

NON-STARCH POLYSACCHARIDES: OVERVIEW, CLASSIFICATION, METHODS FOR ESTIMATION OF NON-STARCH POLYSACCHARIDES AND THEIR PROPERTIES

Abstract

Monosaccharide macromolecules joined by glycosidic linkages are known as polysaccharides. Non-glucan polysaccharides of the plant cell wall make up the majority of non-starch polysaccharides (NSP). They are a diverse category of polysaccharides that vary in size, structure, and water solubility. They are also referred to as hydrocolloids and disperse in water to create viscous dispersants or gels. These gels or dispersants alter colon absorption of nutrients and postprandial blood sugar levels by forming networks or films or by becoming viscouser. NSPs also have a variety of biological functions, including hypoglycemic, immunoregulatory, and antioxidant actions. Plant NSP concentration varies not only by species but also by genotype and cultivar within a given species. Additionally, the NSP content may be affected by the agronomic cultivation circumstances, which include environmental factors before harvesting and storage conditions following harvest. This chapter gives an outline of non-starch polysaccharide, its classification based on solubility and linkage, various methods for estimation of NSPs such as detergent method, chemical method, enzymatic method, gravimetric method and some properties it exhibits such as solubility, water binding capacity, viscosity.

Keywords: Non-starch polysaccharides, β -glucans, arabinoxylans, gravimetric, water-binding capacity

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I. INTRODUCTION

The carbohydrate fractions known as non - starch polysaccharides (NSPs) do not contain starch or free sugars. These polymeric carbohydrates differ from amylose and amylopectin in terms of content and structure. They are the structural equivalents in plants to the skeletal system in the animal realm. NSPs are high-molecular-weight pentose and hexose polymers with β -links that range in molecular weight from 8000 to a million. The majority of cell wall polysaccharides are made up of NSPs, which are also closely related to other polysaccharides and non-carbohydrate materials including lignin and protein [1]. These non-starch polysaccharides (NSPs) cannot be broken down by human endogenous enzymes. They disperse in water to create viscous dispersants or gels, also known as hydrocolloids, which affect intestinal nutrient absorption and postprandial blood glucose concentration by forming networks or films or by increasing viscosity [2]–[6]. NSPs also have a range of biological functions, including hypoglycemic, immunoregulatory, and antioxidant actions [3], [7]. For instance, xanthan gum slows down starch digestion by blocking glucoamylase in addition to absorbing heavy metals like lead, cadmium, and copper [3]. The main NSP found in cereal grains are cellulose, beta-glucan, arabinoxylans (pentosans), and xylans. Pectic polysaccharides are present in modest amounts in the stem and leaves. Only the hull and husk component of leguminous plants contains cellulose and xylans. Legumes have pectic polysaccharide in the cotyledon. Plant NSP concentration varies not only by species but also by genotype and cultivar within a given species. Additionally, the NSP content may be affected by the agronomic cultivation circumstances, such as environmental factors before harvest and storage conditions following harvest.

II. CLASSIFICATION OF NON-STARCH POLYSACCHARIDES

With the exception of α -glucans (starch), a wide range of polysaccharide molecules are referred to as NSP. NSPs have been categorized using a variety of factors. Historically, the process for isolating and extracting polysaccharides constituted the basis for the classification. Cellulose is the residue left over after a series of alkaline extractions of cell wall elements and the portion of the residue that is solubilized by alkali and is known as hemicellulose. Different solubilities led to another classification. Three types of NSP are included in this classification: crude fiber (CF), neutral detergent fiber (NDF), and acid detergent fiber (ADF). Crude fiber are the residues of the plant material after acid and alkali extraction which can contain varying amounts of insoluble NSP. In contrast to ADF, which refers to a portion of insoluble NSP primarily but not exclusively made up of cellulose and lignin, NDF is made up of the insoluble fraction of NSP plus lignin [8].

A more precise division of NSP into three major groups—cellulose, non-cellulosic polymers, and pectic polysaccharides—was suggested by Bailey (1973) (Table 1). Non-cellulosic polymers include arabinoxylans, mixed-linked β -glucans, mannans, and xyloglucan, while pectic polysaccharides are polygalacturonic acids replaced with arabinan, galactan, and arabinogalactan. Another classification is based on the basis of solubility and linkage. Cellulose is not soluble in water as well as in alkali or dilute acids. On the other hand, non-cellulosic polymers and pectic polysaccharides are partially soluble in water [9]. β -(1→4) glycosidic linkage backbones with β -(1→3) linkages are soluble or partially soluble in water whereas long sequences of β -(1→4) glycosidic unit are not soluble in water.

