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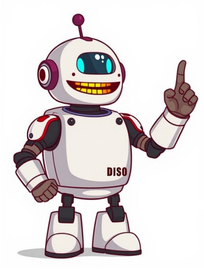

The iodine test is a chemical technique used to differentiate between mono- or disaccharides from specific polysaccharides such as amylose, dextrin, and glycogen. This test has a variant called the starch-iodine test that is performed to identify glucose produced by plants in their leaves. To detect the presence of polysaccharides, primarily starch, The iodine test relies on the fact that polyiodide ions create colored adsorption complexes with helical chains of glucose residue from amylose (blue-black), dextrin (black), or glycogen (reddish-brown). Monosaccharides, disaccharides, and branched polysaccharides like cellulose remain colorless. Amylopectin produces an orange-yellow hue. The reagent utilized in the iodine test is Lugol's iodine, a solution composed of elemental iodine and potassium iodide. Iodine alone is insoluble in water, whereas adding potassium iodide results in a reversible reaction forming triiodide ions, which then react with iodine to form pentaiodide ions. Bench iodine solution appears brown, whereas the iodide, triiodide, and pentaiodide ion are colorless. The structure of glucose chains is crucial to this test. Further, the resulting color depends on the length of the glucose chains. The triiodide and pentaiodide ions formed are linear and slip inside the helix structure. It is believed that the transfer of charge between the helix and polyiodide ions results in changes in the energy levels, which can absorb visible light, giving the complex its color. The intensity of the color decreases with temperature increase and presence of water-miscible organic compounds like ethanol. On heating, the blue color amylose-iodine complex dissociates but is formed again on cooling due to regaining of the helical structure. The blue color reappears on cooling as a result of recovery of iodine binding capacity. 1. Reagent: Lugol's iodine - 5% elemental iodine mixed with 10% potassium iodide Test sample and materials: Take 1 ml of the given sample in a clean, dry test tube. Take control of 1 ml of distilled water in another tube. Add about 2-3 drops of Lugol's solution to both tubes and mix them in a vortex. Observe the appearance of color in the test tubes on heating until the color disappears. Note down the appearance of blue-black or purple color, indicating the presence of starch. If there is no change in color, the result is negative and indicates the absence of starch. This test is used to detect the presence of starch in various samples, similar to identifying glucose production in plants through their leaves. However, this test cannot be performed under acidic conditions as starch hydrolyses under such circumstances. It is a qualitative test that doesn't signify the concentration of starch.The colourless nature of amylopectin and its orange-yellow pigment was studied. The iodine test was performed to determine the presence of starch. ---------------------------------- Lugol's iodine, a 5% elemental iodine and 10% potassium iodide solution, is used in the iodine test. The reagent reacts with iodine ions to produce triiodide and pentaiodide ions, which are colourless. ---------------------------------- A helix shape is crucial for the experiment's success, as it determines the final hue of the resulting ions. The length of glucose chains also affects the colour intensity. # Testing for starch differentiation requires careful consideration of various methods and their limitations. ================================== The solution used to differentiate starch from other polysaccharides involves testing with iodine, which reacts with amylose to form a blue-black complex. This test is primarily used to identify the presence of carbohydrates, particularly starch. The Iodine Test: A Chemical Analysis Tool ================================== Extracting chlorophyll from leaves and observing the change in color to white makes it easier to detect iodine's interaction with starch. This reaction is crucial for determining the presence of carbohydrates in a sample, as starch turns blue upon addition of iodine due to an intermolecular charge transfer component. The iodine test helps identify diseases in rice by diagnosing tungro virus infections. The solution contains iodine and potassium iodide, which are applied to infected leaves in both field and laboratory settings. In the iodine test, starch converts into a blue-black or purple color. References E2%80%93starch_test ==================================The barfoed's test for starch is a qualitative chemical test used to determine the presence or absence of starch in a given sample. In this test, a solution of Lugol's iodine is added to two separate tubes containing the sample and distilled water. The appearance of color in the test tubes indicates the presence of starch. The iodine test is a widely used method to detect the presence of starch in a sample. This test involves the reaction between starch and iodine, resulting in a blue-black colored complex. The objective of this test is to identify the