

Polysaccharides in Agro-Industrial Biomass Residues

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Contributor: Valdir Veiga-Junior

The use of waste biomass to produce biopolymers and nutricosmetic or pharmacological materials is increasing, although still scarcely compared to its great potential, employment, and valorization. Organic waste biomass is a great source of natural polysaccharides such as cellulose, chitin, hyaluronic acid, inulin, and pectin. Biomass from the agricultural sectors is a relevant part of waste generation and commonly comprises leaves, roots, stalks, bark, bagasse, seeds, straw, wood, animal parts, crustacean shells, and others. Polysaccharides are the most abundant biological materials on the planet. This natural abundance contributes to the discovery of their novel applications. Their industrial use is still very modest considering their versatility and great potential, although it has recently seen significant increases.

Keywords: polysaccharides ; agro-industrial residues ; biomass

1. Cellulose

Cellulose is a biodegradable and low-cost biopolymer obtained from renewable resources, e.g., wood and plants cell walls, some bacteria, algae, and (unusually) tunicates (the only known cellulose-containing animals) ^[1]. Its industrial global production is around 1.5×10^{12} tons per year ^[2]. This production is predominantly the result of the cotton and wood industries, which use about 98% and 90% of pulp, respectively ^{[2][3]}. Cellulose is the most abundant polymer found in nature, and it is composed of glucose monomers linked in the β (1–4) position ^{[1][2][3]}. Cellulose has a high molecular weight and a high propensity to form crystalline fibers. Four polymorphic types have been found and are classified as I, II, III, and IV according to the conformation of the carbon skeleton and the hydrogen bonds ^[3]. Cellulose can be found from five main sources: wood, plants and their residues, algae, animals, and bacteria ^[1]. These sources can be classified as: (I) conventional or primary ones, which are usually applied in the manufacturing of textile fibers, paper and wood for civil construction, and the obtainment of ethanol; (II) secondary ones, which are from unprocessed residues and directly obtained from the food industry or are agricultural and forestry residues, such as bark and leaves; (III) tertiary sources, which comprises processed residues from the use, transformation, and conversion of cellulosic biomass, e.g., bagasse and food residues; and (IV) quaternary sources belonging to a small group that includes marine animals, algae, fungi, and bacteria ^{[1][3]}. The first three levels of cellulose sources have common origin, mainly from plants. **Table 1** shows the percentages of cellulose in some common types of agricultural residues, i.e., type III cellulose.

Table 1. Percentages of cellulose composition in plant residues.

Waste	Vegetal Source	Cellulose (%)	Reference
Pseudo stem	Banana	42.4	[4]
Stalk	Garlic	41.0	[5]
Cob	Maize	51.0	[6]
	Grape	19.3	[7]
Bagasse	Carrots	10.0	[8]
	Tomato	9.0	

Waste	Vegetal Source	Cellulose (%)	Reference
Husk	Manioc	18.0	[9]
	Garlic	41.7	[10]
	Onion	41.1	
	Oat	38.7	[11]
	Soybean	45.0	[12]
	Coffee	35.4	[13]
	Maize	29.3	[14]

Many plant residues are promising for the isolation of cellulose molecules due to their chemical composition. However, as shown in **Table 1**, not all agricultural residues are cellulose-rich resources, making its extraction nonviable in some cases such as bagasse. In contrast, pseudo-stems, stalks, maize cobs, and barks that present more than 40% of cellulose are viable sources. Likewise, several aspects of the storage, moisture, presence of contaminants, volume, distance from industrial processing plants, other viable uses (such as fertilizer or to produce compost), local resources, and logistic facilities, among others, can act as enhancements or deterrents to the proper extraction of cellulose for use by biotechnology technologies.

2. Inulin

Inulin is a natural polysaccharide found in more than 30,000 species of plants, with cereals, tubers, and roots being its main commercial sources [15]. The most common residual sources of this polysaccharide are chicory, asparagus, garlic, banana, onion, rye, barley, and wheat, although it can be found in other unconventional sources, e.g., açai seeds [15][16][17][18]. Inulin can also be enzymatically produced and obtained from genetically modified potatoes [19]. On the other hand, food residues may have slightly higher contents of inulin, such as onion with ~18% and garlic with ~28% of dry mass [20].

