2	29.9 ± 0.2	28.6 ± 0.4	27.1^{bcdefg}
Bullet	26.9 ± 0.7	26.8 ± 1.4	26.1 ± 1.2	28.2 ± 1.0	29.6 ± 1.1	27.7 ± 1.1	27.5^{abcdefg}
Dieter	24.8 ± 0.1	25.0 ± 1.4	27.1 ± 0.6	27.9 ± 0.2	28.8 ± 1.3	25.2 ± 0.8	26.5^{efghi}
Hidalgo	25.6 ± 0.1	24.2 ± 2.3	24.7 ± 0.5	26.1 ± 0.8	26.3 ± 0.8	24.8 ± 0.3	25.3^{ijk}
Kara	24.3 ± 1.1	27.3 ± 0.4	25.4 ± 0.9	27.0 ± 1.3	26.1 ± 0.9	28.6 ± 0.4	26.5^{efghi}
Kolosse	27.6 ± 0.2	28.1 ± 0.6	28.2 ± 0.8	28.9 ± 1.4	29.9 ± 1.3	29.4 ± 0.5	28.7^{a}
Nice	24.3 ± 1.2	28.5 ± 0.0	25.3 ± 0.5	26.8 ± 0.9	30.4 ± 0.5	28.0 ± 0.0	27.2^{bcdefg}
Nicolas	26.8 ± 0.9	24.7 ± 0.3	27.2 ± 0.0	30.9 ± 0.1	27.1 ± 0.3	29.4 ± 0.4	27.7^{abcdef}
Noranda	24.5 ± 0.7	27.9 ± 0.5	25.7 ± 1.0	28.1 ± 0.9	29.7 ± 0.5	26.0 ± 1.3	27.0^{cdefgh}
Orrin	26.1 ± 0.5	27.1 ± 0.9	24.8 ± 1.1	26.5 ± 0.7	22.7 ± 0.0	26.3 ± 0.2	25.6^{ijk}
Richmond	25.9 ± 0.1	25.8 ± 1.1	27.1 ± 1.3	28.7 ± 0.8	27.9 ± 0.2	28.3 ± 0.1	27.3^{abcdefg}
Rigodon	28.5 ± 0.5	23.5 ± 0.5	24.1 ± 1.2	27.2 ± 0.2	33.3 ± 1.2	27.5 ± 0.5	27.3^{abcdefg}
Roskens	24.3 ± 0.8	28.4 ± 1.5	26.4 ± 0.6	29.2 ± 0.9	30.7 ± 1.4	28.8 ± 1.3	28.0^{abcd}
Synextra	26.5 ± 0.8	31.0 ± 1.3	26.2 ± 0.9	27.9 ± 0.6	27.9 ± 1.1	28.6 ± 0.9	28.0^{abcd}
Mean	25.5^{D}	25.7^{CD}	26.1^{C}	27.8^{B}	28.7^{A}	27.8^{B}	

[1]Mean values for locations shown with the same uppercase superscripts are not significantly different ($P < 0.05$). Mean values for genotypes shown with the same lowercase superscripts are not significantly different ($P < 0.05$). Data is expressed as mean ± standard deviation.

Analysis of variance (ANOVA) showed that protein, total starch, and amylose content were all significantly ($P < 0.001$) affected by genotype, environment, and GxE interaction (Table 9). The percent contribution to variance was highest for the main effects in relation to the interaction except for amylose content. The GxE interaction contributed the most to variance (~43%) in amylose content, while the effects of environment (~27%) and genotype (~21%) appeared similar. Protein and total starch contents were more strongly influenced by the environment compared to genotype as evident by the percent contribution to variance (Table 9).

Table 9. Analysis of variance of the effect of genotype, environment and their interaction on protein, total starch and amylose content on 30 genotypes across 6 environments[1]

Source	**DF**	**Protein**	**Total Starch**	**Amylose**
Genotype (G)	29	398.7 (24.3)***	1097.6 (18.6)***	411.7 (21.6)***
Environment (E)	5	858.9 (52.4)***	3922.5 (66.4)***	522.3 (27.4)***
GxE	145	364.0 (22.2)***	626.0 (10.6)***	827.7 (43.4)***
Error	180	17.1 (1.0)	257.8 (4.4)	143.9 (7.6)

[1]Data is represented as sum of squares with percent contribution of variance given in brackets.

***Indicates significance at $P < 0.001$. DF= Degrees of Freedom.

3.4.4. Principal Component Analysis

It is crucial to understand in depth the relationships among the main traits analyzed (protein, total starch and amylose) and genotype and environment beyond ANOVA. Complete understanding of oat GxE effects can be beneficial for breeding trials. Therefore, principal component analysis (PCA) was used to explain further in the form of a biplot. PCA was able to display the relationships among genotypes and growing environments. Biplots were utilized to graphically represent the PCs (principal components) corresponding to genotypes and environments associated with each trait analyzed by analysis of variance. For the main traits, a significant proportion of the overall variation was explained by the first and second PC. Between PC1 and

PC2, they explained 85% of the variation in protein, 90% in starch, and 84% in amylose. Growing years and growing locations were affected differently in the sense that growing years were primarily differentiated by PC1, while growing locations were negligibly differentiated by PC1 and PC2. Half of the 30 genotypes were differentiated by PC1, while the other half were differentiated by PC2. The traits were more explained by PC1 and PC2, followed by genotype and environment. For example, protein and starch are differentiated by PC1, and amylose is differentiated by PC2. The results for protein, total starch, and amylose are shown in Figure 4.

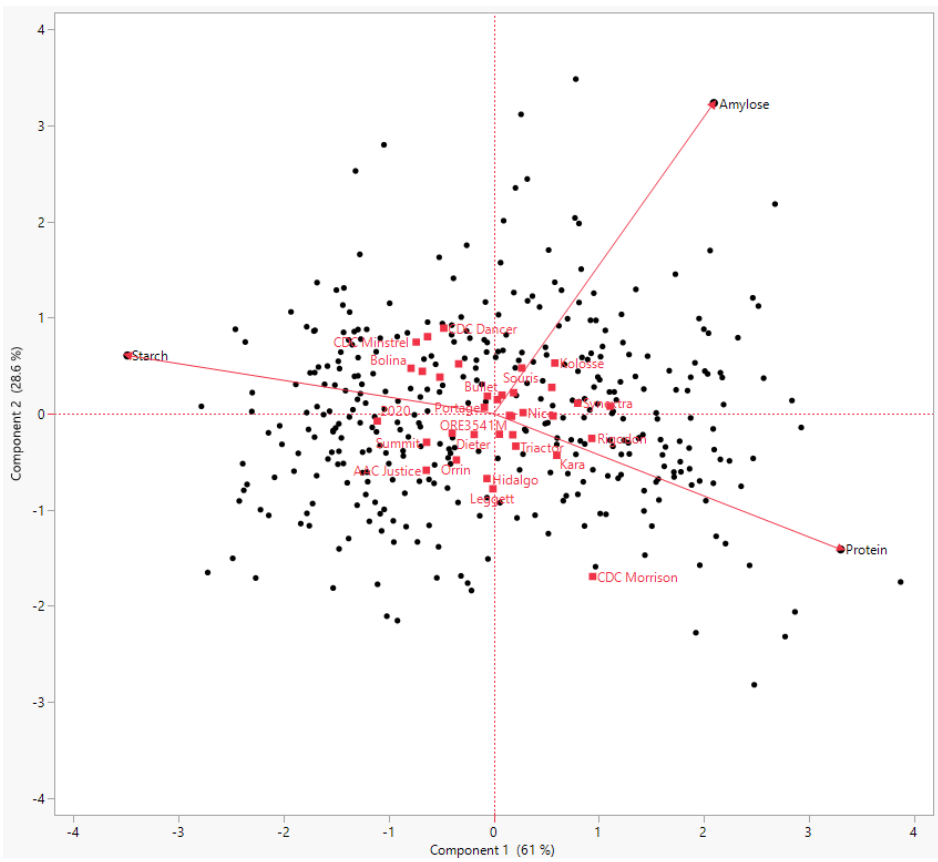

Figure 4. Biplot for protein, total starch, and amylose

PCA could explain approximately 90% in total of the variance of genotype responses to the selected traits in the growing environments. Figure 4 shows that PC1 and PC2 explained 61% and 29% of variance, respectively, indicating that the accuracy of PC1 was higher compared to

PC2. The biplot can be used to understand which genotypes are superior, inferior, and consistent for each trait amongst the growing environments. The origins represent the mean of all genotypes. The distance that a marker extends from the origins signifies the magnitude of the trait, either above or below the mean of all genotypes. In the biplot, genotypes positioned near the origins indicate indistinct interactions among environments and are expected to have trait values close to the means of all genotypes.

Growing locations (Brandon, Lacombe, and Portage) fell close to the origin or intersection of the biplot, an indication that they had a negligible effect on the traits. However, ANOVA showed a significant influence from the environment on all traits, suggesting that the growing year had the greatest effect on traits compared to growing locations. The biplot confirmed ANOVA results by showing that growing years are further from the origin or intersection. Growing years (2020 and 2021) had the same magnitude in opposite directions from PC1. The 2020 growing year fell to the left of PC1 and the 2021 growing year fell to the right. The rainfall pattern in August 2021 was unusually high and greater than in 2020 (Table 5), which could explain the notable variations in analyzed traits. Pearson correlation analysis showed rainfall in August was significantly ($P < 0.05$) and positively correlated (0.896) with protein content (Table 10). In addition, the correlation analysis indicated a negative correlation between starch and August rainfall, implying an inverse relationship between starch and protein. Hence the genotypes positioned to the right of PC1 exhibited higher protein content and lower starch content, whereas those positioned to the left had displayed higher starch content and lower protein content. Pearson correlation showed a significant ($P < 0.001$) negative correlation (-0.810) between protein and starch (Table 11), which confirmed their inverse relationship represented in the biplot (Figure 4). Taking into considering the directions of the protein and starch vectors, a connection with the growing years could be established. The 2020 growing year marker is to the negative side of PC1, similar to the starch vector, while the 2021 growing year marker is to the positive side of PC1, similar to the protein vector. This suggests that groat starch accumulation was higher in 2020 compared to the 2021 growing year and the opposite was true for groat protein accumulation. Tukey-Kramer honest significant difference analysis that was carried out for environmental means, confirmed the result obtained from the biplot for protein and starch (Table 6 and 7).

The biplot also showed that most of the genotypes fell near the center of the plot, indicating stability in biochemical accumulation from environmental stress. The genotypes that had values deviating further from the origin, either in the positive or negative direction of PC1 and PC2, showed less stability for the selected traits and greater susceptibility to environmental influences. For instance, genotypes Akina, ORE3541M and Bullet had low absolute values for PC1 and PC2, meaning they did not exhibit major differences in trait values compared to the means of all genotypes across the growing environments. Tables 6 to 8 show these three genotypes had means for protein, total starch, and amylose that were not significantly different among the growing environments. Hence, these genotypes did not show superiority or inferiority, but provided consistent results for all the tested biochemical traits in this study, regardless of the environment. Cultivar CDC Morrison is a noticeable outlier on the biplot (Figure 4) as it fell to the positive side of PC1 and negative side of PC2, unlike any other genotype. The location of the marker suggests CDC Morrison is above average for protein, total starch, and amylose content. Drawing a straight line from the genotype marker CDC Morrison to the positive quadrant of PC2 would reveal the genotypes with completely opposite traits of CDC Morrison. These genotypes include Bolina, CDC Minstrel, and CDC Ruffian based on the values of the loading matrix. The genotypic mean values obtained for CDC Morrison confirmed that it was above average for all traits given its lowest starch and amylose content and the highest protein content across majority of the growing environments (Table 6-8). However, for genotypes Bolina, CDC Minstrel, and CDC Ruffian, the biplot only accurately depicted them as being above average for total starch and protein but not amylose, which is congruent with the genotypic means (Table 6-8). This is because PC1 explained these three genotypes to a greater extent compared to PC2.

Cultivar Kolosse was above average for amylose content across the six environments (Table 8). However, this genotype did not exhibit the highest value for PC2. According to the PC2 values, cultivar CDC Dancer would have the highest amylose content; however, this was not the case according to the observed mean values (Table 8). The likely cause of these discrepancies is that PC2 can only explain approximately 29% of the variation and is less reliable compared to PC1.

The biplots enabled the identification of genotypes that potentially possess advantageous combinations of traits, making them suitable candidates for breeding trials. For instance, if the goal is to develop a lineage with higher protein and amylose levels, it is advisable to choose a genotype in the right quadrants, as they are more likely to yield the desired traits compared to those in the left quadrants. The genotypes with higher values for PC1 are superior and are influenced to a greater extent by the year they are grown. Those genotypes that were centered near the origin (PC2 = 0) were more stable and less affected by a growing environment. For instance, CDC Morrison and Rigodon both had similar protein values that were above average across all environments (Table 6). Rigodon demonstrated its dependability as a choice for growers looking to cultivate oat with elevated protein content, as it exhibits a more consistent genotype (PC2 ≈ 0.2) compared to CDC Morrison (PC2 ≈ 1.7). This could be confirmed by the higher coefficient of variation (CV) obtained for CDC Morrison (~15%) and the lower CV obtained for Rigodon (~7%) based on the genotypic means (Table 6). However, either Rigodon or CDC Morrison would be a good option if the desired outcome was for high protein.

