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Biopolymers containing in cassava starch (*Manihot esculenta*): Amylose and amylopectin

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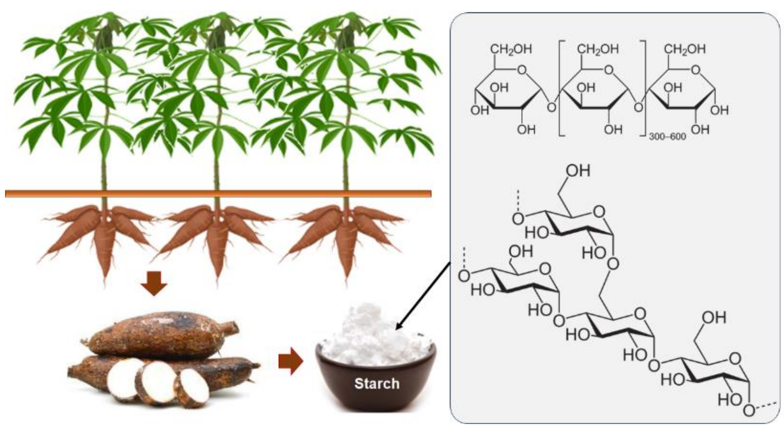
Graphical Abstract



Abstract. Starch is an important raw material for the chemical, pharmaceutical, and textile industries and many other materials. Mainly, into food industry has great relevance as an additive due to its functional properties both native and modified. In addition, it is considered the main energy storage carbohydrate for plants, but also, it is also a highly-hydrophilic polyhydroxylated biopolymer that can be used as a precursor for the making of a numerous material with low-cost, abundance, and availability. Starch is constituted of two biopolymers called amylose and amylopectin influencing its properties such as crystallinity, viscosity, retrogradation, gelation, and stickiness. These properties are essential to give this raw material an industrial use, but they are not determined in terms of its spectral of chemical modifications. Particularly, information related specifically to these biopolymers is dispersed and only these are usually described as parts of complete material. The aim of this review is to give an overview of starch, mainly, taking as executor of the content of its components. This review is of an introductory nature, and allows an overview of amylose and amylopectin, as well as their significance in the properties of starch. In this sense, the growing interest in polyhydroxylated biopolymers, particularly starch, is displayed throughout the document. The importance of delving into the study of starch as a biomaterial is concluded, but also the importance of identifying and characterizing new sources of starch obtained from wild species or resulting from genetic or adaptive modifications.

Keywords: Carbohydrate, starch, polymeric materials, amylose, amylopectin.

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Content

1. Introduction

Agriculture is an essential activity for the development of humanity, it supplies food to almost two-thirds of people in extreme poverty in the world, about 750 million. But also, it supplies raw materials and animal food. At present, food insecurity is on the rise due to the different factors affecting agricultural production, therefore, technological innovation for the production of food from agriculture is a pressing need. These innovations must be directed towards at least four key points: (*i*) the increase in crop yields, (*ii*) the reduction of the environmental impact of agriculture, (*iii*) the reduction of production costs and therefore the reduction of food costs, (*iv*) cope with the challenges: climate change, change in pest dynamics, scarcity of water resources, among others. In Colombia, there are various factors that cause a high in crop yields compared to several countries; however, there are many factors that intervene in agricultural production. An important aspect is the small research and technological development adapted to the current situation of the country, low innovation, and the use of new technologies. But there is also a limitation due to the scarcity of inputs, particularly fertilizers, an aspect that impacts production. In this way, innovation in agriculture, in the field of fertilization, can address a wide variety of aspects, among which are the nutritional requirements of plants, soil fertility, the characteristics and properties of fertilizers, and application technology, among others. The above is decisive for increasing the effectiveness of the nutritional supplement, the nutritional dynamics associated with the soil-plant system, and the environmental impact of crops (FAO, 2017; Ministry of Agriculture and Rural Development, 2019).