presence of starch in a given sample. STARCH-IODINE TESTThe Iodine Starch Reaction: A Diagnostic Tool for Detecting Carbohydrates ================================== Iodide ions, particularly polyiodides (I_3^-, I_5^-, I_7^-), serve as charge donors and form complexes with Amylose, a component of starch. The benchtop Lugol's iodine solution appears brown due to its molecular composition. However, upon complexation with Amylose, the polyiodide ions exhibit blue-black coloration, which is perceived by the human eye. The Iodine Starch Reaction is a widely employed method for identifying starch in samples, primarily due to its distinctive color change characteristics. The reaction relies on the principle of charge transfer, wherein electrons absorb light energy and transition to higher energy levels. This phenomenon results in the appearance of a blue-black hue, which serves as an indicator of starch presence. To conduct the Iodine Test, several materials are required, including test samples, iodine solution or Lugol's reagent, test tubes, a test tube stand, water bath, and a vortex mixer. The process involves adding the iodine solution to the test samples and control samples, followed by heating until the color dissipates. Observation of the resulting colors allows for the determination of starch presence in the sample. A blue-black color indicates a positive iodine test result, signifying starch detection, whereas a brown color or lack thereof suggests a negative result. The Iodine Test has various applications, including distinguishing between starch and other polysaccharides, glycogen, and carbohydrates. However, it is essential to note that the Iodine Test possesses limitations, primarily due to its qualitative nature. The test cannot accurately estimate starch amounts present in the sample, and acidic conditions may lead to incorrect results.the presence of glucose made by plants in the leaves. To detect the presence of polysaccharides, primarily starch. The iodine test is based on the fact that polyiodide ions form colored adsorption complex with helical chains of glucose residue of amylose (blue-black), dextrin (black), or glycogen (reddish-brown). Monosaccharides, disaccharides, and branched polysaccharides like cellulose remain colorless. Amylopectin produces an orange-yellow hue. The reagent used in the iodine test is Lugol's iodine, which is an aqueous solution of elemental iodine and potassium iodide. Iodine on its own is insoluble in water. Addition of potassium iodide results in a reversible reaction of the iodine ion with iodine to form a triiodide ion, which further reacts with an iodine molecule to form a pentaiodide ion. Bench iodine solution appears brown, whereas, the iodide, triiodide, and pentaiodide ion are colorless. It is observed that the helix (coil or spring) structure of the glucose chain is the key to this test. Further, the resulting color depends on the length of the glucose chains. The triiodide and pentaiodide ions formed are linear and slip inside the helix structure. It is believed that the transfer of charge between the helix and the polyiodide ions results in changes in the spacing of the energy levels, which can absorb visible light, giving the complex its color. The intensity of the color decreases with the increase in temperature and the presence of water-miscible organic compounds like ethanol. On heating, the blue color amylose-iodine complex dissociates but is formed again on cooling because the helical structure is disrupted; thereby amylose loses its iodine binding capacity and the blue color. The blue color reappears on cooling due to the recovery of iodine binding capacity due to regaining of the helical structure. The Test for Starch: A Qualitative Approach ================================== The presence of starch in a solution can be detected through a simple test using Lugol's iodine reagent (IKI). When IKI comes into contact with starch, it yields a blue-black color, indicating a positive result. On the other hand, if there is no change in color, the result is negative, suggesting the absence of starch. This test not only helps detect the presence of starch but also provides insight into its process, particularly photosynthesis in plants. However, this test cannot be performed under acidic conditions as starch hydrolyzes under such circumstances. Moreover, this qualitative test does not provide information on the concentration of starch present. It is a useful tool for distinguishing between starch and glycogen, as Lugol's iodine reagent reacts differently with these two polysaccharides. Starch forms helical coils that can bind iodine atoms, resulting in a more intense blue-black color compared to glycogen. The ratio of amylose to amylopectin also affects the binding capacity, with starch having less branching and thus longer helices. To perform this test, 2-3 drops of Lugol's iodine solution are added to 5 ml of the solution to be tested. A blue-black color indicates a positive result for starch, while a brown-blue color suggests glycogen, and a brown-yellow color signifies no starch presence. Various