3.4.5. Correlation Analysis

PCA provided a thorough understanding of how the analyzed traits were affected by genotype, location, and year. In order to enhance comprehension, correlation analysis was conducted (Table 10) to evaluate the reliability of findings derived from PCA and the extent of their significance. From the biplot (Figure 4) it was determined that growing year had a greater influence on oat phenotype thus, the goal was to further analyze how the traits measured were influenced by the changing environmental conditions during the growth period. Means for each environmental condition were analyzed on month basis for the present study, as seasonal means were not sufficient for interpretation of the relationship between environment conditions and traits, particularly for protein. Protein content was the only trait that was significantly correlated with the environmental conditions listed in Table 10. Protein was significantly ($P < 0.01$) and positively correlated (0.932) with mean daily maximum temperature in September. This suggested that higher temperatures occurring later in the growing season may be favourable for higher protein content. Zhang et al. (2022) found that protein content for wheat was positively correlated with daily maximum temperatures, similarly to what was seen in this study. An unusual result that was found was the significant ($P < 0.05$) positive correlation between protein and rainfall in August (0.896), which suggested that higher rainfall towards the end of the grain filling period would favour higher protein content. Literature has reported that protein content in cereal grains typically increased with drought conditions (Csaba et al., 2012; Doehlert et al., 2001), which is opposite to what was observed in the present study. The rate of protein synthesis in a cereal grain kernel is high in the first half of grain filling where about half of the protein has synthesized and will continue until maturity (Spurway, 1988). Drought-like conditions observed in the early stages of grain filling (June to July) for the 2021 growing year compared to 2020 (Table 5) could have promoted the increase in protein synthesis. Thus, the flood-like conditions that occurred in the latter half of anthesis in August of 2021 compared to August of 2020 could explain the positive relationship seen in the biplot between the 2021 growing year and protein (Figure 4). Increased rainfall later in the season is likely to have a lesser influence on protein content since grain filling is almost complete. It may also be of interest to note that the negative correlation, although not significant, seen between total starch and rainfall in August could be explained by the same. As starch synthesis occurs soon after protein synthesis, it is possible that when drought conditions are observed during grain filling (June to July), protein accumulation is favoured leading to less starch accumulation.

Table 10. Correlation of grain traits and monthly environmental conditions across six growing environments pooled from 30 oat genotypes.

	Protein	**Total Starch**	**Amylose**
Growing Month		**Mean Daily Maximum Temperature**	
May	0.553	-0.300	0.115
June	0.436	-0.413	0.377
July	0.482	-0.419	0.355
August	-0.356	0.415	-0.406
September	0.932**	-0.752	0.573
		Mean Daily Minimum Temperature	
May	-0.719	0.639	-0.542
June	0.341	-0.317	0.279
July	-0.043	0.035	-0.028
August	-0.067	0.179	-0.212
September	0.508	-0.411	0.262
		Rainfall	
May	0.116	-0.219	0.151
June	-0.195	0.369	-0.464
July	-0.780	0.667	-0.566
August	0.896*	-0.597	0.463
September	-0.498	0.470	-0.293
		Growing Degree Days	
May	-0.176	0.230	-0.241
June	0.449	-0.434	0.399
July	-0.339	0.086	-0.032
August	-0.014	0.160	-0.225
September	0.740	-0.659	0.434

* Indicates significance at $P < 0.05$. **Indicates significance at $P < 0.01$.

The phenotypic correlation coefficients illustrated the relationships between traits (Table 11). Protein had a significant ($P < 0.001$) highly negative correlation (-0.810) with total starch. This result can be represented by PCA, where protein and total starch were strongly differentiated by PC1, and the vectors present in the biplot (Figure 4) extend from PC1 in opposite directions, explaining their negative correlation. Protein was also found to be significantly ($P < 0.001$) positively correlated (0.630) to beta-glucan, as has been determined previously (Šterna et al., 2018). Unlike protein, majority of the traits that were significantly correlated to beta-glucan content had a negative correlation (Table 11). There was a negative correlation between beta-glucan and both total starch and amylose content. The negative correlation between beta-glucan and total starch has been identified in previous work (Doehlert et al., 2001; Herrera et al., 2016). It could be suggested that the environmental conditions supporting high protein content (Table 10) and consequently low starch content could have also favoured the accumulation of beta-glucan but not amylose content. The significant ($P < 0.01$) positive correlation (0.550) between beta-glucan and oil could suggest that accumulation of oil in oat can also be influenced by the same environmental conditions supporting high protein content, as shown by the negative correlation between total starch and oil content (-0.630).

Table 11. Correlation coefficients among all measured traits[1]

Traits[2]	**Protein**	**Amylose**	**Total Starch**	**Beta-glucan**	**Oil**	***L**(G)**	***a**(G)**	***b**(G)**	***L**(W)**	***a**(W)**
Amylose	-0.200									
Total Starch	-0.810***	0.410*								
Beta-glucan	0.630***	-0.510**	-0.860***							
Oil	0.200	-0.040	-0.630***	0.550**						
*L**(G)	-0.410*	0.320	0.590***	-0.500**	-0.570***					
*a**(G)	0.100	-0.070	-0.020	-0.090	-0.110	-0.360				
*b**(G)	-0.230	0.320	0.350	-0.370*	-0.380*	0.360*	0.010			
*L**(W)	-0.140	-0.030	0.130	-0.200	-0.090	0.210	-0.150	0.410*		
*a**(W)	-0.300	0.410*	0.370*	-0.430*	-0.120	0.090	0.420*	-0.110	-0.350	
*b**(W)	0.330	-0.060	-0.220	0.170	-0.140	0.010	-0.240	0.520**	0.450*	-0.580***

*Indicates significance at $P<0.05$. **Indicates significance at $P<0.01$. ***Indicates significance at $P<0.001$. [1]All traits are based on means of six environments except colour values for oat groat and whole oat, which are based on the means of three environments in 2021. [2]*L**(G) (lightness), *a**(G) (redness/greenness), and *b**(G) (yellowness/blueness) indicate colour values for oat groats, while *L**(W), *a**(W), and *b**(W) indicate colour values for whole oats.

Colour values for oat groat and whole oat were measured to understand if the colour of oat can be used as an estimate for its chemical composition. Correlation was able to demonstrate that the

*L** (lightness) values for oat groats were significantly ($P < 0.05$) correlated with majority of the physicochemical properties, while the other colour values had weak and/or non-significant correlations. *L** values for oat groats were negatively correlated with beta-glucan (-0.500) and oil content (-0.570) and positively correlated with total starch (0.590) (Table 11). This suggested that oat groats that appear to be lighter in colour could have decreased oil and beta-glucan, while total starch content could be higher. Based on the biplot (Figure 4) the growing conditions in the 2020 environments promoted higher total starch values and lower beta-glucan and oil content (Table 11). Therefore, it can be suggested that oats grown in environmental conditions favouring high total starch may result in lighter coloured oat groats making the *L** value a possible predictor for starch content in oat groats. Increased lightness of oat is desirable for producers because it can reflect the quality of the oat grain (Jokinen et al., 2021). Observing the correlation between *L** value and total starch allows the biplot to be a useful tool for selection of an oat genotype that would have a greater chance of producing lighter coloured oat groats based on higher starch content, as predicted by the negative side of PC1.

3.5. Conclusion

Genotype, environment and their interaction were seen to significantly influence protein, total starch and amylose content in Canadian oats. Although all factors were significant, environment generally had the greatest contribution to variation for the selected traits. The biplot from principal component analysis was able to show that the growing year had the greatest effect on total starch and protein while growing locations contributed no distinct differences for the three measured traits (protein, total starch, amylose). Genotypes that were exceptional (highest or lowest percentage for the measured trait) as well as consistent across all growing environments can be identified using the biplot, which allows growers and breeders to select oat genotypes appropriate for the desired application. PCA visually represented the strong inverse relationship between starch and protein, which was further confirmed using correlation analysis. Correlation demonstrated the relationships among all measured traits and with climate conditions for the main traits. Notably, only protein content showed significant correlations with rainfall and maximum temperature. The findings may also allow for the selection of other important traits such as beta-glucan, oil and oat groat colour before genotypes are to be grown in various environments.

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Chapter 4: Pasting, Physiochemical, and Morphological Properties of Native Oat Starch and Citrate-Modified Oat Starch

4.1. Abstract

Starch is widely utilized in the food industry, but its native form may have limitations in terms of functionality and nutrition. This study examined the characteristics of native oat starch and explored its potential for chemical modification by cross-linking, with the aim of gaining a deeper understanding of its functionality. Citrate-modified oat starch (COS) was generated by cross-linking native oat starch (NOS) with citric acid. Chemical analysis revealed distinctions between NOS and COS, notably in terms of resistant starch content, which was elevated in COS. X-ray diffraction results revealed that NOS exhibited crystalline peaks characteristic of A-type starch, distinguishing it from COS. In contrast, COS displayed absent crystalline peaks, attributed to cross-linking. Swelling factor, solubility, and paste clarity along with most of the pasting properties were found to be significantly lower for COS compared to NOS. The scanning electron microscopic images showed how cross-linking could alter the morphology of the NOS. The findings from this investigation highlighted significant distinctions between NOS and COS. The cross-linking process successfully enhanced the resistant starch content and imparted distinctive properties absent in native oat starch. Consequently, there is potential for incorporating modified oat starch as a food ingredient.

4.2. Introduction

Starch is a common material used in many industries. It has wide applications from its use in biodegradable packing to food ingredients. In the food industry native starch is used as thickeners, gelling agents, bulking agents and for its water retention abilities (Liu et al., 2014). However, there are some limitations to the use of native starch. Not only does native starch have high tendencies to retrograde, it is also suspectable to mechanical stress, and has low thermal stability. These characteristics can impart poor quality on final products such as taste and texture (Liu et al., 2014; Marta et al., 2022).Therefore, many native starches are modified for food applications to enhance their functional and nutritional properties. The process of modification brings about structural changes in starch, influencing its physiochemical properties to varying extents and rendering it suitable for a range of industrial applications (Liu et al., 2014).

There are various techniques available for altering starch which encompass both physical and chemical approaches. In the food industry, chemical modification is prevalent, often involving the cross-linking of native starch to fortify the granule structure and limit swelling. This process enhances the functional and nutritional attributes of the starch. The food industry frequently uses citric acid as a cross-linking agent, as it is an ingredient that is generally recognized as safe (GRAS) (Golachowski et al., 2020; Kim et al., 2017). Through its reaction with the hydroxyl groups in starch, citric acid generates a product with a notable degree of substitution. Additionally, this reaction has the capacity to convert hydrophilic hydroxyl groups into hydrophobic ones, preventing retrogradation and thus extending the shelf life of the product (Kim et al., 2017; Marta et al., 2022). From a nutritional point of view, the ability for cross-linked starch to act as a type of resistant starch (RS), provides similar benefits as dietary fibre.

RS is not digested in the small intestines and produces short-chain fatty acids in the large intestine.

Oat starch has unique properties such as elevated viscosity, resistance to shear force, resistance to paste formation, and hydrophobicity in gel formation due to its high lipid content (Zhu et al., 2023). Along with these technological properties, oat starch has naturally higher amounts of amylose compared to other cereals (Zhong et al., 2018; Zhu et al., 2023). High amylose starches have been found to have similar functional and nutritional properties as modified starch (Li et al., 2023). Zhu et al. (2023) found amylose content to range from 33.7-37.7% for Canadian oat starches. However, research has also shown that the amylose content of oat can vary depending on genotype and growing location (Hüttner et al., 2011; Tester & Karkalas, 1996), suggesting that it may be difficult to select an appropriate oat variety to consistently provide high amylose and its associated benefits. Thus, there is an opportunity to mimic the properties of high amylose starch in the form of chemically modified cross-linked starch, which could lead to more consistent results. Previous literature has studied the chemical modification of starches from different botanical sources such as rice, corn, sorghum and sago (Kim et al., 2017; Liu et al., 2014; Marta et al., 2022; Olayinka et al., 2015). However, to the best of our knowledge, minimal research exists on the chemical modification of oat starch, specifically using citric acid. Therefore, the present study aims to produce chemically modified oat starch that has been cross-linked with citric acid to understand its functional and physiochemical properties in comparison to native oat starch for potential use in the food industry.

4.3. Materials and Methods

All reagents were obtained from Fisher Scientific (Ontario, Canada) unless specified otherwise.

Oat starch was extracted from CDC Morrison grown in 2019 in Brandon, Manitoba, Canada. Groats were milled with a Retsch Ultra Centrifugal Mill ZM200 (Retsch GmbH, Haan, Germany) using a 0.5mm sieve to obtain whole oat flour. The groat flour had 17.5% protein, 60.5% total starch, 23.8% amylose, 7.4% oil, and 5.3% beta-glucan.

4.3.1. Oat starch isolation

Oat starch was extracted using alkaline pH, typical of protein extraction (Agriculture and Agri-Food Canada). Oat flour was mixed with water at 1:10 ratio. The suspension was homogenized with a handheld immersion blender (Dynamic MX2000, Memphis, TN, USA) and pH was adjusted to between 9.0 and 10.0 using sodium hydroxide. The mixture was allowed to sediment overnight at refrigerated temperature (0 - 4°C). The top liquid and bran layer was carefully decanted without disturbing the bottom starch bed. The residue was washed three times with RO water. The residue was passed through a 75 μm sieve to remove residual bran particles. The bran residue on the screen was rinsed three times with reverse osmosis (RO) water and the filtrates were pulled together. The filtrate was left to sediment at refrigerated temperature (0 - 4°C). The water was carefully decanted. The pH of the starch slurry was adjusted to 7 with 1 M HCl before transferring to drying pans (66 x 46 cm) for air drying. The starch was dried at room temperature for 24 hours and ground using a nut spice grinder (Cuisinart Model SG-10C) to pass through a 300 μm sieve.