From another point of view, the growth of the world population, the increase in production costs and food prices make agricultural systems limit their availability. These factors impose challenges that are not easy to solve. However, they reveal the multifactorial nature of the problems and their concatenation. For example, as the demand for food increases, due to supply and demand relationships, an increase in costs is experienced, which in turn pushes a demand for the required inputs and a pressure on ecosystem components, among which are included soils and water. In this way, the efforts to increase productivity have led to the implementation of practices based on the excessive use of fertilizers without taking into account the indirect impact of its excessive or inappropriate use. For this reason, it is necessary to adopt alternatives and articulate strategies that, in addition to increasing crop production, imply sustainable development allowing the protection of renewable and non-renewable resources (FAO, 2017; Ministry of Agriculture and Rural Development, 2019).

On the other hand, cassava (*Manihot esculenta*) is an important crop for the agricultural sector. It is cultivated in the 32 departments of Colombia and many other countries (Ministry of Agriculture and Rural Development, 2019). Despite its importance in the food industry, and being a source of supply for a large number of rural and urban families. A crop must nowadays be looked at with a different approach. The traditional view, for example in the cultivation of cassava, conceives its cultivation for a single purpose, the production of the tuber that can later have multiple uses. A sustainable vision of cultivation, and therefore, a disruptive approach, conceives that the crop itself is used for multiple uses, ideally towards obtaining more than one product, generating zero waste, and using minimal external resources. Among the alternatives to increase the productive potential of agriculture is the circular economy approach. Under this approach, the products and/or by-products are directed for their partial or total reuse, depending on what it is. In particular, due to its characteristics, starch can be used not only for food supply, but also for the development of materials for agricultural use, among them, the controlled release of fertilizers (Versino and García, 2014; Versino et al., 2019).

The aim of this review is to give an overview of starch, mainly, mainly taking its components as the executor of the content. This review is of an introductory nature, and allows an overview of amylose and amylopectin, as well as their significance in the properties of starch.

2. Cassava (*Manihot esculenta* Crantz) as a source of starch

Cassava is a plant from Euphorbiaceae family, containing more than 7200 species that are characterized by the development of laticiferous vessels, composed of secretory cells or galactocytes that produce a milky secretion. Its center of genetic origin is the Amazon Basin. Around 98 species of the genus *Manihot* have been discovered, of which only cassava is economically relevant and cultivated. Its allogamous reproduction and its genetic composition are highly heterozygous constituting the main reason for to propagation of it by cuttings and not by sexual seed. The use of this plant is characterized by the consumption of its root, in which a large number of components accumulate, including starch, which is the natural way in which the plant stores energy by assimilation of atmospheric carbon through the chlorophyll present in the plant's leaves. The shell is considered waste as it is hard, woody and inedible. The pulp is hard and even before cooking, furrowed by rigid longitudinal fibers; It is very rich in carbohydrates and sugars, and it oxidizes quickly once it has been stripped of the rind (Sánchez et al., 2009; Otálora et al., 2022).

Starch is considered the main energy storage carbohydrate of higher plants. It can be extracted from various commercial sources, such as cereals, corn, wheat, rice, oats, sorghum, tubers (e.g., potato, roots

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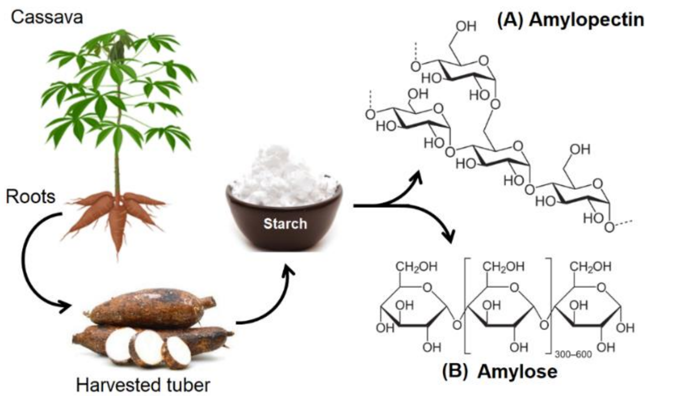

Figure 1. Illustration of the cassava plant, its tuber, and the appearance of the starch that is extracted from them. In addition, the structures of its main biopolymeric components (amylose and amylopectin) are shown.