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Table 1: Classification of Non-Starch Polysaccharide

Category	Cellulose	Non-cellulosic polymers					Pectic polysaccharides			
		Arabinoxylans	Mixed link β -glucans	Mannans	Galactomannans	Glucomannans	Arabinans	Galactans	Arabinogalactans (Type I)	Arabinogalactans (Type II)
Monomeric Residue	Glucose	Arabinose and xylose	Glucose	Mannose	Galactose and mannans	Glucose and mannans	Arabinose	Galactose	Arabinose and galactose	Arabinose and galactose
Linkage	β -(1 \rightarrow 4)	β -(1 \rightarrow 4)-linked xylose units	β -(1 \rightarrow 3) and β -(1 \rightarrow 4)	β -(1 \rightarrow 4)	β -(1 \rightarrow 4)-linking mannan chains with α -(1 \rightarrow 6)-linked galactosyl side groups	β -(1 \rightarrow 4)-linked mannan chain with interspersed glucose residues in the main chain	α -(1 \rightarrow 5)	β -(1 \rightarrow 4)	β -(1 \rightarrow 4) galactan backbone substituted with 5-linked and terminal arabinose	β -(1 \rightarrow 3,6)-linked galactose polymer associated with 3- or 5- linked arabinose residue
Sources	Legumes and most cereals	Sorghum, wheat, oat, rye, barley, rice	Barley, oat	Coffee seed	Guar gum, locust bean gum	Lilies, pulp of sugar beet	Co-products of cereals	Pulp of sugar beet, sugar bean meal	Grain legumes	Cotyledon of rapeseed

Adapted from [8]

- 1. Cellulose:** Cellulose is known to be a complex polysaccharide made up of at least 3000 or more linear unbranched chain of β -(1 \rightarrow 4) linked D-glucose units. Due to the absence of the digestive tract enzyme cellulase in monogastric animals, this bond typically makes cellulose ingestible. About 33% of all vegetable components are composed of cellulose, which is the fundamental structural element of plant cell walls. It makes up more than half of all the carbon in vegetation, making it the most prevalent of all naturally produced organic compounds [8].

The amount of cellulose in whole grains varies depending on the species and is primarily a result of the husk and seed coat thickness. More cellulose in cells results in thicker, more robust cell walls. A well-filled grain should have a low cellulose to starch or any reserve polysaccharides ratio since seed endosperm cells consist of just thin cell walls [10].

The equatorial conformation of the residues of glucose enables the molecule adopt an extended and slightly stiff rod-like conformation since cellulose is a polymer made of straight chains that doesn't have coiling or branching. Numerous hydroxyl groups on the residues of glucose from a particular chain create bonds of hydrogen with oxygen molecules that are on the same or on an adjacent chain. This tightly holds the chains side by side. Cellulose is very insoluble in water due to the ability of the chains to stack up to create stronger microfibrils, nevertheless it can swell in excessive sodium hydroxide solutions. Cellulose is capable of being introduced into solution by using chemicals that disrupt hydrogen bonds, such as N-methylmorpholine N-oxide. Furthermore, through thermal and shear treatments, subsequently followed by alkaline peroxidation and shearing, cellulose-rich maize bran can be transformed into a cellulosic gel for use as a nutritional supplement [11]. In addition to water and matrix polysaccharides such (1, 3, 4)-D-glucans, heteroxylans (arabinoxylans), and glucomannans, cellulose microfibrils may also interact with these substances [12].

2. Non-Cellulosic Polymers

- **Arabinoxylans:** The polysaccharide arabinoxylan (AX), which is found in the cell walls of many cereals including oat, barley, corn, rice and wheat, has a linear backbone consisting of xylose units linked by arabinose units [13]. Despite these polysaccharides only make up a small portion of whole cereal grains, they play a significant role in plant cell walls. Arabinoxylans (60-70%) make up the majority of the thin walls that enclose the cells in the starchy endosperm and the layers of aleurone in the majority of cereals; the exceptions are the endosperm cell walls of rice (40%) and barley (20%) [11]. The pericarp and testa of wheat, in particular, contain a very high arabinoxylan content (64%) [14].