Inulin is a distinctive oligo- or polysaccharide because any sugar ring is part of its backbone, which is characterized by linear fructose chains, joined by glycosidic bonds formed by 2–100 monomeric units [15][16][17]. Its chains are mainly formed by furanose groups, giving more flexibility than pyranose rings [17]. Due to its long polymeric chain and glycosidic bonds in β -(2-1) position, inulin is insoluble at body temperatures and resistant to salivary amylase and intestinal enzymes, thus showing potential for use in the food and pharmaceutical industries [17][19]. Inulin can contain up to 10 fructose units, so it is classified as a fructo-oligosaccharide (FOS) and is considered a prebiotic [15][18]. As it serves as carbohydrate storage in many plants, inulin can also be found in the processing residues of fruits and vegetables such as husks, seeds, stems, and bagasse [21][22]. These by-products are generally side streams from processing steps such as pulping, cleaning, sieving, and food bleaching, which are necessary for food processing and preservation [23].

Industrially, inulin is processed from several raw materials, although its major sources are chicory roots (*Cichorium intybus*) and Jerusalem artichoke (*Helianthus tuberosus*) [16]. Nevertheless, there have been several laboratorial studies scaling up inulin extraction from other raw materials, such as burdock (*Arctium lappa*), dahlia (*Dahlia spp.*), garlic (*Allium sativum*), onion (*Allium cepa*), jicama and agave tequilana (*Agave tequilana* Weber var. Azul). Jicama, also known as “yacon”, is composed of approximately 60–70% of FOS (in dry basis), therefore being a very attractive source of this oligosaccharide [24]. Moreover, the most inulin-rich plants are in both the monocotyledonous and dicotyledonous families. Inulin can also be found in the bulbs, tubers, and tuberous roots of many plants such as *Dahlia pinnata* (Dahlia), *Taraxacum officinale* (Dandelion), *Asparagus officinalis* (Shatwaar), and *Asparagus racemosus* (Shatavari) [25][26].

Inulin extraction can be carried out with different methods mainly based on water, such as infusion in hot water (up to 90 °C), deep freezing followed by precipitation after defrosting, concentration and crystallization steps, and freeze-drying followed by optimized hot water extraction [16][27]. It is important to highlight that the use high temperatures in the inulin extraction process by diffusion in water is crucial, since the solubility of inulin increases with temperature [15][27]. Raw materials such as tubers or roots should be washed, sliced, or milled prior to inulin extraction, followed by filtration and recovery with evaporation or precipitation methods [15][27]. The purification process can involve other steps, e.g., liming, carbonation, filtration, demineralization, and decolorization [15]. Although many studies have investigated alternative sources of inulin extraction, its main source continues to be chicory root, a vegetable residue from the lettuce family known for its bitter taste [15][24]. Inulin extraction from chicory roots is commercially viable due to the high yield of the generated residues from the agricultural cultivation of chicory. When processed as powdered syrup, with concentrations

ranging from 90% to 98%, the market prices for it can range from \$1.00 to \$100.00 per pound depending on the concentration required by the consumer [28]. The extraction market of this polysaccharide is predominantly constituted by Chinese companies [28].

3. Pectin

Pectin is a biocompatible, high molecular-weight, anionic and naturally occurring heteropolysaccharide present in terrestrial plants that is associated with celluloses, hemicelluloses, and lignin [29][30]. It can comprise up to one third of the cell walls in dicotyledonous and some monocotyledonous plants, but it is also found in smaller amount (2–10%) in the cell walls of grass and wood tissue [29]. Pectin is mainly composed of galacturonic acid units, and it can vary in composition, structure, and molecular weight depending of its source and processing conditions [30]. Apple pomace, citrus, sugar beet pulp, grape, pineapple, and mango peel can be cited as residue sources for obtaining pectin [31][32][33][34]. However, the number of sources for industrial use has been limited for the first three materials [35].