4.3.2. Preparation of resistant starch

Citrate-modified starch was prepared from the extracted oat starch using citric acid for chemical modification according to the procedure described by Xie & Liu (2004) with some modifications. The 40% citric acid solution was made by mixing 280 g citric acid with 280 g RO water, and pH was adjusted to 3.5 using 20 M sodium hydroxide. The final volume was adjusted to 700 mL using RO water. Oat starch (350 g) was added to the 40% citric acid solution (350 mL) in a glass bowl and allowed to dry in a forced-air oven (50°C for 12 hours). The pellet was broken into smaller pieces by hand and baked at 140°C for 7 hours in a forced-air oven. The dried citrate-modified starch was washed with RO water until a pH of 7 was reached, to ensure all unreacted citric acid was removed. The washed starch was then dried at 30°C for 16 hours. Dried COS was ground using a nut and spice grinder (Cuisinart Model SG-10C) to pass through a 300 μm sieve.

4.3.3. Proximate analysis

Compositional analysis was conducted on oat starch for moisture, protein, total starch, fat, and ash according to the AACC approved methods 44-15.02, 46-30.01, 76-13.01 (only for NOS), 30-10.01 (only for NOS), and 08-01.01, respectively. Total starch content for COS was measured using a modified version of the AACC approved method (76-13.01), referred to as Rapid Total Starch for samples containing resistant starch (Megazyme International, Wicklow, Ireland). The determination of amylose content was done using the Megazyme kit K-AMYL (Megazyme International, Wicklow, Ireland); with slight modifications for COS. Samples with resistant starch were pre-treated using 1.7 M sodium hydroxide, as outlined in the Rapid Total Starch assay protocol (K-TSTA-100A, Megazyme International, Wicklow, Ireland). Then 12 M hydrochloric acid was added to bring the solution close to neutral pH (6.5-7.0) and the assay was carried out in the same manner using the Megazyme kit K-AMYL. Resistant starch content was measured using the Megazyme K-RSTAR kit (Megazyme International, Wicklow, Ireland). Duplicate analyses were done for all measurements and are presented on a dry weight basis.

4.3.4. Swelling power and solubility index

The swelling power of starch was determined using the method described by Xia et al. (2015) with some modifications. Starch (0.5 g) was suspended in 25 mL of distilled water and kept in a linear shaking water bath (OLS200, Grant Instruments, Shepreth, Cambridge, England) at 80°C for 30 minutes at 100 strokes/min. Samples were then cooled rapidly by placing in an ice bath until they reached room temperature (~25°C). The samples were centrifuged, and top liquid was decanted. The remaining pastes were weighed. Swelling power was calculated using the equation described by Xia et al. (2015).

Solubility index of the starch was measured following a method described by Liu et al. (2014) with only one modification, whereby the supernatant was collected and dried in an air oven at 120°C for 2.5 hours. The solubility index (%) was determined on a dry basis by calculations as described by Liu et al. (2014).

4.3.5. Paste clarity

Clarity of the starch paste was determined according to Liu et al. (2014) with some modifications. Starch was placed into a 10 mL capped test tube with distilled water. These suspensions underwent heating in a boiling water bath for 30 minutes, with intermittent vortexing during entire incubation period. Transmittance (%) was recorded as paste clarity.

4.3.6. Rapid Visco-Analysis (RVA)

Pasting properties of native and cross-linked starch were determined using a Rapid Visco Analyzer (RVA-4500, Perten Instruments, Australia). In brief, 3.5 g of starch was suspended in 25g of distilled water. The slurries underwent standard controlled heating and cooling cycles as described previously (Punia et al., 2019).

4.3.7. X-ray Diffraction

X-ray diffraction (XRD) patterns were observed for NOS and COS using an X-ray diffractometer (D-5000, Bruker/Siemens, Madison, WI, USA) equipped with a Cu X-ray tube. The scattered radiation was detected in the angular range of 3–40°(2θ) using a 0.02° step and 0.5 s dwell time. Degree of relative crystallinity was calculated using OriginPro 2024 (OriginLab Corporation, Northampton, MA, USA) based on the formula described by Zhu et al. (2023).

4.3.8. Scanning Electron Microscopy (SEM)

The morphology of native starch and oat citrate-modified starch granules of oats were observed using a Scanning Electron Microscope (Inspect S50, FEI Company, Hillsboro, OR, USA). Samples were taped down on double sided carbon tape on aluminum stub. Loose particles were removed with compressed air. All imaging was done under an acceleration voltage of 10 kV and magnifications of 2699x and 3953x. Average starch granule size was calculated using ImageJ Free Software (Image Processing and Analysis in Java).

4.3.9. Colorimetry

Colour properties of starches were measured using a Konica Minolta Chromo Meter (model CR-410, Minolta Co., Osaka, Japan) with a 50mm aperture size. The CIE $L^*a^*b^*$ colour space was used where L^* represents lightness, and a^* and b^* represent chromaticity and measure from green to red and blue to yellow respectively. Starch was packed inside the granular material attachment (CR-A50) to obtain CIE $L^*a^*b^*$ colour parameters. Duplicate analysis was performed for each sample.

4.3.10. Statistical analysis

One-way analysis of variance (ANOVA) was performed and when a significant difference was found ($P < 0.05$), the Tukey-Kramer honest significant differences method was used for comparison of means using R software package (R version 4.1.2, R foundation for Statistical Computing, Vienna, Austria). Starch chemical and pasting properties were measured based on duplicate analysis. All results (where applicable) are presented as mean ± standard deviation.

4.4. Results and Discussion

4.4.1. Chemical composition of starches

The proximate composition of native oat starch (NOS) and citrate-modified oat starch (COS) are determined and shown in Table 12. The purity of our extracted NOS is observed to be high, as evident by the total starch content of approximately 95% (Table 12). A significant increase in resistant starch content was achieved (~ 0.4% to 57%) using citric acid for chemical modification (Table 12). Similar results have been reported for rice starch following chemical modification with citric acid (Kim et al., 2017; Remya et al., 2018). The cross-linked structure in the COS resists amylolytic enzyme access. This occurs as a result of the hydroxyl group in the starch structure being replaced with a citrate group through esterification (Kim et al., 2017). When comparing NOS to COS, almost all the compositional characteristics increased in value apart from protein and amylose content, both of which showed a decrease (Table 12). The washing step employed during the formation of COS may have reduced the soluble protein fractions, thus decreasing its protein content. Total starch increased slightly for COS (~96%). Other literature that studied sago and sorghum starch, reported similar results for starch content after chemical modification (Marta et al., 2022; Olayinka et al., 2015).

There was a decrease in amylose content from approximately 25% to 20% for NOS and COS, respectively. However, this difference was not found to be significant due to their coefficient of variation of 7.3 and 12.0% for NOS and COS, respectively. Remya et al. (2018) observed that amylose content in modified starches (potato, cassava and sweet potato) decreased with increasing concentrations of citric acid treatment. The amorphous region of starch, which is mainly composed of amylose, is more susceptible to hydrolysis compared to the crystalline region. Thus, high levels of acid hydrolysis may have degraded the amorphous region, and consequently decreased amylose content. Lastly, ash was seen to significantly increase in the COS (~3%) compared to the NOS (~1%) (Table 12). This could be explained by the presence of any remaining citric acid in the starch that was not washed away.

4.4.2. Colour

The measurement of colour is an indicator of product quality and consumer acceptance. NOS and COS colour values (L^*, a^*, and b^*) were found to be significantly different between the two starches. NOS had higher values for L^* (lightness) and a^* (-green to +red) and a lower value for b^*(-blue to +yellow). This would suggest that the native oat starch is whiter (L^*; ~95), with a slightly higher tendency to red (a^*; ~ -1.0) and lower tendency to yellow (b^*; ~2) compared to COS (Table 12). The higher b^* value that was observed for OCS (~6) indicates it is more yellow than NOS likely as a result of the modification treatment with citric acid, which was also observed by Chaiwanichsiri et al. (2012) for rice flour.

Table 12. Chemical composition and colour of native and citrate-modified oat starch

Analysis		**NOS**	**COS**
Composition	Protein (%)	0.44 ± 0.0[b]	0.18 ± 0.0[a]
	Total Starch (%)	95.3 ± 1.4[a]	96.0 ± 0.4[a]
	Resistant Starch (%)	0.4 ± 0.5[b]	56.5 ± 0.6[a]
	Amylose (%)	25.4 ± 1.9[a]	20.1 ± 2.6[a]
	Oil (%)	0.8 ± 0.5	N.D.[2]
	Ash (%)	1.1 ± 0.1[b]	3.0 ± 0.0[a]
Colour	*L**	94.8 ± 0.3[a]	93.3 ± 0.1[b]
	*a**	-0.9 ± 0.0[b]	-1.3 ± 0.0[a]
	*b**	2.4 ± 0.2[b]	5.6 ± 0.1[a]

Data represented as mean ± standard deviation (n=3).Values with the same letters in the same row do not differ significantly (P < 0.05). NOS = native oat starch; COS = citrate-modified oat starch. [2]N.D.= Not determined.

4.4.3. Crystallinity of Starches

Starch can be categorized by type A, B, and C based on the strongest diffraction peaks observed in their x-ray diffraction patterns. Figure 5 depicts the X-ray diffraction patterns of NOS and COS. The strongest peaks for NOS were observed at Bragg angles of approximately 15, 17, 20, and 23° 2θ (Table 13) and are typical for cereal starches. For each diffraction angle (2θ), a corresponding d-spacing is determined (Table 13), indicating the distances between atomic planes that lead to the formation of diffraction peaks.

The XRD profile showed the strongest peaks at 2θ of 23° and a doublet at 17° and 18° (Figure 5), which are typical for A-type pattern. The d-spacings for NOS were approximately 5.9, 5.1, 4.9, 4.4, and 3.9°A (Table 13). Other literature has reported similar values for starch classified as A-type (Cheetham & Tao, 1998; Irani et al., 2017). Thus, the NOS can be classified as an A-type starch. The smaller peaks observed at 2θ values of 15° and 20° (Figure 5) are typical of higher amylose starches. It has been identified previously that peak intensity at 2θ value of 15° increase with decreasing amounts of amylose in starch and that the presence of a peak at 2θ value of 20° (4.4°A), which was seen in the present study, corresponds to amylose-lipid complexes (Cheetham & Tao, 1998; Taguchi et al., 2023; Zhu et al., 2023). Therefore, the XRD profile suggests that NOS had a greater content of amylose and amylose-lipid complexes compared to cereal starches that have higher intensity peak at 2θ value of 15° and do not show a peak at 2θ values of 20°, such as for rice starch (Kim et al., 2017). The increased peak at the 2θ value of 15°is reflected by the 25% amylose found in NOS (Table 12). The XRD profile for COS is dissimilar from what observed for NOS (Figure 5). This is likely due to the chemical modification of the oat starch, which led to a change in crystallinity as observed by the flattening of peaks at 15, 17, 18 and 23° 2θ that classified NOS as A-type. Moreover, the concentration of citric acid solution used for modification is important, as higher concentrations of citric acid have a greater tendency to destroy the amorphous regions, which alters crystallinity (Zhang et al., 2023). Figure 5 shows only one detectable peak for COS at Bragg angle of 20° 2θ. This angle gave a corresponding d-spacing of 4.4°A, similar to what was seen in NOS. As discussed previously the peak at 2θ value of 20° (4.4°A) corresponds to amylose-lipid complexes. The esterification of the starch molecules that took place during citric acid treatment is likely to increase the amylose-lipid complexes (Shin et al., 2009), which may explain why a peak at 2θ value of 20° is seen for COS (Figure 5).

Relative degree of crystallinity was calculated to be approximately 36 and 13% for NOS and COS, respectively (Table 13). The large decrease in crystallinity observed for COS is explained by the loss in characteristic peaks, as seen in the XRD profile (Figure 5). The fact that the crystalline peaks in the COS were not detected suggests that cross-linking of citric acid occurred in the amorphous and crystalline regions (Kim et al., 2017). The findings regarding the change in crystallinity in our COS (Figure 5) closely resembled those reported by Kim et al. (2017) in their study on rice starch treated with citric acid cross-linking. They attributed the loss in crystalline peaks to the concentration of citric acid utilized in the treatment. This would suggest that the higher concentration of citric used in the present study (40%) may have increased the degree of citric acid substitution, which through the esterification reaction, decreased crystallinity. The decrease in crystallinity may also be attributed to the fact that NOS is an A-type starch. Remya et al. (2018) found that A-type starches are more likely to decrease in crystallinity when treated with citric acid compared to C-type starches, as A-type starches have a less tightly branched structure.

The relative degree of crystallinity for our NOS (~36%) concurs with reports by Hoover et al. (2003) but was considerably higher than what was observed by Zhu et al. (2023). This may be explained by the lower amylose content found in our NOS (~25%), compared to Zhu et al. (2023) who reported a higher amylose content (~35%).