Wheat AXs have been found in endosperm (3–5% of the total endosperm), aleurone, and bran (60–70% of the total cell wall) [15], [16]. Since, they are attached to the cell walls by alkali-labile ester like cross linkages, the majority of the arabinoxylans in wheat grains are insoluble in water [17]. However, arabinoxylans that are not attached to the cell walls have the ability to generate extremely viscous solutions and can hold ten times their weight in water. Arabinoxylans can quickly

form a gel network under the influence of oxidative agents, such as H₂O₂/peroxidase, as the outcome of the restoration of cross-links [18]. Arabinoxylans that are fully cross-linked are capable of holding as much as 100 g of water/g of polymer [16]. AXs make up 10.9 to 26% of all bran fractions in the particular case of wheat bran [19]–[22]. Barley AXs share the same fundamental makeup as wheat AXs, which are polysaccharides mostly made of xylose and arabinose. Though far less researched than AXs from barley or wheat, corn also serves as an ideal source of AXs [23]–[25]. AXs have been found in about 51% of maize bran, or 67% if residual starch is not taken into account [23]. Other researchers, however, have noted lower AX yields from corn bran (approximately 35–40%) [26].

Cereals can have AXs extracted from them utilizing a variety of methods from various grain sections. Cereal brans, which account for between 10% and 25% of all bran, are the most prevalent source from which AXs are derived [19], [21], [22], [27]. Enzymatic treatments, chemical treatments, water treatments, mechanical treatments, or a combination of these procedures can all be used to extract AXs [16], [28]–[31].

- **Mannans:** The four types of mannans that are naturally occurring are linear mannan, glucomannan, galactomannan, and galactoglucomannan [32]. Linear chains of D-mannose residues connected by β -1,4-glycosidic linkages make up linear mannan. The linear mannan backbone is water-insoluble like cellulose because of its similarity [33]. Contrarily, glucomannan is made up of D-mannose and D-glucose residues joined together by β -1,4-glycosidic linkages. Cereal grains have been shown to contain trace amounts of glucomannans. Mannose and glucose units make up these NSPs. In glucomannans, the M: G (mannose: glucose) ratio ranges from about 1.5:1 to 4.2:1 [34]. Since, they are hydrophilic, glucomannans are very soluble in water [33]. On the other hand, galactomannans are made of linear chains of D-mannose residues that have had their galactose residues replaced by α -1,6-glycosidic linkages. Their mannose to galactose content ratios are different. The endosperm of the seeds of many dicotyledonous plants, particularly those belonging to the Leguminosae family, is where galactomannans are primarily found. The gums made from carob (27-33%), fenugreek seed (25.5-32.8%), and guar (28.6-34.6%) contain the highest concentrations of galactomannans and is also a contributor of the gum's viscosity and ability to form gels [32], [33]. Cereals' galactomannan content has not been documented. The most complicated mannans are galactoglucomannans, which have a glucomannan backbone with D-galactose replacements. According to reports, the molar ratio of the mannose, glucose, and galactose residues in galactoglucomannan is 3:1:1. Depending on where it comes from of the polysaccharide, galactoglucomannans are able to be acetylated at the C-2 and C-3 positions of mannose residues to varying degrees [35].

3. **Pectic Polysaccharides:** Galacturonic acid molecules are the building blocks of pectic polysaccharides. These molecules are connected by α -(1→4) glycosidic linkages, frequently with rhamnose residues attached, but they can also contain glucuronic acid, galactose, fucose, arabinose, or xylose. In grains of wheat, corn, and rice, pectic polysaccharides typically make about 0.24–0.25% of the total grain weight [36]. It is 4.68% in quinoa grain and 2.41% in barley grain [37]. Two forms of pectins—high

methoxyl and low methoxyl—are characterized by the presence of certain carboxyl groups in the galacturonic acid chain as methyl esters. Protopectin is also found, as well [38]–[40]. Low methoxyl pectins have less than 50% methanol-esterified carboxyl groups, while high methoxyl pectins have more than 50% [41]. Among cereal grains, high methoxyl pectins make up 28.7–36.4% of the overall pectin content, while low methoxyl pectins make up 30.9–35.2%. Protopectins, which are water-insoluble pectic compounds, make up the rest of the pectin content (32.3–36.5%) [42].

III. METHODS USED FOR ESTIMATION OF NSPS

1. Gravimetric Methods: After non-fiber materials have been chemically or enzymatically solubilized, gravimetric methods quantify the insoluble residue. Gravimetric techniques are simple to use and don't need any specialized tools [1].