analytical methods and techniques exist for characterizing complex carbohydrates like glycogen and starch. This article provides an overview of commonly used methods, including isolation, purification, and fragmentation procedures. However, the lack of a single all-encompassing method has led to the use of multiple techniques, each providing valuable information but also resulting in partial loss of specific details. A combination of methods and overlapping approaches is necessary for extensive characterization of glycan samples like starch and glycogen. Key differences in analytical workflows for starch and glycogen are essential due to their similar chemical properties but distinct physical characteristics. By understanding these differences, researchers can tailor their approach to suit the specific requirements of each polysaccharide.polymers amylopectin and amylose glycogen serve as important reserve polysaccharides for storage of carbon and energy many species among Eukaryota Bacteria and Archaea [1,2]. Glucan polymers consist of α-D-glucosyl residues connected via α 1,4 and α 1,6 glycosidic bonds. α 1,4 glucan chains are connected via α 1,6 linkages. While starch and glycogen are chemically identical major differences in their physicochemical properties related to molecular organization of glucan chains within molecules. In starch branching points clustered unlike glycogen resulting in longer linear glucan chains that can form double helices and water excluded. Organization of double helices within amylopectin results mainly two crystalline allomorphs type A and B (see also Figure 1) [3,4,5,6,7]. As general consequence starch and glycogen differ in water solubility. Starch consists branched water insoluble semi-crystalline amylopectin and nearly linear amylose probably interspersed within amorphous regions of amylopectin [8,9,10]. Glycogen mostly watersoluble. Starch shows relative high density approximately 1.5 $g cm^{-3}$ Consequently isolation methods for starch and glycogen also differ (see Section 2).starch storage in various organs and tissues exhibits a heterogeneous nature due to the significant differences between these tissues, making homogenization necessary [16,17,18,19,20,21]. The extraction process often involves chemical or enzymatic treatments to remove proteins, lipids, and other non-starch carbohydrates [19,22,23], while avoiding the generation of artificial glycogen and starch species through partial destruction. Additionally, inactivating endogenous starch enzymes, such as those inhibited by detergents, is recommended to prevent alterations to the glucan structure, necessitating tissue-specific adaptations [24,25,26]. Following homogenization, the starch can be separated from most contaminants using aqueous extraction and centrifugation, often supplemented with filtration via mesh and density gradients like Percoll [24,25,26]. However, it remains unclear whether this treatment also affects granule size selection or loss of structural information at the surface of starch grains [27]. Consequently, data obtained on starch granules should be scrutinized critically. The isolation of glycogen is more complex due to its solubility in water, similar to potential contaminants like proteins and other metabolites [28,29]. In mammalian tissues, such as liver or muscle, trichloroacetic acid (TCA)-based isolation procedures or ultracentrifugation combined with sucrose gradients are commonly employed [30,31]. In contrast, bacterial glycogen can be isolated by sonification or a French press, followed by centrifugation and precipitation of the supernatant with ethanol or its combination with KCl or LiCl [32,33]. In certain cases, glycogen may become insoluble, such as in Lafora disease, affecting subsequent isolation procedures. Similarly, phytoglycogen, found in plants with reduced starch debranching enzymes, exhibits a high degree of similarity to glycogen but has distinct characteristics, necessitating specialized isolation methods [34,35,36,37,38,39]. The determination of starch or glycogen content in specific tissues can be achieved through various enzymatic and non-enzymatic procedures, including iodine staining for visualization [40]. However, this method may yield false positives due to the presence of other glucosyl residues like maltodextrins, emphasizing the need for additional analyses. Commercial kits, such as the Megazyme Total Starch Assay Kit, rely on enzymatic or chemical hydrolysis to produce glucose monomers, which are then converted into NADPH through a series of enzyme-catalyzed reactions [41].The morphology of starch granules can be analyzed using various microscopic techniques. These methods are useful for understanding the structure and characteristics of starch granules in different species and storage organs. ================================== The water insoluble starch granules can be easily observed under a microscope, providing information about their morphology at a specific level. The size of starch granules isolated from different species varies greatly, ranging from below 1 µm to several hundred