such as cassava and yams), and legumes (chickpeas, soybeans, lentils, and beans). Starch is packed on itself forming discrete particles generically known as granules. The size and morphology of the starch granules vary depending on the plant source. It is observed that the granules are relatively dense and insoluble in cold water, although they may give rise to suspensions when dispersed in water. When starch is present in an aqueous solution and is heated, the hydrogen bonds are broken and the grain absorbs water and swells. Chemically it is a polysaccharide constituted by two biopolymers, amylose, and amylopectin (see **Figure 1**), that results from the polymerization of D-glucose molecules (Robyt, 2008; Wang et al., 2022). Structurally, starch is formed by carbon, hydrogen, and oxygen in a ratio of 6:10:5 [$C_6H_{10}O_5$], it belongs to a class of organic carbohydrate compounds, so it is considered a polymer with bonds between glucose units that are formed during condensation. Each of these glucose molecules is linked by an oxygen atom, which connects with the carbon atom, through bonds (1-4) of one glucose unit with the C4 atom of the next glucose unit, thus forming a long chain of interconnected glucose units. In addition, cassava starch contains ash (0.03-0.29 %), protein (0.06-0.75 %), lipid (0.01-1.2 %), phosphorous (0.0029-0.0095 %), and fiber contents (0.11-1.9 %) (Wang et al., 2022).

3. Amylose

As indicated above, amylose is one of the constituent biopolymers of starch, its content is an important quality attribute of starch and determines various of its properties (e.g., solubility, water absorption, and viscosity). In addition, depending on the amylose content of starches are usually referred to as waxy starch, regular starch, and high-amylose starch. Thus, amylose content varies between 0 and 15 % for waxy starch, 10–40 % for normal/regular starch, and higher than 40 % for high-amylose starch. However, most of the common cassava starches are normal/regular starches (Cruz-Benítez et al., 2019; Wang et al., 2022). Contents of amylose from different sources are shown in **Figure 2**.

Compared to amylopectin, its molecular weight is lower (amylose from cassava starch has a molecular weight among ~$2.5 \pm 0.2\times10^2$ kDa whereas the molecular weight for amylopectin is $5\text{-}40\times10^3$ to 10^5 kDa), however, both biopolymers are formed by chains of D-glucose units joined by α-(1-4) glucoside bonds. Glycosidic units are characterized by having primary and secondary hydroxyl groups capable of forming hydrogen bonds between them, which also spatially reorients the chains, giving them a helical-type spatial configuration. These no-covalent links are also responsible for its high hydrophilicity and the high degree of packing and molecular association giving it greater resistance to digestion; however, hydroxyl groups give amylose high hydrophilicity and act as the main reaction centers for carrying out chemical modification reactions aspects as its solubility in water are a consequence of linear-chain structure and relatively low-molecular weight (Berthaller and Hollmann, 2014; Kitamura and Suzuki, 2020; Wang et al., 2022). Details of amylose structure are shown in **Figure 3A**.

On the other hand, the interest in this biopolymer is evidenced by the number of publications made about it. As an example, taking as a reference the ScienceDirect database, which contains all its journals and books associated with Scopus, which is one of the most important databases of scientific documents worldwide, allows for establishing the growing number of publications directly related to amylose. Only in 2023, 2311 documents have been published, being characteristic that in the first decade of this century, the number of documents published in this database was 7716 (i.e., in 2023 almost a third of the documents were published). on amylose that were published in the first 10 years of this century). Likewise, the number of documents accumulated on the subject in the first 20 years was 22,194 documents, evidencing a nascent interest around the year 2013 (Science Direct database) (see **Figure 3B**). If the search is restricted in the same database by imposing the keywords "cassava" and "amylose" in the title, the number of publications is drastically reduced to 13 (of which only 12 correspond to research articles). This can be explained by the specificity of the search that involves amylose directly with the source from which it is obtained (Science Direct database). Below is given a brief description of publications about amylose.

Freitas et al., in 2004, quantified the amylose contents in yam (36.2 %) and cassava (24.2 %) starches by studying the kinetics of gelatinization. The approach is based on the structural characteristics of amylose with respect to amylopectin (Freitas et al., 2004). In general, as the molecular weight of a water-soluble polymer increases, its solutions will have higher viscosity as the molecular weight and/or concentration increase. Likewise, it can be generalized that a branched polymer, of the same molecular weight and at the same conditions of concentration and temperature, will have a higher viscosity than its linear analog.