The numerous biotechnological applications reported for pectin have allowed for the recent growth of interest in this area. Due to its good safety and low toxicity profile, pectin can be applied with simple methods, such as ionotropic gelation, for gel formation [29][30][32]. In this case, pectin concentration and molecular weight are proportional to the viscosity of the formed gel since more reaction sites are found in longer molecular chains [29]. The pectin market represents 45,000 tons yearly, and around 6300 tons of commercial pectin are used worldwide in the food industry, where it can be supplied in solid and particulate forms, similar to conventional sugar [32][36].

It can also be used in the pharmaceutical and food industries as a thickening agent, rheology modifier, and an emulsifier in the production of jams, sweets, and desserts [29][30]. Generally, pectin has similar applications to other polysaccharides to produce pharmaceuticals, cosmetics, and food coating products with functional, antimicrobial, biological, and active properties [30][37]. Pectin is capable of associating with other biopolymers to form microparticles, nanoparticles, or filmogenic solutions for food packaging design due to its anionic characteristic and gelling properties [38][39][40]. In biomedical products, pectin acts as a carrier for the delivery of certain medications used in wound healers and tissue engineering, in cholesterol reduction, as a probiotic agent, in anti-cancer therapies, in the manufacturing of contact lens membranes, in artificial corneas, and in catheters [30][41][42][43]. Pectin can also be applied to treat or prevent poisoning by toxic substances, e.g., mercury, because the functional groups present in its polymer chains can form complexes with metals, thus allowing for its use as a bio(nano)sorbent for the selective elimination of heavy metal [30][44].

Chemically, pectin chains can be divided into anionic and non-ionic side chains known as “smooth regions” and “hairy regions”, respectively [31]. Pectin is a linear poly-galacturonic acid with different esterification degrees, either highly esterified by a methyl group (HMP—high methoxyl pectin, >50%) or only partially esterified (LMP—low methoxyl pectin, <50%), that can influence its emulsifying, texturizing, and gelling properties [31][29]. HMPs have been commonly used in food industries as thickeners and gelling agents, where large amounts of sugars are required for gelation, while LMPs are used to form low sugar-content jams [31]. Moreover, pectin can be divided into homogalacturonan and rhamnogalacturonan I and II, which differ due to the sugar linked in the β -1,2 and β -1,4 chains [31][29]. The primary structure of pectin defines the interactions of this macromolecule with solvents, though when it is switched into a helical structure, the intermolecular interactions lead to the formation of a different arrangement and result in complexes and viscous solutions formed by secondary structures [31]. The presence of carboxyl and hydroxyl groups confer hydrophilic and biodegradable characteristics to this polysaccharide [31][29]. The main functional groups in pectin chain are hydroxyl, amide, carboxyl, and methyl, and the hydrogen and ionic bonds interchain interactions have important roles in gel formation [31][29].

Pectin is easily obtained through simple extractive methods such as the use of hot water and a solvent with an acidic pH of around 2 that generates a fibrous concentrate, which can be pressed and washed to obtain a crude extract [35]. Extraction yields can be around 70%, though other methods such as superfluid critical extraction, ultrasound-assisted extraction, and microwave-assisted extraction can be used to achieve higher values [35]. The species, extraction method, and storage conditions can change the chemical composition of pectin [31][29][35]. Compared to cellulose and chitosan, pectin has a more complex chemical structure that contributes to its biomedical effects. The researchers analyzed journal publications from the last 20 years and found that chitosan has a higher number of publications (55%), followed by cellulose (18%), and pectin (3%). The number of patents filed followed a similar sequence: cellulose (38%) > chitosan (24%) > pectin (6%) [33].

4. Chitin/Chitosan

Food residues of animal origin are relevant sources of bioactive chemical substances. Seafood processing, especially that of crustaceans such as shrimp, crab, and lobster, generates a volume around 50–70% of waste ^[45]. Worldwide, the annual production of residues from the harvest of crustaceans is estimated at about 3.14 million tons ^{[46][47]}. Chitin is the second most abundant polysaccharide found in nature after cellulose ^{[46][48]}. Chitin's non-toxicity and biocompatibility contribute to its application in different fields ^{[49][50][51]}. In addition to seafood, chitin/chitosan can also be extracted from insects and some fungi species ^{[52][53]}. Mainly, to produce one kilogram of chitin with conventional chemical processes, close to 30 kg of shrimp shells (on a wet basis) are needed, whereas to produce one kilogram of chitosan, up to 1.5 kg of chitin, together with several acid and alkaline substances, are necessary ^[46].