- **Crude Fiber Method:** The crude fiber method is the oldest and most widely used method for fiber analysis. Since some of the structural polysaccharides such as cellulose and lignin can be solubilized, this approach can only measure a small portion of the fiber's constituents. The insoluble fraction is isolated using the crude fiber technique, which alternates between acid and alkaline digestion. In some parts of the world, as well as in the food sector, the crude fiber method is still in use. However, due to the loss of some insoluble polysaccharides, all soluble polysaccharides, some lignin, and the addition of some nitrogenous material to the residual residue, its utility is severely constrained.
- **Detergent Method:** Van Soest and associates pioneered the application of detergents to dissolve protein in the 1960s. Although these techniques were initially created for pasture fiber, detergent techniques are now more frequently employed for concentrate feeds. Strong acid is used in the Acid Detergent Fiber (ADF) process, which was used for animal feeds. All polysaccharides are hydrolyzed safeguard cellulose and lignin, which are the only components in ADF. This method, like the crude fiber method, has limitations regarding its applicability to human nutrition because it excludes other cell wall polysaccharides. Van Soest created the Neutral Detergent Fiber (NDF) method, for assessing all insoluble cell wall material, in response to the requirement to define and include additional cell wall elements. This turned out to be a more accurate predictor of the dietary fiber content of animal meals than the crude fiber technique. The NDF approach started to be used in human nutrition in the 1970s, but its applicability was still constrained because it did not incorporate soluble fiber components or completely eliminate starch. The fiber that is insoluble in acid detergent (ADF) and neutral detergent (NDF) can be measured using the detergent method in a more detailed way. While ADF only measures cellulose and lignin, allowing for the determination of hemicellulose by difference, NDF measures hemicellulose, cellulose, and lignin. Since both water-soluble and water-insoluble NSP might be lost during the NDF process, starch and protein can contaminate the NDF residue, and hemicellulose may remain in the ADF fraction, this computation may not provide a precise measurement of NSP. The difference between total dietary fiber and NDF can be used to determine NSP.

- 2. Colorimetric Method:** Strong acids that include orcinol, carbazole, and anthrone, which are generally selective for pentoses, uronic acid, and hexoses, respectively, cause carbohydrates to condense in a reaction. This reaction results in the production of spectrophotometrically measurable colored compounds. Methods were enhanced in the 1950s by adding a number of extraction processes, followed by polysaccharide hydrolysis and monosaccharide component colorimetric analysis. Southgate noted that removing all of the starch was essential since any remaining starch would cause the amount of dietary fiber derived from glucose to be overestimated. The Fourth Edition of McCance and Widdowson's *The Composition of Foods* included the United Kingdom nutritional tables when the Southgate technique was adjusted for human nutrition in the 1970s. Southgate acknowledged that a colorimetric assay did not distinguish individual monosaccharides but suggested that gas chromatography (GC) or high-performance liquid chromatography (HPLC) be used because the method established significant information on monosaccharide groups (pentoses, uronic acids, and hexoses). Additionally, there were still issues with the removal of starch, which resulted in exaggerated values for numerous dietary products with high starch contents, including grains, legumes, and starchy vegetables.
- 3. Chromatographic Method:** There are numerous chromatographic techniques used to estimate NSPs. A method for measuring non-starch polysaccharides using Gas Chromatography that extends Southgate's work was published by Englyst and colleagues. The technique required more thorough starch removal and made it possible to identify the various monosaccharides that make up dietary fiber in processed foods. Additionally, it enabled the separation of soluble from insoluble polysaccharides and cellulose from non-cellulosic polysaccharides. As a result, the approach offered a great deal of information about the polysaccharide components of diets for humans. The Englyst method is an upgraded version of McCance and Southgate's enzymatic-chemical approach for determining NSP. The enzyme completely breaks down starch, and NSP are calculated as the total of the component sugars liberated during acid hydrolysis. Gas-liquid chromatography (GLC), high-performance liquid chromatography (HPLC), colorimetric analysis, can all be used to assess the presence of carbohydrates. Data for total NSP, soluble NSP, and insoluble NSP can be determined. Cellulose can also be evaluated independently with a modest modification to the procedure. With its colorimetric version, the Englyst method can determine total, soluble, and insoluble NSP in 8 hours, or in one and a half days when using chromatographic techniques. NSP is calculated using GLC as the total of neutral carbohydrates, while uronic acids are calculated independently. Neutral sugars and uronic acids are the components of NSP as measured by HPLC. Monosaccharides and oligosaccharides are two examples of individual sugars that can be separated using thin layer chromatography (TLC). It is most frequently used for oligosaccharides in the raffinose series, including raffinose, stachyose, verbascose, etc. The various sugars are recognized, recovered, and then estimated spectrophotometrically from the ethanol extract that contains a combination of sugars and is spotted on the TLC plate (cellulose coated).
- 4. Enzymatic Method:** The nineteenth century saw the invention of using enzymes to extract readily available carbohydrates by German researchers. In order to address issues with applying the approach to items with a high starch content, where the starch is not