A modification with analytical fines of starch was proposed by Charoenkul et al., in 2006, by fluorescent labeling of starch and a system based on a size exclusion chromatographic column, for the simultaneous determination of amylose and amylopectin distribution in four cassava varieties (Charoenkul et al., 2006).

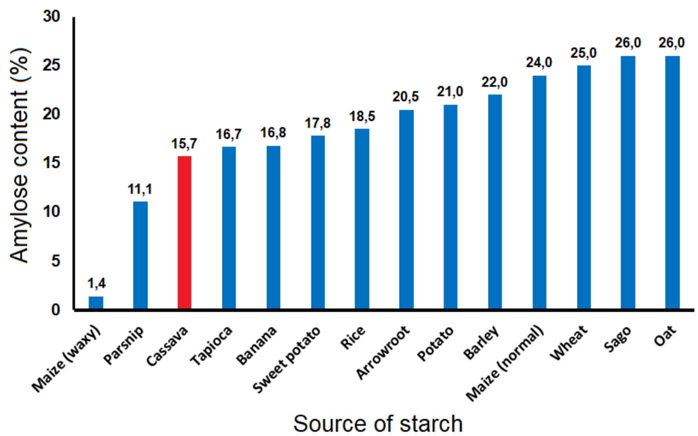

Figure 2. Content of amylose in different sources of starch (Adapted from Mottiar and Altosaar, 2011).

Dias et al., in 2007, studied the effect of enriching starch films by adding glycerol (between 20-45%) and amylose (between 6.3 and 25%). The main effect of the enrichment was the modification of the barrier properties and mechanical properties. In general, glycerol acts as a plasticizer, favoring water vapor permeability, ultimate stress, Young's modulus, and mechanical strength (Dias et al., 2007).

Some investigations have been directed towards the discovery of a natural source of cassava starch without amylose, this clearly more agronomic approach was motivated with the objective of developing a new series of crops that allows adaptation to new and different environmental conditions (Morante et al. others, 2016).

Pulido et al., in 2017, studied the thermal properties of cassava starch, establishing mechanical relaxation temperatures between 29-38 °C for wax starch without amylose (0 %), high crystallinity (40 %) and consequently characterized by a more organized structure (Pulido et al., 2017). These conclusions are consistent with the linear and branched nature of starch-constituting biopolymers. The branched nature of amylopectin would be associated with the formation of more amorphous structures, and consequently, the presence of amylose would affect these mechanical properties by influencing the packing of starch granules.

França-Lemos et al., in 2019, characterized the amylose contents in different types of starches using different techniques, including thermal techniques such as thermogravimetric analysis and differential scanning calorimetry. Their results reveal that amylose can be described as semicrystalline polymorphs, and highlight the selective precipitation of amylose with 1-butanol (França-Lemos et al., 2019).

Cruz-Benítez et al., in 2019, studied different cassava starches with different amylose contents, which were identified as normal and waxy. The purpose of this study was focused on the encapsulation of microorganisms, specifically, *Lactobacillus pentosus*. They report the modification by acid hydrolysis, the use of phosphates (sodium tripolyphosphate), and succinates (octenylsuccinamate). Among the starch modifications, the crosslinking of starch with sodium tripolyphosphate has been reported, which produces starches with greater solubility, greater water absorption capacity, and better freeze-thaw stability; acid hydrolysis in order to produce starches with lower viscosity; and esterification with octenyl succinate, which produces starches with hydrophobic properties and gives it emulsifying properties (Cruz-Benítez et al., 2019).