Table 13. X-ray diffraction properties of native and citrate-modified oat starch

	X-ray diffraction angle (2θ) and d-spacing (Å)						
Sample[1]	**15**	**17**	**18**	**20**	**23**	**Type**	**Crystallinity (%)**
NOS	15.07 (5.87)	17.32 (5.11)	18.02 (4.92)	20.17 (4.40)	23.08 (3.85)	A	35.8
COS	–	–	–	20.08 (4.42)	–	N.A.[2]	13.1

[1]NOS = native oat starch; COS = citrate-modified oat starch. [2]N.A.= not applicable.

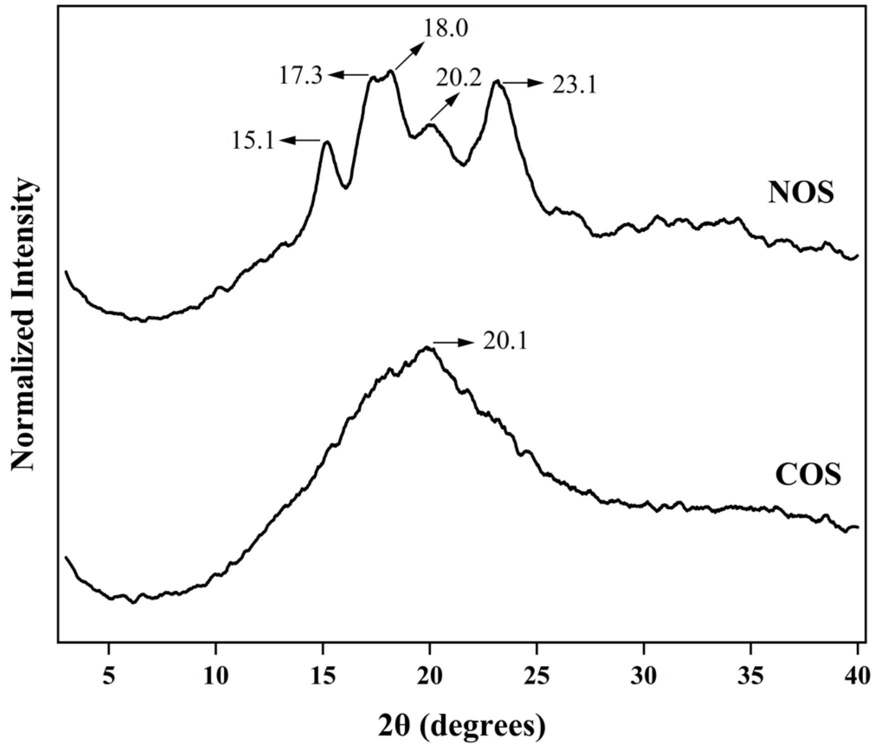

Figure 5. X-ray diffraction patterns for oat starch. NOS = native oat starch; COS = citrate-modified oat starch.

4.4.4. Starch paste properties

Changes in viscosity of starch can be measured through controlled heating and cooling cycles using an RVA. Parameters obtained from the RVA profile can give insight into the starch functional characteristics, which helps understand its application as a food ingredient. Figure 6 shows the RVA curves for NOS and COS and the corresponding results for the measured parameters are presented in Table 14. Significant differences for all RVA parameters were seen (excluding peak time) between NOS and COS, which is evident by their pasting curves. COS had a curve that remained flat for the entire heating and cooling cycle compared to NOS, which exhibited a curve with distinct fluctuations (Figure 6). For all RVA characteristics that were significantly different, NOS showed significantly higher values than for COS. The high peak viscosity for NOS (~4300 cP), demonstrates the ability of the starch granules to swell compared to COS (~19 cP), which did not swell. The significantly lower final viscosity observed for COS shows the inability for citric acid modified starch to form a paste. The pasting properties that were obtained for NOS were comparable to what has been previously observed for oat starch (Zhu et al., 2023). In comparison to starch from sorghum and rice, our NOS exhibited a higher pasting temperature and final viscosity (Olayinka et al., 2015; Remya et al., 2018). The higher pasting temperature may be a result of the higher crystallinity and more amylose-lipid complexes, as observed by higher peak intensity at 2θ value of 20° (Table 13). A greater extent of amylose-lipid complexes can reduce amylose leaching, thus effecting pasting temperature. Similar results were observed by Hoover et al. (2003) for oat starches.

The RVA profile obtained for the COS is comparable to what was observed by Kim et al. (2017) for citric acid-modified starch. This occurs due to the strengthening of starch chains through cross-linking with citric acid. This cross-linking restricts water absorption and swelling, resulting in the observed flat RVA profile in the current study (Figure 6).

Table 14. Pasting properties of native and citrate-modified oat starch

Analysis		**NOS**	**COS**
Swelling factor (%)		6.4 ± 0.3^a	4.4 ± 0.1^b
Solubility Index (%)		2.8 ± 0.1^a	1.9 ± 0.0^b
Paste clarity (%)		11.4 ± 0.0^a	1.6 ± 0.0^b
RVA	Peak (cP)	4263.5 ± 75.7^a	18.5 ± 0.7^b
	Trough (cP)	2911.5 ± 30.4^a	14.5 ± 0.7^b
	Breakdown (cP)	1352.0 ± 45.3^a	4.0 ± 0.0^b
	Final viscosity (cP)	5090.0 ± 50.9^a	17.0 ± 1.4^b
	Setback (cP)	2178.5 ± 20.5^a	2.5 ± 0.7^b
	Peak Time (min)	6.4 ± 0.0^a	3.3 ± 3.1^a
	Pasting Temperature (°C)	93.3 ± 0.7	N.A.[2]

Values represented as mean ± standard deviation (n=2). Values with the same letters in the same column do not differ significantly ($P < 0.05$). NOS = native oat starch; COS = citrate-modified oat starch. [2]N.A = Not applicable.

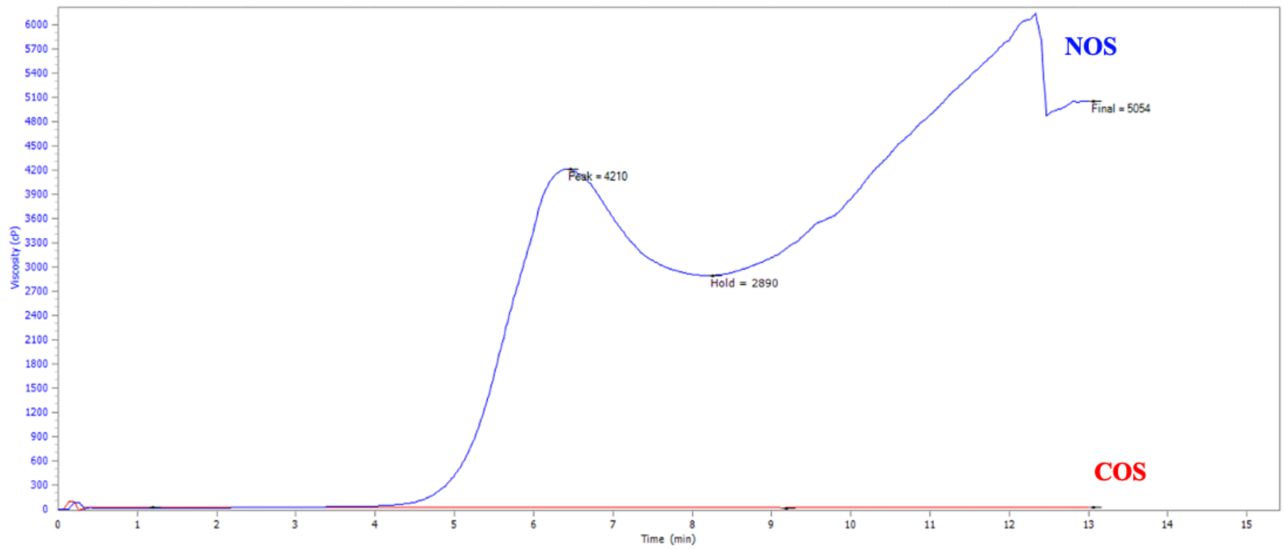

Figure 6. Pasting properties of oat starches. NOS = native oat starch; COS = citrate-modified oat starch.

4.4.5. Swelling power and solubility index

Swelling power for COS was significantly lower compared to NOS (Table 14). The decreased swelling power for COS (~4%) is typical for starches that have been cross-linked with citric acid. It is due to the strengthening of the starch granular structure from the citric acid cross-linking, which limits swelling (Kim et al., 2017). The swelling power of NOS (~6%) is similar to what has been reported previously for oat starch (Zhu et al., 2023); however, some reports have suggested higher swelling power for oat starch (Hoover & Vasanthan, 1992). Granular swelling is thought to be impacted by phospholipid content since phospholipids form amylose-lipid complexes that restrict swelling (Irani et al., 2017). Thus, it is likely that our NOS and COS had increased amylose-lipid complexes that resulted in decreased swelling power (Table 14), as evidenced by the 20° 2θ peak in the XRD profile (Figure 5).

Solubility index was found to significantly decrease for COS (~2%) compared to NOS (~3%) (Table 14). This observation agrees with previous findings that studied rice and corn starches

(Kim et al., 2017; Liu et al., 2014). The cross-linking enhances the bonding among starch polymers thereby increasing their integrity and affecting their solubility. Solubility may also be impacted by amylose and fat content in the starch. Similar as to swelling power, solubility is affected by amylose-lipid complexes, as they can decrease solubility (Zhu et al., 2023). XRD data obtained for NOS and COS support this observation (Table 13).

4.4.6. Paste clarity

Paste clarity has a direct relationship with starch swelling, as paste clarity increases with the decreasing amounts of undissolved starch granules after heating. Thus, a high paste clarity indicates increased swelling of starch granules (Zhu et al., 2023). Paste clarity for NOS was approximately 11% which was significantly higher than for COS (~2%) (Table 14). Similar results have been observed previously for rice and corn starches (Kim et al., 2017; Liu et al., 2014). Citric acid-modification of starch increases the density of granules and restricts swelling, as observed in Table 14. The density of granules will impact the light transmittance, as a dense granule reflects rather than absorbs (Irani et al., 2017). This density along with the low swelling power of COS can explain its low paste clarity. NOS had a lower paste clarity than what has been determined for oat starch previously (Zhu et al., 2023). Irani et al. (2017) found that more amylose-lipid complexes resulted in decreased paste clarity for canary seed starch. Thus, it is likely that oat starch with more amylose-lipid complexes, as supported by our results, yields pastes with decreased clarity.

4.4.7. Scanning Electron Microscopy

The micrographs shown in Figure 7A and 7B were used to obtain information on the size and shape of NOS and COS granules, respectively. Scanning electron microscopy (SEM) was able to identify the small and large granules typically found in oat starch, which were more apparent in

NOS compared to COS. The average size of the granules was determined to be 6.45 µm for NOS and 7.05 µm for COS. On average the COS granules were slightly larger; however, the distribution of granule size was greater for NOS. The NOS granules ranged from 1.72 to 16.86 µm in which the average size of the small granules was 3.02 µm and the larger granules were 9.80 µm (Figure 7A). Literature has reported oat starch granule size to range on average from 6-10 µm (Hoover & Vasanthan, 1992; Zhu et al., 2023), which is a smaller range than what was observed in the present study. This could be due to formation of starch clusters, making it difficult to measure granule size accurately. In comparison, the COS granule size ranged from 2.47 to 15.09 µm with an average of 4.18 µm and 9.93 µm for the small and large granules, respectively (Figure 7B).

The classification of starch granules can be made based on size and shape. The micrograph showed that the oat starch granules are small and large and are shaped as mostly irregular, polygonal, and ovoid and that they have the tendency to form clusters instead of distinct individual granules (Figure 7A and 7B). This morphology is unique compared to other cereal starches, such as wheat, making it more difficult to classify them into A-type (>10 µm and disc-like or lenticular) and B-type granules (<10 µm and spherical or polygonal) (Irani et al., 2017). However, it can be suggested that the NOS had A-type and B-type granules based on the micrograph image (Figure 7A) and predicted granule sizes. The peaks observed in the XRD profile classifies NOS as type-A; however, type-B granules are not reflected in the profile. This could be due to the tendency of the granules to cluster, making it difficult to differentiate between type A and B and possible bias in granule size during SEM analysis. Some differences can be observed in the starch granule structure following treatment with citric acid (Figure 7B). Most noticeably, the granules in COS became more jagged as opposed to being smooth and there is loss in the smallest granule size, making COS appear more uniform in size. This suggests that the formation of COS modified the starch granule structure by eroding the smooth surface and reducing the number of small granules. These morphological alterations may explain the differences seen in pasting properties and XRD profiles of COS compared to NOS.