fully solubilized, Schaller added the amylase treatment in the neutral detergent technique developed by Van Soest & Wine. Mongeau and Brassard modified the technique and created a quick gravimetric approach with great precision, but it had the drawback of being unable to entirely extract the starch and/or protein in some samples. The non-enzymatic gravimetric method of Prosky and other more exact NSP analytical techniques, such as the enzymatic-chemical method, or Englyst method, have emerged as the primary methods for NSP assessment. Furda, Schweizer and Würsch, as well as Asp and Johansson separately created the first gravimetric techniques for measuring the soluble and insoluble parts of NSP. Together with DeVries, Prosky, and Harland, these writers created the first iteration of the AOAC's enzymatic gravimetric method. Later, the procedure was modified to work with both soluble and insoluble fractions before being made simpler by employing 4-morpholine-ethanesulfonic acid-TRIS buffer. The AOAC modified Prosky and Asp's approach. By enzymatically removing accessible starch and solubilizing and extracting a portion of the protein, the method calculates the overall amount of dietary fiber. The rest of the residue is then dried, weighed, and its crude protein and ash levels are adjusted. If there is at least 10% of fat present, a preliminary step is added to eliminate it. The procedure can be completed quickly and easily, and it has been mechanized to allow for the evaluation of many samples at once. Many nations have made it their official way of analyzing dietary fiber. This procedure used protease, amyloglucosidase, and thermostable amylase to break down the gelatinized material. Alcohol is used to precipitate the undigested fraction. With Nx6.25 and ash, the residue is corrected. The drawback of the AOAC method is that hydrolyzed byproducts stay in solution. Alcohol cannot dissolve the Na and Ca salts found in the samples and buffers, which results in an increase in ash. There exists a slight overestimation of polysaccharides at 525 °C due to the depletion of volatile components. Due to these issues, the process was altered to include urea-based dialysis, which forgoes the heat treatment and dialysis-based byproduct removal. The method's fundamental component is the unique activity of a thermostable enzyme in an 8M urea solution. Southgate and Bach Knudsen modified an approach created by Asp for the analysis and characterisation of dietary polysaccharides groups for feed.

IV. PROPERTIES OF NON-STARCH POLYSACCHARIDE

NSPs' fundamental characteristics include their solubility, ability to bind to water, ability to create very viscous solutions, and capacity to cross-link and lower surface tension [1].

- 1. Solubility:** The molecular structure of NSPs affects how soluble they are. Higher solubility is caused by any structural trait that prevents intermolecular interaction, such as molecular branching or the existence of carboxyl, sulfate, or phosphate groups [43]. The existence of linear chains within the molecule, high molar mass, and other common structural traits are examples of structural characteristics that encourage intermolecular interaction and cause poor solubility [44], [45].

AXs are often divided into two fractions: those that are soluble in water and those that are not. The degree and pattern of substitutions, the proportion of arabinose to xylose, and their molecular weight all affect how soluble AXs are in water. It has been

established that the AX chain's solubility decreases when arabinose residues are removed [46]. AXs that are insoluble in water are coupled to components of the cell wall such as cellulose, lignin, or proteins [46], [47]. The surface of the cell wall contains loosely attached water-soluble AXs [17], [46]. AX is typically 30–40% [48] soluble in wheat flour, compared to 18–23% [49] soluble in whole-grain rye flour. AXs were 10–18.5% [50] soluble in grain of barley. It should be noted, nonetheless, that the observed variations in the AXs' calculated solubility may be the result of different extraction procedures, preparations with different compositions, or solubility measurement techniques [49], [50].

The ratio of tri-saccharide to tetra-saccharide units influences the solubility of β -glucans; a smaller ratio indicates a higher solubility [51]. Since oat grains' β -glucans are more soluble in heated water than in cold one, food processing procedures that call for both heat as well as moisture are likely to do the same [52]. It has been demonstrated that baking bread increases the solubility of β -glucans as well [53]. However, it has been found that foods kept frozen for prolonged periods of time cause a reduction in the soluble capacity of β -glucans [54]. It was demonstrated that when the molecular weight of β -glucans decreased (from 2,200,000 g/mol to 400,000 g/mol), so did their solubility. Low molecular weight (120,000 g/mol) β -glucan molecules can, nevertheless, form insoluble clumps [55], [56]. Oat grain β -glucans are 27–78% soluble, whereas barley grain is 53–63% soluble [57], [58].