Chemically, chitin is a long-chain polymer similar to cellulose but with acetyl amine groups replacing hydroxyl groups, thus making it a direct precursor to chitosan ^{[46][48][49]}. Chitin is a water-insoluble polysaccharide due to the intra- and intermolecular hydrogen bonds formed by the oxygen of acetamido group with adjacent –NH or –OH functions groups ^[53]. Moreover, the N-acetylation degree among chitin's chains also has a significant effects on water-insolubility, limiting swelling properties and thus its industrial applications ^[53]. Chitin can occur as α and β allomorphs, though the α -chitin form is the most abundant and the β -chitin form is rarely found to be associated with proteins in squid pens and synthesized by pogonophoran and vestimentiferan worms ^[54]. The three different crystalline phases differ in the orientation of their microfibrils, making their applicability versatile for the production of different functional materials, such as for pharmacological, anti-inflammatory, antimicrobial, and immunological purposes ^{[46][48][49]}.

Chitin's conventional extraction and deacetylation to form chitosan have many drawbacks, especially linked to harmful effects in the physico-chemical properties of both biopolymers, leading to diverse molecular weight and acetylation degrees that can affect their functional properties; these include the utilization of harmful/toxic solvents, low processing yields, the generation of wastewater effluent containing toxic chemicals, and processing costs ^{[46][54]}. To overcome these difficulties, green techniques, such as those based on the use of deep eutectic solvents and ionic liquids, have been used as sustainable alternatives ^{[46][54]}. As described for other polysaccharides obtained from residues, the molecular weight and the degree of deacetylation play important roles in the biological functions of chitin, e.g., higher degrees of deacetylation promote greater responses. The physicochemical characteristics of chitin and chitosan can vary by source and seasonality of raw materials, as well as other factors intrinsic to processing methods ^[54].

Chitosan, on the other hand, is a deacetylated derivative of chitin that can be processed into different materials such as membranes, sponges, gels, nanoparticles, and nanofibers ^{[46][48]}. The differentiation of chitin and chitosan can be seen in the degree of deacetylation: if it is higher than 50% mol, it is classified as chitosan ^{[46][54]}. Chitosan is water-soluble at a mild acidic pH of around 4.5, which facilitates its biological application and solubilization, while chitin is only soluble in concentrated acid solutions ^{[46][48][49]}. Due to the limited applications of pure chitosan, techniques can be used to improve its selectivity, functionalizing its structure with chemical reactions and cross-linking to several molecules for industrial uses ^{[46][48][49]}.

Both chitin and chitosan are known in biomedical areas for drug delivery. Both biopolymers act in the removal of allergenic proteins from some foods, the controlled transfer of drugs, and food supplements with hypocholesterolemic effects, among others ^{[46][48][49]}. They are also used as antibacterial, anti-inflammatory, sunscreen, and anti-aging cosmetics, as well as in tissue engineering where they aid in the reconstructive and healing processes of epithelial tissues ^{[55][56][57]}. In food technology, chitosan has a wide range of applications, such as acting in the extension of food shelf-life, e.g., for bread and apples, because it can reduce starch retrogradation and inhibit microbial growth in both food preparation and the production of coating packaging with bioactive properties ^{[46][58][59]}. The production of coatings occurs due to the association of chitosan with other compounds with biotechnological action, and this is mainly pursued to overcome some of chitosan's technological limitations, such as its brittleness, low elongation, and flexibility ^[46]. For example, by combining chitosan with poly(vinyl) alcohol and lignin nanoparticles or with other polysaccharides to form composite coatings, greater plastic resistance compared to that of individual components can be achieved ^{[55][60][61]}.

For benchmark market values of these isolated compounds, those of the "GTC Bio Corporation" company in China can be highlighted, with diverse prices and different levels of quality. The price of chitin is around \$23.4/Kg, and it can range from \$21.06 to 52.65/Kg for chitosan due different purity degrees ^[62]. In the production of functional materials, chitin has stood out in the manufacturing of hydrogels, aerogels, membranes, films, and fibers. Since chitosan has a quick biodegradability (less than 1 month) in different sites compared to the 300 years of the conventional plastic use, it can replace products that use petroleum-based plastics ^{[46][62][63][64][65]}.