A) **B)**

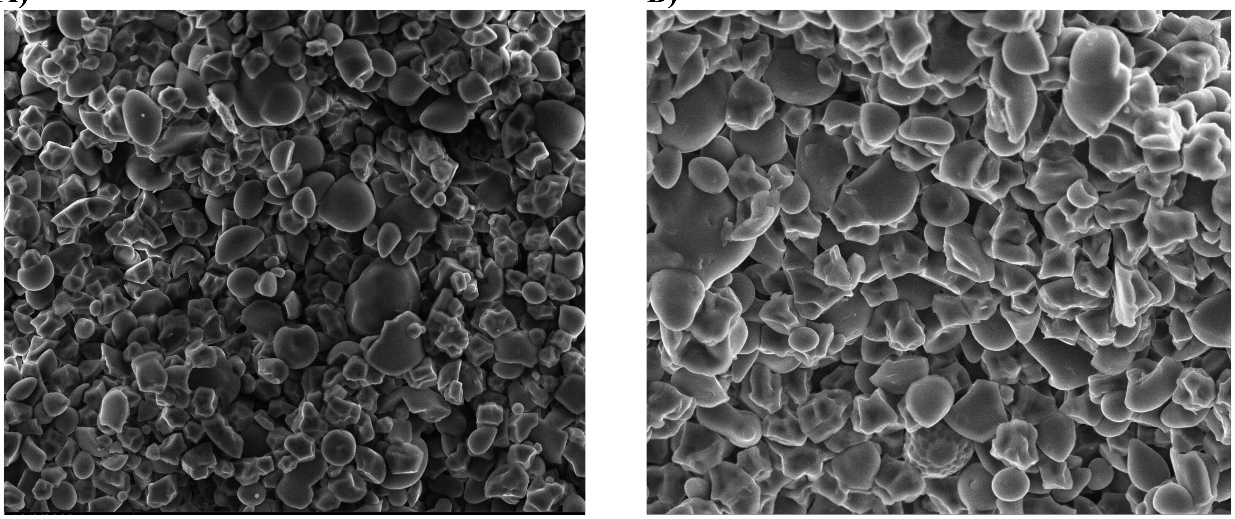

Figure 7. Scanning electron micrographs of oat starch granules. A) native oat starch (x 2700); B) citrate-modified oat starch (x 4000).

4.5. Conclusion

The present study investigated the physicochemical, pasting, and morphological properties of native oat starch and modified oat starch cross-linked with citric acid. The examination of both starch types indicated that the application of citric acid for oat starch modification resulted in changes in the chemical composition, particularly for resistant starch, ash and protein contents. Citric acid cross-linking successfully led to an increase in resistant starch content. X-ray diffraction patterns revealed that our NOS is classified as A-type starch, with a higher crystallinity typical of lower amylose starch. A loss of major diffraction peaks for COS showed that starch was highly cross-linked from the citric acid treatment as evident by the decrease in crystallinity. There was a noticeable difference in pasting profiles and a decrease in paste clarity, solubility, and swelling when oat starch was modified with citric acid. Morphology of granules obtained using SEM showed NOS had smooth and both large and small granules, while COS was observantly more jagged and there was a loss in the very small granules. These results confirmed that cross-linking oat starch with citric acid gives it unique physicochemical, pasting, and morphological properties dissimilar from native oat starch. These findings form a solid scientific foundation for the utilization of chemically modified oat starch as a food ingredient.

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Chapter 5: Exploring the Noodle Making Potential and Digestibility of Native Oat Starch and Citric Acid Cross-Linked Resistant Oat Starch.

5.1. Abstract

Oat starch, a significant by-product of Canada's plant protein industry, is often overlooked for human consumption. This puts emphasis on the necessity of reintegrating the starch into mainstream food production. Oat starch holds promise as a versatile food ingredient given its unique properties. To address persistent concerns regarding the health implications of starch consumption, chemical modification of starches present potential nutritional advantages when incorporated into food products. Consequently, ongoing research aims to enhance the utilization of oat starch, particularly in the production of starch noodles.

The optimized oat starch noodle preparation method was subsequently used for substituting varying levels of native oat starch (NOS) with citrate-modified oat starch (COS) (15%, 20%, 25%, and 30%). Results indicated that cooking quality, texture, and colour remained consistent across different levels of substituted COS. However, significant differences emerged between COS and NOS noodles concerning cooking time, texture attributes, and colour values. COS and NOS noodles exhibited similar cooking loss and colour values to commercial noodles with significant distinctions observed in other measured parameters. Noodles with a 25% substitution of COS demonstrated the most favorable quality characteristics, indicating the potential for incorporating this amount of COS without compromising quality. In terms of *in vitro* digestibility, noodles with COS substituted at 25% of NOS released the least amount of glucose over the 300-minute digestion period compared to NOS and commercial noodles.

The results from this study showed the potential of oat starch by-products for human consumption through starch noodle preparation. Our findings highlight the benefits of

incorporating chemically modified oat starch to enhance nutritional value through improved digestibility of the noodles. Therefore, we have presented potential avenues for the utilization of oat starch within the food industry following separation of fibre and protein fractions which are beneficial economically.

5.2. Introduction

The valorization of industrial by-products for human consumption is a valuable opportunity for the food industry to increase sustainability and achieve responsible consumption goals. The plant protein industry is one sector in the food industry where this approach can be implemented. Starch, a by-product of the plant protein industry, is typically discarded or underutilized (Zhu, 2017; Zhu et al., 2023). Research into the utilization of the starch by-product is driven by the goal of enhancing production sustainability and finding novel ways to reintegrate this starch into the mainstream food system. Thus, it is crucial to study various applications whereby these starches are economically and optimally utilized.

Oats (*Avena sativa L.*) are a major global cereal crop, with Canada ranking second in production after Russia (Shahbandeh, 2023; Zhu et al., 2023). Comprising approximately 60% starch and 10-20% protein, oats offer a high-quality nutritional profile (Malunga et al., 2022). Current research is focused on developing protein concentrates from oats for food applications, a reflection of the trend towards plant-based protein-rich diets (Gell et al., 2021; Immonen et al., 2021). However, oats hold potential beyond their protein content, particularly in terms of their starch. To maximize the benefits of oats, it is crucial to leverage all their components in creating nutritious food products.

Starch noodles, prevalent in Asian cuisines, provide an excellent platform for utilizing the starch component of cereal grains including oats (Spaen & Silva, 2021; Tan et al., 2009). Traditionally, starch noodles are made from mung bean starch and, in some cases, rice starch (Tan et al., 2009). Mung bean starch noodles are used as the quality standard for starch noodles due to their excellent eating quality and low cost. Other starch from sources such as sweet potato, potato, cassava, pea, sorghum and wheat have been used for the production of noodles which are classified as coarse grain starch noodles (Tan et al., 2009). However, these alternative starches can result in undesirable properties of the final noodle products. For example, noodles made from potato starch have inferior cooking properties and texture (Bangar et al., 2023). Therefore, there an opportunity to utilize alternative starches, such as from oat, which are still to be investigated for starch noodle production.

Starch, the primary glycemic carbohydrate in many cereal grains, raises concerns for its use in food since it has been linked to obesity, type II diabetes, and heart disease. However, the composition of the two glucans (linear amylose and branched amylopectin) in starch, particularly the amount of amylose, affects its digestion properties. Studies indicate that starch with higher amylose content tends to have more slowly digestible (SDS) and resistant starch (RS), both of which are associated with lower glycemic responses (Xu et al., 2017). Oat starch, known for its high amylose content, has gained attention in the food industry, though conflicting reports exist on its actual levels depending on oat genotype (Rhymer et al., 2005; Xu et al., 2017; Zhu et al., 2023). This prompts exploration of modified starches for managing glycemic responses in starch-based products like noodles.

The presence of modified starches in noodles has been associated with increases in both SDS and RS (Bangar et al., 2023). Researchers have explored how chemically modified starches can mimic/create RS and improve properties such as viscosity and swelling capacity. (Kim et al., 2017; Liu et al., 2014; Remya et al., 2018). Kim et al. (2017) used citric acid to chemically modify rice starch and it resulted in improved resistant starch content of the rice starch. Citric acid is regarded as the best cross-linking agent since it is nutritionally harmless, better accepted by consumers, and more economically viable (Kim et al., 2017; Punia et al., 2019). There has been some research on the application of cross-linked starch for noodles (Punia et al., 2019;

Yousif et al., 2012) and to the best of our knowledge, there is no literature on the chemical modification of oat starch with citric acid and its application for starch noodle production. Thus, there is opportunity to expand the existing knowledge on oat starch and investigate novel methods for its application for human consumption.

The first objective of this study was to optimize the method for noodle production and investigate the noodle-making potential of native oat starch. The second objective was to examine the quality and digestibility of native oat starch noodles as affected by addition of citrate-modified oat starch at substitution levels of 15, 20, 25, and 30%.

5.3. Materials and Methods

Native oat starch (NOS) was isolated using an alkaline extraction method, and a portion was chemically modified to produce citrate-modified oat starch (COS), following the methods described in experimental chapter two. Chemical composition for NOS and COS were also determined (Figure 8) following the methods described in experimental chapter 2. The NOS was used to make gel and noodles for method optimization. Noodles with added COS were made by substituting NOS with differing amounts of COS. For comparison purposes, commercial rice noodles obtained from a local grocery store (President's Choice), contained 85% starch as displayed on the nutrition label and in agreement with previous findings for white rice flour (Farooq et al., 2021; Kraithong et al., 2018; Ronie et al., 2022).

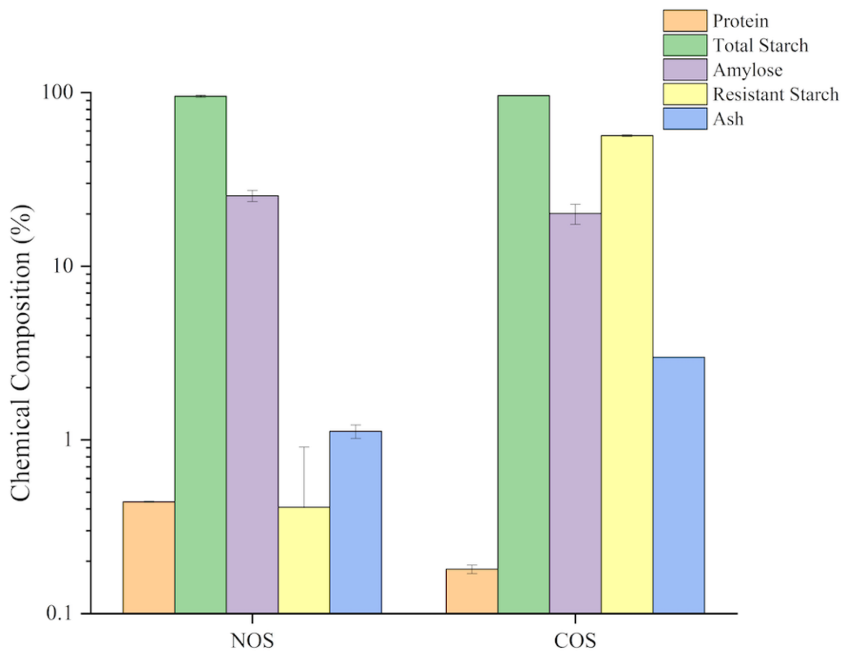


Figure 8. Chemical composition of native oat starch (NOS) and citrate-modified oat starch (COS) presented as mean ± standard deviation from duplicate analysis.

5.3.1. Optimization of Noodle making method

Native oat starch was used to optimize a starch gel preparation method described by Lee et al. (2005). The ratio of starch to water for gel preparation were tested and optimized by preparing slurries of native oat starch (300 mL) with varying solid contents (30, 32.5, 35, 37.5, 40%). Reverse osmosis (RO) water and starch were mixed to obtain a slurry, which was left to equilibrate at room temperature for 1 hour. The slurry was heated in a stainless-steel bowl in a water bath set to 50°C. Once the slurry reached 50°C it was stirred manually for 6 minutes. The heated slurry was then poured into a dark coloured metal baking tray (33 x 23 cm) to a thickness of approximately 1.5 mm high. The metal tray was immediately transferred to a steam oven (99.4°C) (Accutemp Products, INC., Fort Wayne, IN, USA) and cooked for 4 minutes. Starch gel was cooled at room temperature for 10 minutes and then covered with aluminum foil. It was left to age at refrigeration temperature (0-4°C) for 18 hours.

Aged starch gel was extruded through a noodle processor (Sercom, Montpellier, France) using a 60-hole die (#49522), instead of a roller cutter. Extruded noodles were placed in a pasta drier (Italpast Lab Dryer EAC-LAB, Fidenza, Italy) for 4 hours at 30°C and then left in the drier with no air movement at room temperature (~25°C) for another 20 hours.

5.3.1.1. Texture analysis of gels and noodles

Texture was measured on starch gel and test noodles using a TA.XT.plus texture analyzer (Texture Technologies Corp., Scarsdale, NY, USA) equipped with a 5-kg load cell. The starch gel was left in the pan then compressed using a cylindrical probe (TA-23) with the following test parameters in compression mode: 1 mm/sec pre-test speed, 1mm/sec test speed, 20 mm/sec post-test speed, 1.5 mm target distance, and trigger force of 5 g. Parameters calculated included gel firmness and work of compression.

Dried noodles were cooked following the TA.XTPlus Application: Method for Firmness of Irregularly Shaped Pasta (2012) with some modifications. Dried noodles (25 g) were cooked in 900 g of distilled water for one minute. Noodles were immediately rinsed with cool water for 30 seconds then submerged into a pot of cool water for 3 minutes to halt the cooking before straining for 20 seconds. Approximately 80 g of cooked, cooled noodles were transferred into the 300 mL beaker. The triple cutting ring attachment (A/TRCS) was used to measure texture of noodles. The texture analyzer settings used were according to the TA.XTPlus Application: Ramen Noodles with SMS' Triple Cutting Ring System (2020). A return to start, compression test was employed with the following parameters: 3.0 mm/sec test speed, 10.0 mm/sec post-test speed, 49.5 mm target distance, button trigger from a calibrated probe height of 50 mm from the bottom of the beaker. Parameters measured included noodle firmness and work of shear.