The degree to which mannose chains are replaced with galactose residues determines how soluble galactomannans are. The molecules are more soluble when there is a greater degree of substitution. Galactomannans, which have galactose residues linked to every mannose molecule, provide such a significant steric barrier that they disintegrate in cold water. In cold water, galactomannans, which have a galactose residue connected to every fourth mannose molecule, are difficult to dissolve, but in hot water, they dissolve with ease. The main reason galactomannan molecules are more soluble is that they contain more side chains, that maintain the primary mannose chains sufficiently apart. On the other hand, due to their lengthy sections of unsubstituted mannose units, galactomannans with less side chains (higher mannose-to-galactose ratio) are able to interact with other polysaccharides [59], [60]. Previous research studies have not provided information on the extent of solubility of galactomannans from cereal grains. However, a test of locust bean gum's solubility revealed a range of 62.7–82.7% [61].

The most common form in which arabinogalactans are extracted as NSPs is arabinogalactan peptide. About 92% of the polysaccharide is made up of arabinogalactan peptide, and the remaining 8% is made up of peptide [12]. There is no information on the solubility of arabinogalactans in cereal grains.

Pectins' degree of polymerization, quantity, as well as distribution of carboxyl groups all affect how soluble they are. Pectin's solubility rises as its molar mass falls and the esterification of its carboxyl groups increases. pH and temperature of the solution have a definite impact on pectin solubility as well [62]–[64]. The soluble state of cereal pectins is yet to be established.

Although xyloglucans have the ability to dissolve in water, there is no information on how xyloglucans in cereal grains are soluble [65].

- 2. Water Binding Capacity:** NSPs possess hydrophilic characteristics that allow them to bind to water. The ability to bind water is the result of interactions between water and NSP molecules that produce in bonds of hydrogen or dipole interactions [66], [67].

Particularly the water-soluble AXs exhibit a great affinity to bind water [68]–[70]. In comparison to wheat flour with no inclusion of these polysaccharides, 11–12% more water was bound when water-soluble AXs in the range of 0.5–1.3% were added [70]. AXs that are water-soluble and made from wheat flour can bind, on average, 0.38 g of water per gram of dry basis [69], but AXs made from rye grains can bind, on average, 1.58 g of water per gram [68]. Additionally, it has been demonstrated in other research that AXs from flour made from wheat are capable of binding even 15 g of water/g [71]. AX from wheat bran, which is cellulose-rich, may bind 13.3 to 16.13 g of water [72].

The amount of water bound by β -glucans from barley grain varied depending on the extraction technique, from 2.91 g/g d.b. employing enzymatic treatment to 3.79 g/g d.b. employing hot water extraction [73]. According to other research in the literature, barley grain's β -glucans have a water holding capacity of 6.1 to 6.74 g water per 1 g of β -glucans [57]. The usage of additional barley grain kinds is likely what caused the large variances in the results that were achieved.

It is important to keep in mind that the outcomes of water-binding capacity that have been determined are closely related to and significantly influenced by the ways in which NSPs are extracted, as well as by variations in the procedures employed to assess this parameter.

- 3. Viscosity:** The length of the molecule chains, their molar mass, the amount and sequence of arabinose substitution in the main chain (backbone), which is made up of xylose residues, and the presence of connected ferulic acid all affect how viscous water solutions of AXs are [46]. The soluble AXs come in two different varieties: arabinoxylan I and arabinoxylan II [74]. The first one has a series of xylose residues with over 50% replaced at the O-3 position by arabinose residues. Arabinoxylan II is made up of a series of xylose residues having 60–70% of their O-2 and O-3 locations replaced by arabinose residues [74]. Compared to arabinoxylan type I molecules, arabinoxylan type II molecules exhibit a greater association with viscosity. AXs' aqueous solutions' inherent viscosities range from 1.96 dL/g to 4.23 dL/g [75], [76].

Additionally, β -glucans have the power to make aqueous solutions viscous. According to the literature, the extraction technique, molar mass, temperature, solution pH, concentration, and aggregation capacity all have an impact on the viscosity of β -glucans [44], [77]–[79]. Typically, β -glucan water solutions have inherent viscosities that range from 0.28 dL/g to 7.2 dL/g [80], [81].