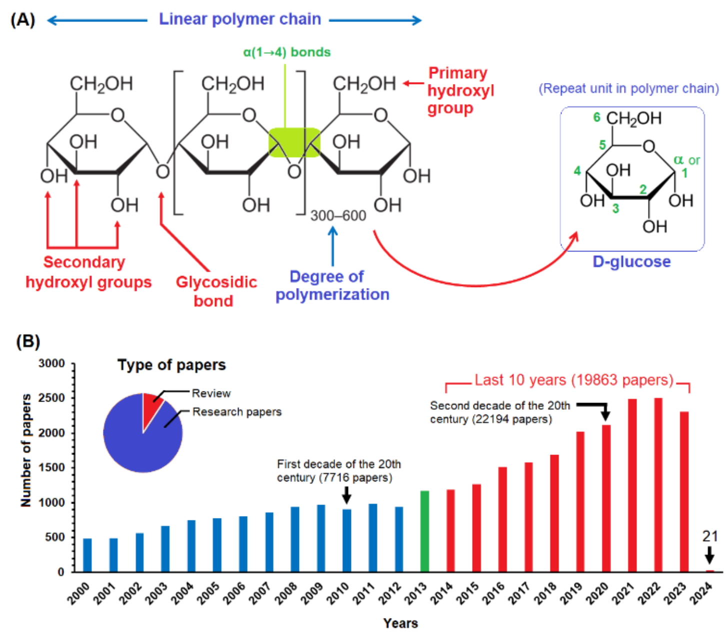

Figure 3. (A) Details of the chemical structure of amylose taking into consideration aspects related to: (i) polymer nature (blue and green annotations) and (ii) chemical nature (red annotations). (B) Evolution of a number of scientific papers related to amylose: last 10-years (red), inflection point (green), from 2000-2012 (blue) (Science Direct database, 2023).

Ekeldo et al., in 2023, described different drying methods on the rheological profile of amylose obtained from yellow-fleshed cassava flour. They show different types of drying including: *(i)* drying using sodium metabisulfite, *(ii)* drying using citric acid, *(iii)* flash drying with citric acid. The analysis of this approach focuses on food applications because high-quality cassava flour is characterized by being creamy, non-fermented, gluten-free, rich in carotenoids, and has a high energy value provided by carbohydrates. However, due to its high moisture content, the roots of this tuber tend to deteriorate after harvest. Key aspects related to amylose include its spectrophotometric determination at 620 nm by iodometry (Ekeledo et al., 2023).

On the other hand, within the quantification methods of amylose content in starches, it is usual the formation of complexes and the subsequent quantification by iodometry measurements, either through conventional titration (using some indicator systems), spectrophotometric titration (since complex formed with iodine produce a characteristic color) or amperometric titration (by changes of electrical properties resulting of complexing with iodine) (see **Figure 4**). Other methods are selective precipitation using concanavalin A (which is a lectin-type protein that was initially ex-

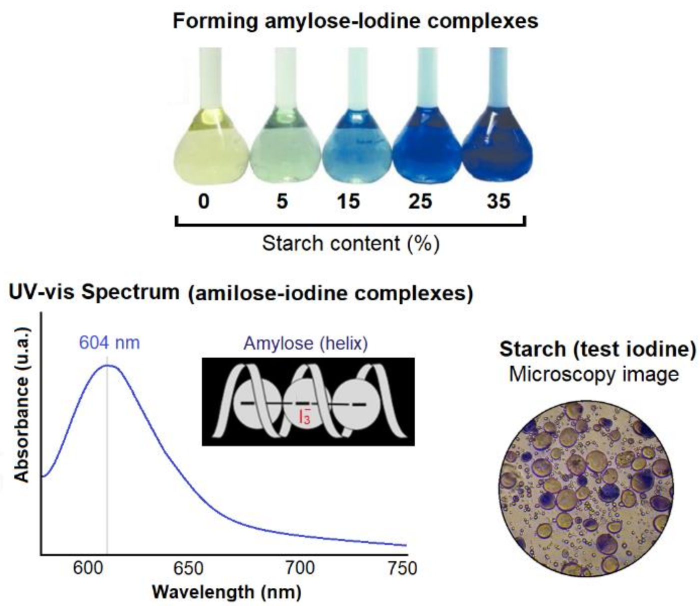

Figure 4. Illustration of colorimetric determination of amylose: visualization of color formed for starch in contact with iodine (upper), ultraviolet-visible spectrum, and microscopic image of starch granules during the formation of amylose-iodine complexes.

tracted from the *Canavalia ensiformis* plant, which is characterized by forming chemical bonds with certain carbohydrates), the use of chromatography, for example, high-throughput size exclusion, and fluorophore labeling techniques (Charoenkul et al., 2006).

In the food industry, removal of amylose by fractionation methods is common, including selective leaching using hot water (60 °C) to bring the starch above its gelatinization temperature, promote the swelling of the granules and their molecular reorientation in order to unwind them, and destroy the packaging, under this sequence of events, the amylose diffuses through the amylopectin chains generating the separation. Other methods are complexation and fractional precipitation by salts. In general, the purification of amylose has a high cost and to reach food standards it is common to use low molecular weight alcohols (Dias et al., 2007).