5.3.2. Effect of citrate-modified oat starch on oat starch noodles

Noodles were prepared according to the optimization method stated above. Briefly, a starch slurry with 40% solids was prepared by blending RO water (300 mL) and native oat starch (120 g). Of the 40% solids, a portion of the NOS was substituted with COS. In these blends, COS accounted for 0, 15, 20, 25, and 30% of NOS. The starch slurry was prepared according to the optimization method stated above. In general, the slurry rested for 1 hour at room temperature and then heated to 50°C while stirring manually for 6 minutes. The heated slurry was poured into a metal pan and steamed for 4 minutes at 99.4°C. Once the gelatinized mass was cooled to room temperature, it was allowed to age for 18 hours at refrigeration temperature. Aged starch was then extruded through the noodle processor and noodles were dried in the pasta drier for 4 hours at 30°C and for an additional 20 hours at room temperature.

5.3.3. Characteristics of noodles with added COS

5.3.3.1. Cooking time, cooked weight, cooked loss

Cooking time, cooked weight and cooked loss of starch noodles were determined following the method established by Wang et al. (2012) with some modifications. Noodles (~5 g) were placed into 250 mL of boiling distilled water in a metal pot, which was then covered with a lid to reduce evaporation losses. Every 30 seconds one noodle strand was taken out of the boiling water and squeezed between a plastic petri dish. The cooking time was determined when the opaque core was no longer visible.

The cooked weight was assessed by weighing 5 g of dried noodles, which were prepared for the predetermined cooking time. After cooking, the noodles were drained using a sieve, rinsed with 50 ml of distilled water at room temperature, and allowed to drain for 5 minutes. The cooked weight was then calculated according to Wang et al. (2012). Cooking loss was determined by evaporating the combined cooking and rinsing water to dryness at 100 °C for 24 hours and calculated as described by Wang et al. (2012).

5.3.3.2. Noodle Texture

Texture of NOS, COS and commercial noodles were measured using the same procedure that was followed as outlined mentioned above. Parameter's measured included noodle hardness, work of shear, and adhesiveness.

5.3.3.3. Colorimetry

Colour properties were determined for dried oat starch and commercial noodles using a Konica Minolta Chromo Meter (model CR-410, Minolta Co., Osaka, Japan) with a 50 mm aperture size. The CIE *L*a*b** colour space was used where *L** represents lightness, and *a** and *b** represent chromaticity and measure from green to red and blue to yellow respectively. Triplicate analysis was performed for each treatment.

5.3.4. *In Vitro* Digestion

The *in vitro* digestibility of noodles made with NOS and 25% COS (COS-25) was determined and compared to commercial noodles, using the Englyst et al. (2000) method modified by van Kempen et al. (2010) and with additional slight modifications. Briefly, noodles were cooked according to the predetermined cooking method and time, then noodles were macerated by hand in a plastic bag for 30 seconds. Noodles (500 mg) were added to a capped 15 mL test tube and 2.5 mL of pepsin (P-7012; Sigma) guar gum solution then added along with a stirring bar. Triplicate samples were prepared. These contents were vortexed and incubated in a 37°C water bath for 30 minutes with constant stirring. Tubes were removed and 2.5 mL of 0.25 M sodium acetate solution was added. A solution (1.25 mL) of pancreatin (P-7545; Sigma), amyloglucosidase (E-AMGDF; Megazyme) and invertase (P-14504; Sigma) was added at one-minute intervals (starting from when the first test tube was placed back in the water bath). After 20 minutes, the following was completed in one-minute intervals between samples, the first sample was taken out of the water bath, vortexed, and 100 µL was removed and added to a 15 mL conical tube containing 5 mL of 100% ethanol and vortexed. The sample was placed back into the water bath and next sample was removed when one minute was reached. All sample-ethanol mixtures were centrifuged at 300 x *g* for 2 minutes. Removal of 100 µL aliquots of sample was repeated in the same manner for 40, 60, 120, 240 and 300 minutes. The sample (100 µL) and 3 mL of glucose oxidase/peroxidase (K-GLUC; Megazyme) were added to a 10-mL test tube. A blank (100 µL of 100% ethanol) and four glucose standards (100 µL of glucose) were prepared and all test tubes were incubated for 20 minutes at 50°C. Absorbance was read at 510 nm against blank.

Two-stage moisture was completed on remaining cooked noodle samples to determine glucose content (g/100 g, dry basis) according to the AACC method 44-15.02.

5.3.5. Statistical analysis

Results were analyzed using one-way analysis of variance (ANOVA) ($P < 0.05$) followed by Tukey-Kramer honest significant difference method to compare means using R software package (R version 4.1.2, R foundation for Statistical Computing, Vienna, Austria). All treatments (starch gel and noodles) were prepared in triplicates, and duplicate analysis was done on each treatment unless stated otherwise with results reported as mean ± standard deviation. Graphs were prepared using OriginPro 2024 (OriginLab Corporation, Northampton, MA, USA).

5.4. Results

5.4.1. Method optimization for noodle preparation using NOS

The native oat starch noodle preparation method was optimized using texture analysis for the prepared NOS gel and noodles at the various levels of solid content. The maximum amount of solids tested was at 40%. Based on literature, up to 45% solids could be used for gel preparation with sweet potato starch (Lee et al., 2005). However, in our preliminary research (data not shown), use of 45% oat starch solids resulted in a starch slurry too viscous and not pourable after heating. Thus, using a solids greater than 40% was not applicable for optimization of the noodle production method using oat starch.

Texture profile analysis revealed that hardness of the NOS gel increased with increasing amounts of starch and the same trend was observed for compression work (Table 15). Hardness was taken as the peak force, while compression work was the calculated area under the force-time curve. Noodle hardness decreased with increasing amounts of starch, while work of shear (area under the curve) for noodles increased from 30% to 35% starch solids and then decreased (Table 15). Work of shear for noodles exhibited a different trend from the other measured parameters, where there was a significant difference between two groups, those containing 30, 37.5 and 40% and those containing 32.5 and 35% starch solids content. There was also a significant difference ($P < 0.05$) among samples with 30, 37.5 and 40% starch solids for hardness values of starch gel and noodles and work of compression for starch gel, which reflected the amount of starch added. Pearson correlation showed a significant ($P < 0.001$) negative correlation (-0.918) between starch gel hardness and starch noodle hardness. This indicated that the strength of the gel is translated to noodle firmness and thus the slurry with 40% solids was optimal for further analysis based on highest gel and lowest noodle hardness values.

Table 15. Textural parameters of NOS gel and noodles

	NOS[1] gel		NOS noodles	
Samples (%Starch)	**Hardness (g)**	**Compression work (g.sec)**	**Hardness (g)**	**Work of shear (g.sec)**
30	1623 ± 32.2^{c}	115 ± 20.2^{c}	6747 ± 185.3^{a}	5397 ± 32.1^{b}
32.5	241 ± 7.4^{bc}	164 ± 3.6^{bc}	4223 ± 113.1^{b}	8078 ± 502.5^{a}
35	279 ± 68.4^{bc}	192 ± 41.3^{bc}	4206 ± 147.8^{b}	7492 ± 41.3^{a}
37.5	382 ± 49.6^{b}	256 ± 29.8^{b}	3425 ± 173.6^{c}	5617 ± 44.1^{b}
40	590 ± 9.0^{a}	390 ± 13.9^{a}	1790 ± 25.5^{d}	4703 ± 59.5^{b}

Data represented as mean ± standard deviation (n=4). Values with the same letters in the same columns do not differ significantly ($P < 0.05$). [1]NOS=native oat starch.

5.4.2. Quality characteristics of noodles substituted with COS

The quality of NOS was investigated through cooking characteristics, texture and colour and compared to commercial noodles. NOS noodles were also similarly compared to noodles with different levels of added COS (15, 20, 25 and 30%). COS at 30% level was found to be the maximum amount that could be substituted for NOS, given the inability for COS to form a paste (experimental chapter 2). Moreover, previous literature showed that only up to 20% citrate starch substitution using barley starch was acceptable during sensory evaluation (Punia et al., 2019).

5.4.2.1. Cooking quality

Cooking time, cooked weight and cooking loss were analyzed to investigate the cooking quality of starch noodles (Table 16). Cooking time of noodles prepared with NOS was approximate 2 minutes. This was a slight reduction in cooking time compared to commercial rice noodles (~3 min). Cooking times for noodles with added COS were significantly lower than NOS noodles indicating the influence of COS on cooking time. The cooked weight of NOS noodles (~3%) was significantly lower than that of commercial rice noodles (~4%). Cooked weight ranged from 3.3 to 3.5% for noodles with added COS and no significant difference was found when compared to

NOS noodles. No significant difference was observed for cooking loss among all noodle samples.

5.4.2.2. Texture analysis

The variations between starch noodles were revealed through texture analysis (Table 16). For hardness and work of shear, commercial noodles gave values significantly higher than NOS noodles, while adhesiveness was not significantly different. Comparing the texture of NOS noodles to noodles with added COS showed significantly higher work of shear excluding noodles substituted with 25% COS (COS-25), which showed no significant difference. Adhesiveness ('stickiness') of noodles with added COS was found to be significantly higher than for NOS noodles, displaying the adverse effects COS had on noodle stickiness. Hardness of noodles with added COS was not significantly different from NOS noodles. Considering the levels of substitution for noodles with added COS, texture, in general, was not heavily influenced by the degree of COS substitution, based on absence of significant differences among them for most measured parameters.

5.4.2.3. Colour

Colour values for starch noodles are represented in Table 16. The lightness (L^*) of NOS noodles was comparable to commercial noodles; however, a^* (greenness) and b^* (yellowness) values were significantly different between the two. L^* values of NOS noodles was comparable to noodles with added COS at all levels excluding COS-25 noodles, where L^* was significantly higher for the latter. For a^*, almost all noodles had values close to zero (neither green nor red), except for NOS noodles, which had a significantly lower a^* (greenness) value compared to all noodles with added COS. Noodles that were substituted with COS had b^* (yellowness) values that were significantly higher than noodles made with NOS, showing the tendency for noodles with added COS to be more yellow.

Table 16. Characteristics of NOS noodles and those with added citrate-modified oat starch and commercial rice noodles

			Oat Starch noodles[1]				
Properties		**COM[2]**	**NOS**	**COS-15**	**COS-20**	**COS-25**	**COS-30**
Cooking characteristics	Cooking Time (min)	3.33 ± 0.3[a]	1.58 ± 0.1[a]	0.95 ± 0.1[b]	0.89 ± 0.0[b]	0.86 ± 0.1[b]	0.92 ± 0.1[b]
	Cooked weight (%)	4.1 ± 0.0[a]	3.1 ± 0.1[b]	3.3 ± 0.1[b]	3.4 ± 0.2[b]	3.5 ± 0.1[b]	3.4 ± 0.0[b]
	Cooking loss (%)	9.5 ± 1.8[a]	13.4 ± 6.5[a]	9.0 ± 1.4[a]	9.9 ± 0.8[a]	11.3 ± 0.7[a]	12.1 ± 3.1[a]
Texture	Hardness (g)	6099 ± 385[a]	2701 ± 632[b]	1991 ± 356[b]	1804 ± 64[b]	1913 ± 118[b]	1919 ± 64[b]
	Work of shear (g.sec)	15834 ± 752[a]	6949 ± 500[b]	5183 ± 444[c]	4507 ± 114[c]	5627 ± 286[bc]	5037 ± 23[c]
	Adhesiveness (g.sec)	-169 ± 9[a]	-158 ± 17[a]	-255 ± 22[b]	-251 ± 24[b]	-266 ± 7[b]	-295 ± 18[b]
Colour	L^*	65.4 ± 2.3[ab]	64.2 ± 0.5[b]	69.1 ± 1.9[ab]	69.5 ± 0.6[ab]	71.4 ± 0.6[a]	69.5 ± 0.6[ab]
	a^*	0.5 ± 0.1[a]	-0.9 ± 0.1[b]	0.3 ± 0.1[a]	0.1 ± 0.2[a]	0.1 ± 0.1[a]	0.0 ± 0.2[a]
	b^*	14.1 ± 0.3[a]	7.2 ± 0.2[c]	11.2 ± 0.5[b]	10.9 ± 0.3[b]	11.9 ± 0.7[b]	10.8 ± 0.8[b]

Data represented as mean ± standard deviation (n=3). Values with the same letters in the same rows do not differ significantly (P < 0.05). [1]NOS=native oat starch; COS-15: oat starch blended with 15% citrate-modified oat starch; COS-20: oat starch blended with 20% citrate-modified oat starch; COS-25: oat starch blended with 25% citrate-modified oat starch; COS-30: oat starch blended with 30% citrate-modified oat starch. [2] COM= commercial rice noodles.