At relatively low concentrations, galactomannans can produce extremely viscous aqueous solutions that are only marginally influenced by pH, ion concentration, and

treatment with heat. Galactomannan solutions have high viscosity and are highly stable over a broad pH range (1–10.5), mostly because their molecules are neutral [59], [82]. Galactomannan aqueous solutions mentioned in the literature have inherent viscosities that range from 9.7 dL/g to 14.3 dL/g [83], [84].

The intrinsic viscosity of arabinogalactans in aqueous solutions ranged from 0.045 to 0.062 dL/g [75].

The pH, molar mass and structure, ionic strength, and polymer content of pectin polysaccharides all affect their capacity to generate very viscous solutions. As the molar mass of pectins increases, so does their viscosity in aqueous solutions [85]. Additionally, pH has an impact on the viscosity of pectic polysaccharide solutions. This is because the electrical charge of polysaccharides influences a shift in the molecular structure of the chains of those molecules [86]. The electrostatic repellent forces between the pectin chains are weaker in an acidic environment due to the reduced dissociation of the carboxyl groups that are part of the galacturonic acid residues, and as a result, the viscosity of an aqueous solution of such polysaccharides will be higher than that of an aqueous solution of polysaccharides with a neutral or alkaline pH [87]. High-methylated pectic polysaccharides gels at acidic pH (3.6), whereas low-methylated pectic polysaccharides gel at a wide pH range (3.5-8.5) [88], [89]. Fruit-derived pectic polysaccharides have intrinsic viscosities that range from 0.64 dL/g to 5.9 dL/g in aqueous solutions [90], [91]. The viscosity of aqueous solutions of xyloglucans, pectic polysaccharides, and arabinogalactans, from cereal grains is not well-documented in the literature.

- 4. Cross Linking:** Nearby AX molecules can establish a covalent bond with ferulic acid residues under oxidizing circumstances and in the occurrence of free radicals, leading to cross-linking [92], [93]. The peroxidase/hydrogen peroxide systems (both of which exist natively in flour and yeast) or an enzyme called laccase are the two substances that are most frequently used to cross-link AX [93]. Monomeric ferulic acid is oxidized and transformed to the isomers of dehydrodiferulic acid or dehydrotriferulic acid during the enzymatic coupling reaction [94]. The molecular structure of AX, or their molar mass, the degree to which the xylan skeleton has been replaced by arabinose residues, and the amount and distribution of ferulic acid within the molecules all have an impact on how effectively they cross-link. More covalent cross-links are capable of being produced if there are more ferulic acid residues present in the polysaccharide backbone. It is estimated that the ferulic acid concentration of AX molecules derived from cereal grains ranges from 0.4 to 450 mg/100 g [31], [95]–[100]. Cross-linking produces NSPs with large molar masses and hence high viscosities [101]. Horseradish peroxidase and hydrogen peroxide cross-linked AX has a weight average molar mass that ranges from 191,000 g/mol to 720,000 g/mol [97], [102]–[104], while laccase cross-linked AX has a weight average molar mass that ranges from 159,000 g/mol to 508,000 g/mol [105]–[107].

By employing ethylene glycol diglycidyl ether (EDGE) in a mixture of 4% sodium hydroxide, β -glucans from euglenin cells and a strain of the bacteria *Alcaligenes faecalis*, kurdlan, were cross-linked to produce substances with higher hydrophilic

properties (better capacity to form a gel). By severing the hydrogen bonds between the molecules' chains, cross-linked β -glucans are produced, which results in the formation of new hydroxyl groups [108].

There are currently no reports on the cross-linking of pectic polysaccharides, arabinogalactans, or xyloglucans, of cereal grain origin.

- 5. Surface Tension:** Cohesive interactions among water molecules produce surface tension. The surface tension of water is reduced as a result of active surfactants' disruption of the interactions of cohesive forces between water molecules [109]. This is crucial since the adhesion of dough, food emulsions, and liquid foods to packaging, processing machinery, and leftover wastes results in large financial losses [110].

According to research, adding AX to water in concentrations ranging from 0.2% to 1.6% lowers its surface tension from 72 mN/m to roughly 52 mN/m [75].

Water's surface tension is somewhat decreased by β -glucans, but not significantly. The surface tension drop is more apparent at larger concentrations of β -glucans. Surface tension decreased by about 10 mN/m in the case of 1% β -glucans, but it decreased by less than 1 mN/m in the the existence of 0.5% β -glucans [111].

The surface tension of water dropped from 72 mN/m to 61 mN/m in the presence of 0.4-0.5% galactomannans derived from fenugreek seeds [112].