4. Amylopectin

As previously indicated, amylopectin is the second and main biopolymer contained in the starch (it constitutes more than 75 % of the granule). Its main structural difference lies in the branches of its chain, that is, while amylose is a linear polymer, amylopectin is essentially a branched polymer formed from the same repeating units as amylose: D-glucose molecules (see **Figure 5**). It is important to highlight that the polymeric nature of amylose and amylopectin leads to the definition of starch as a mixture of miscible polymers since they are in a high degree of intimate contact at the molecular level. Thus, it is possible to state that amylose and amylopectin are linked to each other by hydrogen bonds, forming

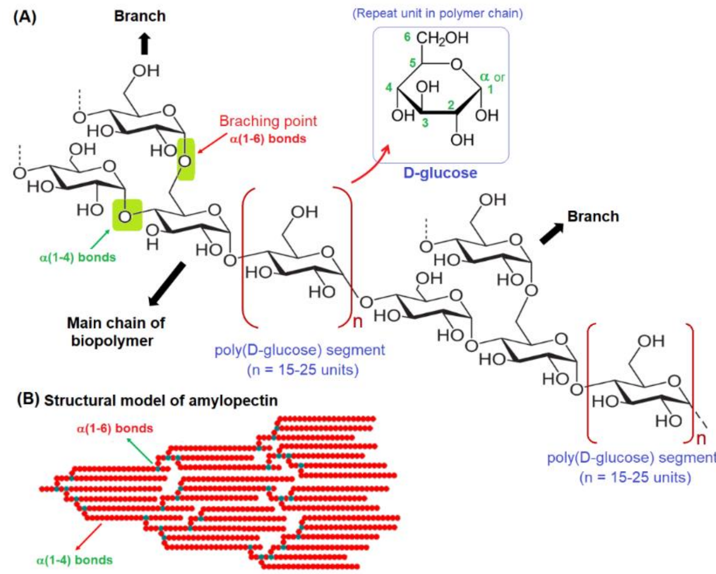

Figure 5. (A) Illustration of the chemical structure of amylopectin and (B) three-shape structural model of amylopectin.

radially-oriented crystalline zones (Ismail et al., 2013; Bergthaller and Hollmann, 2014; Kitamura and Suzuki, 2020; Wang et al.,2022). Although the molecular architecture is unknown, it is accepted that the relationship of chain lengths, frequency, and branching pattern is consistent with a tree-shaped structure, in which groups of chains are formed with regular interruptions along the entire axis of the molecule. Branches of amylopectin are attached to the central trunk (similar to amylose) by α-D-(1,6) bonds, located every 15-25 linear glucose units and represent about 5-6 % of these branching bonds (**Figure 5**) (Rolland-Sabaté et al., 2012; Wang et al., 2022).

Rolland-Sabaté et al., in 2012, carried out the characterization of cassava starches with low and high amylose contents; in addition, they reported different sizes for the molecular organization: (i) granular shape (0.1-200 µm), (ii) growth rings (120-400 nm), and (iii) crystalline lamellae (9-10 µm), therefore, amylopectin is the component responsible for crystalline domains of starch granule. This description proposed by them is based on the packing of double helices formed from short and grouped branches of amylopectin molecules within a thin lamella. Thus, amylopectin is described from starch as type A, B, or C. Amylopectin obtained from “type A starches” is characterized to contain shorter average branched chains, while amylopectin obtained from “type B starches” contains longer average branched chains. Finally, “type C starches” are described as those that contain both types of amylopectin, i.e., amylopectin with long and short branched chains (Rolland-Sabaté et al., 2012). The internal structure of amylopectin has been a topic studied by several researchers, in this way, amylopectin’s internal molecular structure has been correlated with several physicochemical properties of starch (Manners, 1989; Zhu, 1980).