5. Hyaluronic Acid (HA)

Hyaluronic acid is a naturally occurring polymer commonly found in animal tissues, including residual ones such as vitreous humors, chicken crests, and fish eyeballs, or in the cell walls of some bacteria such as *Streptococcus zooepidemicus* [66][67][68]. Chemically, it is composed of a major glycosaminoglycan macromolecule with alternating β -1,3 and β -1,4 glycosidic linkages from a family of linear, anionic, hetero-co-polysaccharides [67][68]. The repeating units of β (1,4)-glucuronic acid (GlcUA)- β (1,3)-N-acetylglucosamine (GlcNAc) in HA generally adopt a stable chair conformation, while in solution highly flexible regions may exhibit a random coil structures [69]. Besides hyaluronic acid, the main glycosaminoglycans largely diffused in the epithelial, connective, and nervous tissues of vertebrated animals are chondroitin sulfate, dermatan sulfate, keratin sulfate, heparin sulfate, and heparin [66]. HA is highly hydrophilic due to the presence of hydroxyl groups among chains that can bind water molecules, and this trait is linked to its greatest asset: allowing for tissue flexibility [67]. As a result, it can also be applied in the development of pro-drugs with better physicochemical properties, stability, and optimized therapeutic efficacy compared to free drugs [66][67].

Industrial extraction predominantly consists of the isolation of HA from chicken crests residues, although some microbial fermentation is also applied [67]. For the purity of this substance, free from contaminating agents, chickens should be selected to avoid cross-contamination [67][68]. It is estimated that the global market for HA will reach a worldwide value of around US \$15.4 billion by 2025, and it will be dominated by nine international brands, mainly due to its cosmetic applications [66][70].

Table 2 presents different concentrations of HA extracted from livestock industry residues. The studies referenced in this table were initiated back in the 1980s and used optimization methods to industrially obtain HA from slaughtered chicken crests. Over a period of 10 years, an academic effort to experimentally improve the extraction performance of HA was noted; however, not all livestock residues were found to have significant volumes and have been used for the extraction of hyaluronic acid.

Table 2. Hyaluronic acid content from different sources.

Source	Residues	Content	Reference
Swine		0.04 g L ⁻¹ of vitreous humor	
Swordfish		0.055 g L ⁻¹ of vitreous humor	[71]
Sharks	Eyes	0.3 g L ⁻¹ of vitreous humor	
Tuna fish		0.42 g L ⁻¹ of vitreous humor	[72]
Bovines		0.47 g L ⁻¹ of vitreous humor	[73]
		1.0 mg g ⁻¹ of crest	[74]
Chickens	Crests	39.8 mg g ⁻¹ of crest	[75]
		15.0 mg g ⁻¹ of crest	[76]

The biomedical applications of hyaluronic acid include its use in ocular and plastic surgery, the treatment of osteoarthritis, corneal xerosis in anti-aging products, and tissue culture, as well as in carriers for different osteo-inductive or osteogenic components [66][67][75]. HA has been also applied for a wide range of pharmaceutical purposes, such as the design of nanoparticles, microparticles, microspheres, gels, polyplexes, liposomes, micelles, and implants [67][75]. Good experimental results were found when HA was applied as wound dressings and chronic ulcers in the form of nanocomposites combined with different biopolymers, highlighting HA's biomedical application [74][75]. Applications in drug delivery include matrices for the treatment of skin infections, cancer therapy, and controlled release of proteins, antiseptics, and antibiotics via different routes, e.g., skin, ocular, topical, nasal, and oral routes [77]. Additionally, from a therapeutic point of view, HA is among the most studied biomaterials for cartilage regeneration [66][75][77]. A potential approach to cartilage repair is the transplantation of autologous chondrocytes or stem cells into cartilage defects using a natural or synthetic framework [77]. To perform the cartilage repair function, the component must be biocompatible with cartilage tissue, biodegradable, non-toxic, and non-immunogenic, which are relevant characteristics of biopolymers such as HA and chitosan [74][75][77].

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