Surface tension was similarly reduced by pectin polysaccharides from orange peel and pigeon pea (likely arabinogalactan) [113]. Surface tension dropped from 72 mN/m to roughly 50 mN/m with the incorporation of 1.5% pigeon pea polysaccharides, and from 72 mN/m to 53 mN/m with the inclusion of 1.5% citrus peel pectin [113].

There are no known effects of pectic polysaccharides, arabinogalactans, or xyloglucans, from cereal grains on water's surface tension.

- 6. Molar Mass:** In terms of molar mass, NSPs derived from natural origin are polydisperse, meaning that the size of their molecules varies [114].

Size Exclusion Chromatography (SEC) is the primary method used to determine the weight average molar mass of cereal AX, which is typically between 197,800 and 2,000,000 g/mol for rye, 176,000,000 and 381,000 g/mol for wheat, 276,000 and 1,220,000 g/mol for barley grains, 244,000 and 491,000 g/mol for corn, and 24,400 and 232,000 g/mol for rice 6, 7, 9, 76, 92, [27], [102], [115]–[118].

Oat β -glucans have a molar mass ranging from 172,000 g/mol to 2,300,000 g/mol [77], [78], [119]–[125], while barley β -glucans have a molar mass ranging from 187,000 g/mol to 2,340,000 g/mol [57], [126]–[129]. It is estimated that the weight average molar mass of β -glucans in wheat grain ranges from 487,000 to 635,000 g/mol, while it is 970,000 g/mol in rye grain [122], [130], [131].

It has not been possible to determine the molar mass of pectins, xyloglucans, and arabinogalactans from cereals. Galactomannans produced from fenugreek seeds are thought to have an average weight molar mass of 1,170,000–1,810,000 g/mol [112], [132], [133]. Arabinogalactans had an average molar mass of 16,000 g/mol in Mongolian larch and 141,000 g/mol in *Polygonatum sibiricum* rhizome [104], [134]. The weight average molar mass of xyloglucans in azuki bean seeds ranges from 98,000 to 420,000 g/mol, while it is 480,000 to 2,400,000 g/mol in tamarind and *Detarium senegalense* seeds [65], [135], [136]. Fruit pectins' typical molar masses have been found to range from 61,000 g/mol to 247,000 g/mol [90], [137].

V. INFLUENCE OF NSP ON HUMAN BODY

As a result of their significant contribution to the quick movement of digestive contents, NSPs have a positive impact on how well the digestive system functions [138]. In addition, the fermentation of NSPs produces short-chain fatty acids (SCFAs), which have a number of health-promoting qualities, such as preventing or treating diarrhea and reducing the growth of pathogenic organisms [139], [140]. Because they influence the growth of bifidobacteria, or probiotic bacteria, in the colon, both AXs and β -glucans from cereal have a prebiotic impact [141], [142].

As immunomodulators, AXs and β -glucans have an impact on both innate as well as acquired immunity [143]–[146]. Immune cells (including granulocytes, macrophages, monocytes, and natural killer cells) have pattern recognition receptors that can attach to β -glucan molecules [147]–[149]. They exhibit anticancer action as a result of this property [150], [151]. Furthermore, AX molecules with ferulic acid in their molecular structure might have antioxidant properties [152]. Clinical studies have shown that the therapeutic immunostimulatory characteristics of β -glucans from fungal cell walls include antibacterial, anti-inflammatory, anti-tumor, and faster wound healing [153], [154]. NSPs show promise in the management of diabetes and obesity, two common disorders in contemporary society.

AXs reduce postprandial levels of blood glucose, control insulin response, and promote the release of postprandial ghrelin, also known as "the hunger hormone" and produced by gastric cells [141], [155], [156]. Because AXs make aqueous solutions more viscous, bread that contains these polysaccharides in the baking process has a lower glycemic index [157].

In both the pharmaceutical and food sectors, morel (*Morchella esculenta*) galactomannans have the potential to operate as an immunomodulator.

VI. CONCLUSION

This chapter summarizes the overview of non-starch polysaccharides, different types of non-starch polysaccharides, different ways to estimate them and their various properties. This also gives an insight of influence of non-starch polysaccharides on human body. A lot of literature on starch polysaccharides have been reported but very less literatures on non-starch polysaccharides have been reported till now. So, there is a scope for non-starch

polysaccharides as they have some important physiochemical properties that can be used as a thickening, stabilizing, gelling and emulsifying agent in preparation of food products.

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