Berfort et al., in 2010, analyzed the organization of the amylopectin

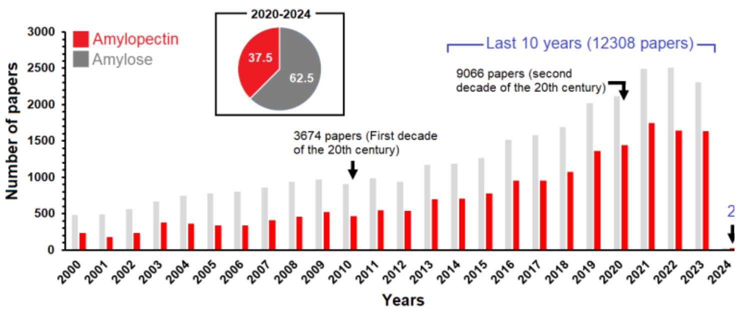

Figure 6. Number of publications about amylopectin compared to that related to amylose among 2000-2024 (Science Direct database).

chains, specifically for the starch obtained from the cassava root. The study was conducted through microbiological hydrolysis using *Bacillus subtilis*, later, the characterization of the hydrolyzed fragments was carried out (Berfort et al., 2010).

Similarly, to the bibliometric study carried out for amylose, in the case of amylopectin the number of publications is relatively less, although it can still be considered significant and of growing interest with 12,308 articles in the last 10 years, in the consulted database. A comparison of the number of publications is shown in **Figure 6** (Science Direct database). Below is given a brief description of publications about amylopectin.

Wang et al., recently studied enzymatic modification of starch granules for their use in the food industry. They quantified the crosslinking density by studying the kinetics of enzymatic debranching of starch using two glycosyltransferases and Michaelis-Menten kinetics. The novelty of the proposal made by the researchers lies in the fact that procedures for the same purpose have been developed for solubilized starch (amylose) and not for branched starch (amylopectin) (Wang et al., 2024).

An et al., in 2023, studied the retro-degradation mechanisms of starch, an aspect that impacts the quality and durability of products from the food industry that contain starch. Retro-degradation has been associated with increased crystallinity, viscosity, and turbidity of highly hydrated starch mixtures; furthermore, it is associated with gel formation and decreased digestibility. Their study was based on a spectroscopic analysis using infrared and Raman spectroscopy (An et al., 2024).

Javaid et al., in 2023, developed polyurethanes for the release of ciprofloxacin, a fluoroquinolone-type antibiotic, from amylopectin, chitosan, isophorone diisocyanate (i.e., cyclic and non-aromatic diisocyanate) and polycaprolactone diol. From their experiments, they concluded that the release of the drug is greater in the individual systems synthesized from chitosan and amylopectin compared to the system obtained from the mixture of the polymers (Javaid et al., 2023). The strategy used in this study is well known, it is based on the crosslinking reaction that occurs between the polymers in the reaction system, resulting from the reaction of the hydroxyl and amino groups with the isocyanate groups. As there are multiple reaction points, the matrix cross-links forming a mixed polymeric matrix that will also be characterized as being hydrophilic due to the enormous number of hydroxyl groups with respect to the number of isocyanate groups. This strategy can be generalized as "by-polycondensation crosslinking" from urethanization reactions (see **Figure 7**). Another example is "by-polycondensation crosslinking" from esterification reactions. For example, Kim et al. recently dispersed starch in ethanol, under heating and in the presence of phytic acid (myoinositol-hexakisphosphate), citric acid, and a mixture of both polyacids (Kim et al., 2024). In this other approach, starch provides multiple hydroxyl groups while polyacids, having more than one carboxylic group per molecule, act as crosslinking agents while being precursors for ester formation (See **Figure 8**).

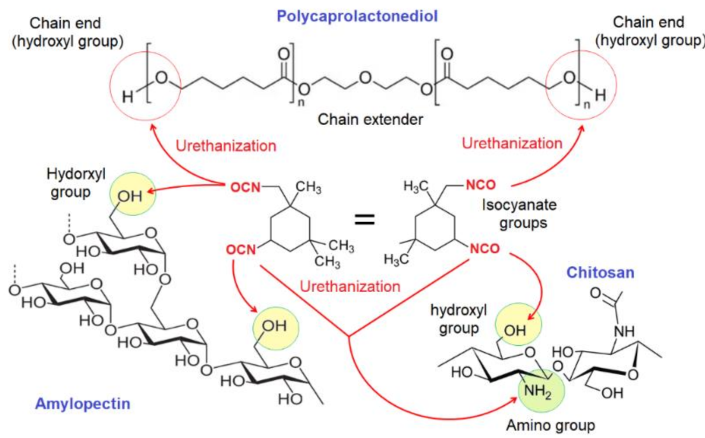

Figure 7. Chain urethanization-based by-polycondensation crosslinking.