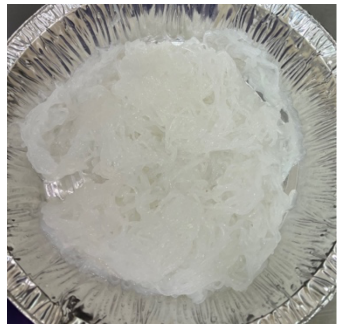
5.4.3. Digestibility of Noodles

In vitro digestibility was used to analyze amount of glucose released in NOS, COS-25, and COM cooked noodles (Figure 10) over a period of five hours (300 minutes), as shown in Figure 9A and B. Amount of glucose released is displayed based on 100 g of noodles (Figure 9A) and corrected to amount of starch in each noodle type (Figure 9B). For glucose released based on 100g of sample (not corrected to starch), no significant differences were observed among the three types of noodles, except at the 40 and 240-minute marks, where the glucose levels in NOS and COS-25 were significantly ($P < 0.05$) lower compared to the commercial noodles (Figure 9A). In 100 grams of noodles, the amount of glucose released was rapid during the first hour of digestion for all noodle types. The average amount of glucose that was released after 60 minutes was 47.9, 45.4 and 44.5 g/100g for commercial, NOS and COS-25, respectively. For the remainder of the digestion time (60 to 300 min) the amount of glucose released slowed for all noodles with the slowest release from NOS and COS-25, at 32.5 and 27.0 g of glucose, respectively. In comparison, glucose released for commercial noodles from 60 to 300 minutes was 36.7 g. The final amount of glucose released was measured at 300 minutes and was found to be 84.7, 77.9 and 71.9 g for commercial, NOS and COS-25, respectively.

Considering the amount of starch in each sample resulted in slightly altered digestion curves (Figure 9B). The high amount of starch determined for COS (~96%) caused the amount of glucose released in the COS-25 noodles to decrease compared to NOS noodles, as well as commercial noodles. In a similar manner, the comparably high starch in NOS (~95%) resulted in a decrease in the amount of glucose released, compared to commercial noodles. In Figure 9B, it can be observed that the release of glucose reached a plateau at the 240-minute mark for all noodles, following the steep increase from 20 to 120 minutes, mirroring the trend seen in Figure 9A. Glucose released at the 300-minute mark was higher for adjusted glucose values, at 95.2, 83.9 and 76.9 g for commercial, NOS and COS-25 noodles, respectively (Figure 9B).

i)

ii)



iii)

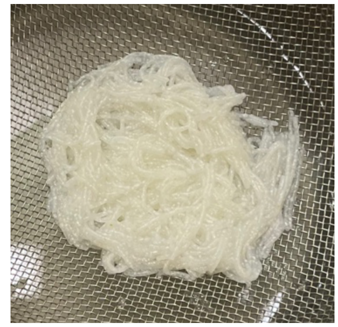


Figure 9. (i) Commercial rice (COM), (ii) native oat starch (NOS), and (iii) 25% citrate-modified oat starch (COS-25) cooked noodles.

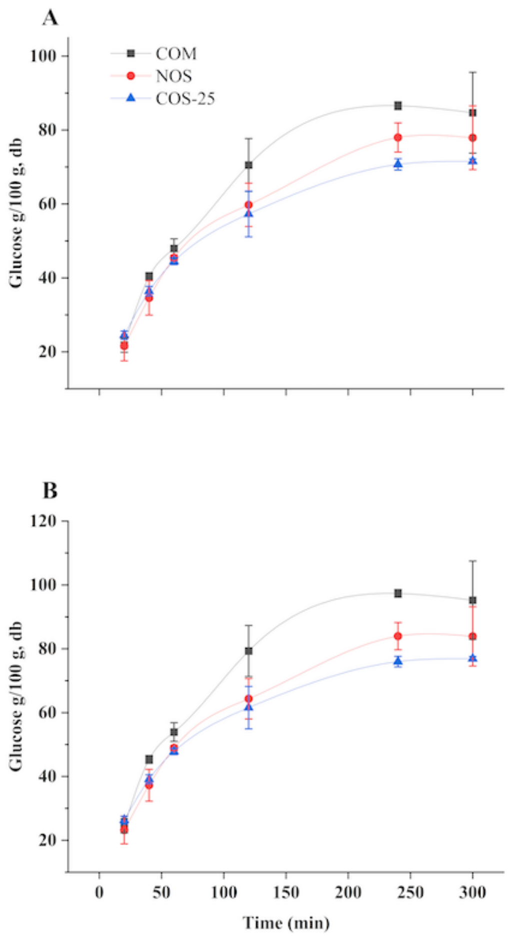


Figure 10. Release of glucose in 100 g of noodles (A) and release of glucose in 100 g of noodles adjusted to amount of starch in each sample (B) represented as mean ± standard deviation from triplicate analyses.

5.5. Discussion

The present study was designed to optimize a starch noodle production method specifically for oat starch, based on what has been optimized for alternative starches Lee et al. (2005). The findings obtained through optimizing the methodology were applied to create noodles using oat starch alone and combinations of NOS with COS. This was done to gain insights into the factors influencing the quality of noodles produced from oat starch and from those where modified oat starch was incorporated. A single Canadian oat genotype (CDC Morrison) was selected for starch isolation based on parameters identified in experimental chapter 1, which provided control over this variable. Thus, the variations exhibited in noodles are solely based on starch physiochemical properties and the level of COS substitution.

Optimization of the starch noodle making was determined through the analysis of the five different starch slurries with different solid contents (30, 32.5, 35, 37.5, 40%) and results demonstrated which level of starch addition is optimal for oat starch. Based on texture parameters of prepared starch gels and associated noodles, it could be seen that gel hardness was reflected in the hardness of noodles (Table 15). This was due to the increasing amount of solid content in the gel, as the slurry with 40% solids gave the highest gel firmness. When gel firmness increased, noodle firmness decreased, and this relationship is demonstrated through the significant ($P < 0.001$) negative correlation (-0.918) between the two. This finding is different from what was reported by Lee et al. (2005), who observed a positive correlation between starch content and noodle hardness using sweet potato starch. Swelling power of starch may have a role in the hardness of noodles, as it has been found that starches with low swelling power produce noodles with increased hardness (An et al., 2022; Singh Gill et al., 2004). Oat starch was found to have a low swelling power, as seen in our previous work (Experimental chapter 2), thus it would be expected that noodle hardness would be higher. This trend was observed in the starch gel but was not conveyed in the extruded noodles (Table 15). The discrepancies may be due to excess starch in the gel matrix that upon extrusion, would when weaken the noodle structure. However, the stronger gels were beneficial for noodle processing, as it produced noodles that were less sticky after extrusion, similar to what has been previously reported (Lee et al., 2005). Compression work, which is the work (force over time) required to deform the starch gel, was directly correlated with gel hardness (0.99, $P < 0.001$), as determined by Pearson correlation. The starch slurry made with 40% solids required the most work to compress, which indicates that a firmer gel would require more work to deform the structure. This was evident by the ease of handling the prepared gel made with 40% solids. Work of shear describes the force required to cut through a bulk sample. It was observed that noodles made from a slurry of 40% solids required the least force to cut through, which is reflected in the decreased hardness of these noodles (Table 15). No relationship between work of compression for gel and work of shear for noodles was observed. Considering all observations discussed it was determined that the slurry made with 40% solids would be best suited for starch noodle production. This is due to the resultant firmer starch gel, which improved gel handling and noodle extrusion; however, the softer noodle texture needed improvement. This was investigated through the addition of citrate-modified oat starch (COS).

Oat starch noodles were prepared to examine the impact of adding COS. Using the pre-determined solid content of 40%, some of the NOS was substituted with COS and characteristics of these oat starch noodles were measured to understand the implication of the addition of COS in terms of noodle quality. Characteristics of NOS noodles was also compared to purchased commercial rice noodles. Cooking characteristics were measured on all noodles (Table 16). The significant increase in cooking time for noodles containing COS compared to NOS noodles, implies that the incorporation of COS leads to a reduction in noodle cooking time. Nevertheless, the extent to which COS is added to the noodles did not significantly impact the cooking duration. A lower cooking time is most desired by consumers due to convenience (Bangar et al., 2023), therefore, the decreased cooking time observed for noodles with added COS is desirable. The observed significantly lower cooked weight for NOS noodles compared to commercial noodles demonstrates the lower water uptake during cooking (Table 16). Kim et al. (2017) found that native rice starch had a swelling capacity of approximately ~16%, which is more than double what was observed for oat starch in our experimental chapter 2 (~6%). This may explain why a lower cooked weight was obtained for noodles made with NOS. In contrast, noodles with added COS were comparable to NOS noodles for cooked weight. This suggests that a difference exists between the water uptake of rice starch and oat starch, but addition of COS did not have a major influence on water uptake. Cooking loss refers to the quantity of solids lost during the cooking process, resulting from the dissolution of loosely bound gelatinized starch and the robustness of retrograded starch structures (Wu et al., 2015). The increased standard deviation noticed in NOS noodles might be a result of their starch properties. This higher variability in cooking loss for NOS noodles could stem from more pronounced distinctions in solubility and amylose leaching during cooking. However, non-significant differences in cooking loss among all noodle was observed and suggests that NOS noodles and noodles with added COS are comparable to commercial starch noodles (Table 16). With the commercial starch noodles being used as a standard for acceptable noodle quality, it can be suggested that the cooking loss observed for noodles made with NOS and COS would be within an acceptable range.

Pasting properties of COS are likely to influence the cooking characteristics of noodles. In our previous work (Experimental chapter 2) it was reported that COS had significantly different pasting profile compared to NOS, which may explain the variation in some cooking characteristics. Cooking time can be influenced by the gelatinization temperature of starch (Bangar et al., 2023). Hence, the low, almost negligible gelatinization temperature of COS, may be reflected in the lower cooking time in agreement with previous findings (Obadi & Xu, 2021; Wang et al., 2015). Cross-linking can inhibit swelling as evidenced by lack of peaks in the pasting profile (Experimental chapter 2) which in turn, may reduce the amount of amylose that leaches out of the noodles during cooking, thus contributing to the observed lower cooking loss for noodles made with COS compared to NOS noodles (Table 16).

Texture analysis of noodles revealed that there were more differences between NOS noodles and commercial noodles than there were between NOS noodles and noodles with added COS. Hardness and work of shear for NOS noodles were not comparable to commercial noodles. This may be attributed to the restricted granule swelling and higher amylose content of NOS (~25%) (Figure 8) compared to rice starch (~18%) (Luo et al., 2021) thereby preventing formation of a firm gel and consequently resulting in softer noodles (Punia et al., 2019). The commercial noodle product had a higher firmness/toughness compared to NOS noodles. Lack of significant difference between NOS noodles and those with added COS for hardness, indicated that addition of COS does not compromise the hardness of cooked noodles when compared to NOS noodles. Punia et al. (2019) reported a decrease in noodle hardness with increasing amounts of cross-linked starch, which translated to poor consumer perception during sensory analysis. No distinct trend could be seen in the present study suggesting that COS can be incorporated into noodles up to 30% and hardness of noodles would remain acceptable when compared to NOS noodles.

Work of shear demonstrated that noodles made with COS-25 were similar to NOS noodles in terms of the force required to cut through the bulk sample of noodles. Adhesiveness ('stickiness') of the noodles was taken as the negative area under the force-time curve and could be used to describe stickiness. During texture analysis, noodles that stuck to the probe upon its return after reaching target force were depicted in the negative area under the curve. Visual observation during analysis led to the conclusion that the more noodles stuck to the probe, the more increase in the size of the negative area, resulting in adhesiveness/stickiness of the noodles being interpreted as greater. The degree at which COS was added to the noodles did not impart major differences in noodle adhesiveness, however, adhesiveness was greater than what was seen for NOS and commercial noodles. Kang et al. (2017) found that adhesiveness of noodles was associated with increased viscosity and lower water absorption. Therefore, the cooked weight of oat starch noodles may be able to predict adhesiveness. However, Pearson correlation confirmed that there was no significant ($P > 0.05$) relationship between adhesiveness and cooked weight (0.231).

Colour of dried starch noodles showed only small variations in $L*$ (lightness), $a*$ (redness/greenness), and $b*$ (yellowness/blueness) values (Table 16). Lightness of noodles prepared with NOS had the lowest lightness; however, they were comparable to commercial noodles. NOS had an $a*$ value that was different and negative from commercial noodles and noodles with added COS, meaning they appeared greener. The significantly lower $b*$ value observed for NOS noodles shows that they appear less yellow than commercial noodles, which had a higher $b*$ value. The biggest difference was seen between NOS noodles and noodles with 25% COS, where COS-25 noodles had an $L*$ value significantly higher than NOS noodles. For $a*$ values, all noodles made with COS exhibited values close to zero meaning they did not exhibit a strong red nor green colour. In terms of $b*$ values, noodles made with COS showed non-significant differences among themselves; however, they were significantly different from NOS noodles. In general, increased whiteness and translucency are most desirable for consumers when purchasing starch noodles (Tan et al., 2009). Thus, noodles made with COS-25 would be more preferred based on lightness ($L*$), while NOS noodles would be more preferred based on transparency ($a*$ and $b*$).

The cooking characteristics, texture, and colour of noodles produced with NOS were comparable to commercial noodles for most aspects, with the most differences seen in texture. NOS noodles and those with added COS exhibited the most differences in terms of texture and colour; however, their cooking characteristics were comparable. It was also observed that the degree of COS substitution into noodles did not have major influences on the cooking characteristics, texture and colour. Producing noodles with COS up to 30% was achieved; however, noodles with 25% COS exhibited notable distinctions in certain features such as adhesiveness, work of shear, lightness, and cooking time. Hence, it is proposed that incorporating COS up to 25% could uphold satisfactory noodle quality. Including COS is expected to result in noodles containing more RS, as indicated by our initial investigations (experimental chapter 2), potentially enhancing their slowly digestible properties. Therefore, we focused on examining noodles prepared with COS-25 as the maximum level of COS incorporation that still maintains satisfactory quality, to determine whether there are improvements in digestibility when compared to NOS and commercial noodles.