Starch has been chemically modified with dithiocarbamate, citric acid, sodium trimetaphosphate, and sodium hypochlorite for adsorbent applications, particularly heavy metal adsorption (Garces et al., 2017; Kim et al., 2024). In general, one of the aspects that makes amylopectin relevant is the fact that it is harmless (i.e., non-toxic), biocompatible, biodegradable, inexpensive, and its inherent condition as an edible material. This makes it an attractive biopolymer for the manufacture of drug delivery systems, food additives, and by modifying its properties, a material with fine structures (Javaid et al., 2023; Palencia et al., 2017).

5. Conclusions and final remarks

Starch is the main reservoir of chemical energy in plants. Depending on their biological nature, they may have organs that accumulate starch to a greater or lesser degree. Among the main starch-accumulating plants are tuberous plants and cereals. Being part of the human diet, we are all familiar with starch to some extent. However, starch has great complexity when looked at in detail. It is not a pure substance, but rather a mixture of two structurally different biopolymers, even though they are made up of the same repeating unit.

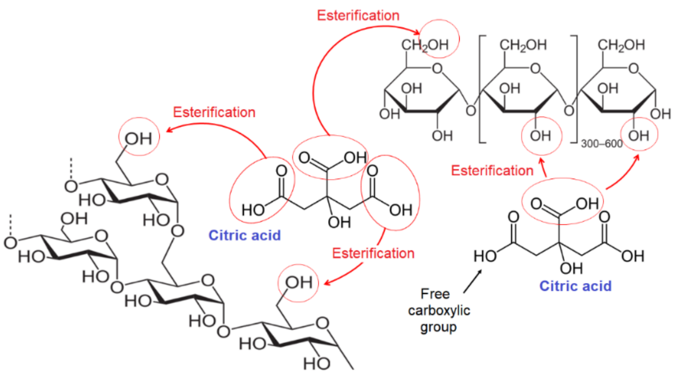

Figure 8. Chain esterification-based by-polycondensation crosslinking.

Given their biopolymeric characteristics, these polymers differ in their properties as shown throughout the article, but it is clear that many of these differences can be understood and modeled from polymer chemistry. From a chemical point of view, starches are essentially hydrophilic and polyhydroxylated molecules, with different architectures and a single stereospecificity. This latter aspect is evident from the stereochemistry of the D-glucose molecule. Its polyhydroxylated nature, in addition to its peculiarities as a biomaterial, makes it ideal for many applications, as well as being susceptible to modifying its properties through the different reactions of its hydroxyl groups.

On the other hand, if starch is looked at not as a whole, but is fractionated in such a way that we focus our interest on its components, i.e., amylose and amylopectin, the growing interest in these is undoubtedly increasing. Therefore, papers that address the chemistry and properties of individual starch components are highly desired.

It is important, in this context, to keep in mind that the similarity of polymers in terms of their repeating unit does not imply the similarity of their behavior in the solid state, solution, or even their chemistry. Therefore, it is important to show that the identification of new sources of starch, extraction, characterization, modification, and combination is a goal to which one must strive.

As a final comment, it is clear from the information shown here that a systematic review of starch and its components should be undertaken. Only in the last 10 years, more than 30,000 documents have been generated in a single database, for the current year, in that same database the documents add up to 3943, and even at the date of writing this document, already there are 45 published articles (using "amylose" and "amylopectin" as search criteria, given that if the search criteria are "starch" the article number in this database dated next year, 2024, is 156 documents). Consequently, two aspects should be highlighted:

(i) the speed of generation of information on starch and its components is growing at such a dizzying rate that a review focused only on advances in this topic is necessary with a relatively high frequency, and

(ii) the fact that starch appears in publications since the dawn of the 20th century has produced that much essential information is outdated, forgotten or simply omitted to give way to the application, almost in a standardized way, of analysis techniques.

Conflict interest. The authors declare that there is no conflict of interest.

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