Digestibility of starch noodles revealed that there were some differences in the amount of glucose released based on the type of noodles when assuming the same amount of starch present. Greater differences in glucose released was observed when values were corrected to actual amount of starch present in the noodles, as it accounted for starch available for digestion in each sample. For both digestion graphs, amount of glucose released is based on 100 grams of noodles, as this would be most representative of a typical serving size and therefore, results better reflect how glucose would be released when consumed.

During the initial phase of enzyme digestion (20-60 minutes), there was a consistent release of glucose across all types of noodles. However, beyond the 60-minute mark, a greater variation in glucose release became evident, particularly with commercial noodles. Glucose release was notably higher from 120 to 300 minutes for commercial noodles, while NOS and COS-25 noodles showed a slower and less pronounced increase. The rate of glucose release started to plateau at the 240-minute mark for all noodle types (Figure 9A), with the commercial noodles showing the highest amount of glucose released over 300 minutes when compared to NOS and COS-25 noodles. The amount of glucose released follows a similar trend when corrected to amount of starch in the different noodles; however, there is a greater amount of glucose released for all noodles (Figure 9B). As amount of starch in the noodles went up, the amount of glucose released at each time decreased. This is explained by the fact that COS-25 noodles had the highest starch content (~96%), while commercial noodles had the lowest starch content (~85%). The digestion curve for COS-25 is shown below those of commercial and NOS noodles. Correction of glucose to starch content differentiated NOS and COS-25 noodles from commercial noodles to a greater extent, highlighting their different digestibility behaviours.

The digestibility of starch by digestive enzymes is influenced by its accessibility. When starch is chemically modified with citric acid, it undergoes esterification, replacing some hydroxyl groups with ester-linked citrate groups in its structure. This modification renders the starch less accessible to digestive enzymes. Consequently, the reduced release of glucose observed during enzyme digestion of COS-25 noodles could be attributed to this cross-linking, which hampers enzyme access to starch, thus conferring resistance to enzymatic breakdown, similar to resistant starch (RS). This outcome also implies that COS demonstrates resilience against the mechanical stresses encountered during noodle processing and consumption. *In vitro* digestion was conducted on noodles that were subjected to all processing stages, from extrusion to cooking. The findings suggests that COS maintains its structural integrity throughout processing, thereby enabling it to resist enzymatic degradation. Glycemic response is correlated to the rate of starch digestion since the amount of glucose available for absorption in the small intestine guides the intensity of glycemic response (Englyst et al., 2000). Based on the digestion curve of COS-25 noodles, it can be inferred that their glycemic index would likely be lower compared to our NOS and the commercial noodles, owing to the slower and reduced release of glucose (Figure 9).

5.6. Conclusion

The successful preparation of native oat starch (NOS) noodles was achieved through method optimization, with texture analysis aiding in predicting noodle hardness. Comparing NOS noodles to commercial rice noodles revealed similarities in terms of cooking characteristics, adhesiveness and lightness. Substituting NOS with citrate-modified oat starch (COS) at varying levels demonstrated that while some quality parameters were affected, particularly texture, others remained largely unaffected. In addition, the degree of COS substitution did not have a major effect on overall noodle quality. Noodles with 25% COS substitution (COS-25) were selected for *in vitro* digestion analysis, revealing a lower glucose release over 300 minutes compared to NOS and commercial noodles, indicating enhanced resistance to digestive enzymes. Our findings suggest that NOS holds potential for starch noodle production as compared to commercially available rice noodles and that inclusion of up to 25% COS is recommended to retain acceptable quality and potentially enhance the glycemic index of starch noodles.

5.7. References

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Chapter 6: Final conclusions and future directions

Production of starch noodles is a beneficial option for the reintegration of the oat starch by-product for human consumption. A thorough understanding of Canadian oat cultivars, can aid in the selection of oat with optimal characteristics to help meet the demands of the plant protein industry and improve marketability of the starch by-product through noodle production. The modification of native oat starch was possible and showed how this can increase resistant starch content and improve resistance to processing requirements; however, pasting was significantly affected by modification of oat starch. These qualities were reflected in the noodles made with NOS and added COS. Where noodles with added COS exhibited a softer and stickier texture, with improved digestibility due to resistance towards digestive enzymes. Nonetheless, starch noodles can be made with native and citrate-modified oat starch.

6.1. Experimental chapter conclusions

The study on screening oat cultivars can be summarized using the following conclusions:

- Means of the thirty Canadian oat genotypes grown in six environments ranged from 12 to 24%, 49 to 67%, 21 to 33%, 3-7%, and 5-10% for protein, total starch, amylose, beta-glucan, and oil, respectively.
- All main traits (protein, total starch, and amylose) were significantly affected by genotype, environment, and their interaction.
- The main effects of genotype (G) and environment (E) contributed the most to variance for protein and total starch content, while their interaction (GxE) had a greater contribution of variance to amylose.
- Principle component analysis (PCA) showed that growing location did not have a strong influence on the main traits, while growing year had a larger influence.

- A strong inverse relationship between protein and starch was observed in the biplot, which was confirmed by their negative correlation.
- The biplot demonstrated the relationship between growing year and protein and total starch, such as that groat starch was higher in 2020 and groat protein was higher in 2021.
- High August rainfall was positively correlated with protein content and negatively correlated with total starch content.
- Correlation demonstrated the positive relationships between beta-glucan and protein and oil, while total starch and amylose had a negative relationship with beta-glucan.
- Analysis of colour values (*L*, a*, b**) showed that *L** (lightness) of oat groats could be a predictor for higher total starch due their positive correlation.
- Akina, ORE3541M and Bullet were the genotypes that showed the most consistency for the measured traits, as their means were similar to all genotypes across all environments.
- CDC Morrison and AAC Rigadon are the best choice for high protein genotypes, with AAC Rigadon exhibiting more consistency.

The results showed that growing environment had a stronger influence on physiochemical properties of Canadian oat varieties and that the close relationship between protein and starch, can aid in the selection of other important traits due to their interconnectivity.

The study on properties of native oat starch and citrate-modified oat starch can be summarized using the following conclusions:

- Production of resistant oat starch by cross-linking with citric acid was successful as evident from the increase in RS content from ~0.4 to 57%.
- The chemical composition of NOS was altered by citric acid cross-linking, whereby COS had decreased protein and amylose and increased RS, total starch, and ash content.

- The colour characteristics (*L*, a**, and *b**) of COS differed significantly from those of NOS, with COS appearing darker (lower *L**), exhibiting more green tones (higher *a**), and more yellow hues (higher *b**).
- NOS was categorized as type-A based on the XRD angles of 15, 17, 20, and 23° 2θ and COS showed a loss in majority of prominent peaks, with only one peak at 20° 2θ.
- XRD patterns reflected the change in crystallinity due to the high degree of cross-linking of starch with citric acid and was explained by the differences in percent relative crystallinity of NOS (~36%) and COS (~13%). The high relative degree of crystallinity suggested NOS had increased amylose-lipid complexes and lower amylose contents.
- The RVA parameters revealed that COS remained stable through heating and cooling cycles; however, it lacks the ability to form a viscous paste as evident by the low final viscosity (17 cP) compared to NOS (5090 cP).
- Swelling power, solubility index, and paste clarity were significantly lower for COS compared to NOS, which was predicted to be a result of the strengthened starch structure from the citric acid cross-linking. Amylose-lipid complexes were suggested to be the factor impacting the solubility and paste clarity of NOS.
- SEM images identified that NOS had both large and small granules (1.72 to 16.86 μm) that were smooth, irregular and polygonal in shape while COS lost some of the very small granules (2.47 to 15.09 μm) and were jagged and irregular shaped. This suggests that chemical modification of starch alters the morphological properties.

In general, there were notable distinctions in the chemical, pasting, and morphological characteristics when oat starch undergoes cross-linking with citric acid. The significant increase in RS content and distinctive pasting attributes of COS position it as a promising option for utilization as an innovative food ingredient.

The study on starch noodles made with native and citrate-modified oat starch can be summarized using the following conclusions:

- Method optimization for starch noodle production revealed that texture analysis of starch gel can be used to predict hardness of noodles, allowing for the selection of ideal solids content for production of starch noodles.
- Preparation of starch gel with 40% solids produced noodles that had improved handling during processing and extrusion, which made it ideal for further analysis of noodle quality.
- Starch noodles were successfully made with NOS and found to be comparable to commercial rice noodles in terms of cooking characteristics, adhesiveness and lightness.
- NOS was successfully substituted with different levels of COS (15, 20, 25, 30%) for noodle production. Most quality characteristics were unaffected by the addition of COS, besides texture, which exhibited more discrepancies. This was likely due to the inability of COS to form a viscous paste as determined in study 2, resulting in softer noodles.
- No significant differences were found among all levels of COS substitution for cooking characteristics, noodle hardness and most colour values. This suggested that the degree of COS addition to starch noodles does not have a major impact on noodle quality.
- Quality characteristics of noodles with added COS showed that NOS could be substituted up to 30% COS; however, COS substituted at 25% revealed optimal characteristics compared to the other levels in terms of cooking time, cooked weight, work of shear, adhesiveness, and lightness ($L*$).

- *In vitro* digestibility of NOS, COS-25 and commercial noodles showed differences in amount of glucose that was released over a 300-minute digestion period.
- Amount of glucose released showed a similar trend for the three noodle times analyzed; however, during the entire digestion period the highest amount of glucose released was observed for commercial noodles and lowest was for COS-25 noodles. Suggesting that the COS is acting in a similar manner to RS by making the starch less accessible to digestive enzymes, as a result of the cross-linking by chemical modification. Thus, addition of COS may have improved the glycemic index of noodles compared to noodles made with native starch.

Overall, native oat starch demonstrates promise for the production of starch noodles, with many quality attributes comparable to commercially available starch noodles. Although some variations were noted when COS was introduced to noodles made with NOS, incorporating COS at 25% still showed potential for enhancing NOS noodles. This integration proved advantageous for digestibility, as citrate-modified starch helped reduce glucose release during digestion.

Based on the results from the studies conducted, there is an opportunity to select for optimal oat genotypes that would be of interest to the plant protein industry. This subsequently allows for the reintegration of the oat starch by-products back into the mainstream food system in the form of starch noodles with the possibility of enhanced glycemic index.

6.2. Future directions

The focus of the current study was centered around reintegrating underutilized starch by-product from the plant protein industry for human consumption by developing low glycemic starch noodles using oat starch. The results of the study indicated that oat starch has potential for making starch noodles. However, further investigation is necessary to explore the nutritional value and consumer preferences of the resulting noodles, including dynamic digestibility and sensory evaluation. Additionally, it would be crucial to examine the microbiological quality of the starch noodles.

In the first two studies, oat groats and extracted oat starch were thoroughly investigated. This facilitated the identification of optimal oat varieties for specific purposes and provided insights into the technological properties of oat starch for use in the food industry. The second study introduced the utilization of chemically modified oat starch, revealing its distinct physicochemical characteristics. The cross-linking of oat starch with citric acid proved successful in augmenting resistant starch levels and enhancing oat starch's resilience to mechanical strain. Nevertheless, there remains scope for exploring alternative methods of chemical modification that could enhance paste clarity, swelling, and viscosity. Techniques such as oxidation, esterification, and etherification warrant investigation to ascertain the most suitable chemical modification approach for starch noodle production, building upon the findings of the current study.

Findings from the third study lay the groundwork for enhancing the quality attributes of oat starch noodles, including those made with chemically modified starch. While some quality characteristics mirrored those of starch noodles already available in the market, sensory analysis is the subsequent essential step to fully comprehend the acceptability of the produced oat starch noodles. Notably, microbial quality of oat starch noodles was not explored in the current study, which is pivotal for advancing towards consumer acceptability. Noodles need to undergo testing for potential foodborne pathogens like Shiga-toxigenic E. coli, Salmonella, and Listeria monocytogenes.

Lastly, the findings of study three regarding *in vitro* digestibility could be further explored through dynamic or semi-dynamic *in vitro* digestion models. The results from the current study already demonstrated the potential for noodles supplemented with citrate-modified oat starch (COS) to reduce glucose released during digestion, which typically correlates with *in vivo* glycemic response. This observation could be reinforced by employing a computer-controlled dynamic multi-compartmental TIM-1 system, although challenges persist due to cost and operational complexity. Consequently, future research could also consider utilizing *in vivo* methods to validate the outcomes obtained from the *in vitro* enzymatic digestion model employed in the present study.

Considering all aspects discussed, future studies should prioritize leveraging the insights obtained from the present study to refine oat starch-based noodles. Ensuring the selection of the most suitable starch modification method to optimize both quality and nutritional characteristics of the noodles is recommended. The long-term goal would be to have oat starch noodles commercially available. Hence, it is imperative to conduct microbiological investigations and sensory analyses to achieve this ultimate goal. The current study has set the foundational groundwork that is needed to progress towards the industrial level production of noodles using value-added oat starch by-products.

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