µm. For example, transitory starch granules are typically small, flat, or discoid in shape. In contrast, starch granules from storage organs like potatoes and maize have varying sizes and shapes. It is worth noting that the term "A-type" and "B-type" starch particles refers to specific types of starch granules found in endosperm, not allomorphs or inner structures. Different techniques such as light microscopy, confocal microscopy, and staining with pseudo-Schiff propidium iodide can provide information about starch morphology. Transmission electron microscopy (TEM) and scanning electron microscopy (SEM) are commonly used to analyze starch granules in situ. TEM allows for the analysis of starch granules inside plastids and cells but is limited by the sectioning process, resulting in two-dimensional data. SEM is useful for analyzing isolated starch granules and can provide information about internal structures through energy dispersive X-ray analysis (EDX) or wavelength dispersive X-ray analysis (WDX).Looking at starch analysis [73,76,77,78,79,80,81,82,83], AFM helps determine the surface properties of starch granules, including modifications. However, because of three-dimensional characteristics and altitude differences in z-axis, only parts of entire granules can be analyzed (Figure 2E–G). Starch granule sizes are also of interest, which can be relatively accurately determined by methods like multisizer [16,84,85,86,87,88,89,90]. The electrical sensing zone method used here measures the increase in electric resistance when particles pass between electrodes. It is unaffected by particle color, shape, composition, or refractive index. Flow cytometric analyses of starch granules have been reported [91,92,93], while microscopic methods allow individual or multiple granules to be described. In contrast, multisizer gives an overview of the size distribution of a population of starch granules, but the determined sizes are approximations. Isolation of starch granules is strictly necessary for multisizer and SEM analyses. Therefore, critically review the isolated procedure, especially considering partial rupture and size-selective isolation. The accuracy is also sensitive to isolation methods. For glycogen molecules, direct determination is possible only after isolation using separation techniques like liquid chromatography and field flow fractionation. Coupling with multi-angle laser light scattering allows for precise determination of molecular weight [30,94,95,96,97]. However, the accuracy is very sensitive to isolation. Nuclear magnetic resonance (NMR) can also be applied for starch analyses. Solid-phase NMR can analyze entire starch granules [90,98,99,100,101], but in most cases, the starch is solubilized or further degraded prior to NMR analyses. The accuracy of structural levels 3 and 4 depends on the isolation method. Different methods exist for solubilization, such as heat treatment which eliminates semi-crystalline nature and results in a disordered state. Analyses of thermal properties allow comparison of starches but will not determine inner starch structure in detail. Additional procedures include enzymatic or chemical treatments with various solvents like DMSO, NaOH, KOH, urea/NaOH, and $ZnCl_2$. The separation of glucans, particularly starch and glycogen, requires the use of specific enzymes. Differentiating between enzyme application for solubilization and structural analysis is crucial due to varying linkages in these molecules. Starch, a complex carbohydrate composed of α1,4 and α1,6 bonds, necessitates the use of enzymes targeting only these linkages. In contrast, glycogen, another polyglucan, also contains these specific bond types, thus relying on the same enzyme application. Regardless of solubilization methods employed, treatment time plays a critical role in achieving partial or total solubilization, enabling subsequent separation and isolation of the two polyglucans: amylopectin and amylose. Common techniques for fractionating starch include exploiting differences in physicochemical properties such as solubility, diffusion, hydrodynamic, and complexing properties due to degree of branching and molecular weight. Storage starches can be dissolved using organic solvents or alkaline solutions, often accompanied by heating, which causes granule swelling and destruction of crystallinity. Heating starch below amylopectin's melting point selectively leaches amylose from the solution, resulting in two distinct polyglucans. Centrifugation separates pelleted amylopectin from leached amylose. The efficiency of this procedure is highly dependent on variables like starch concentration, temperature, heating and cooling rates, and duration. Leaching amylose can be improved by precipitating it with hydrophobic substances or ethanol, allowing for subsequent recovery through precipitation steps. Concanavalin A-based methods have been used to separate amylopectin and amylose, taking advantage of the lectin's affinity for non-reducing glucan ends. This technique has been applied across various starch types. Additionally, size exclusion chromatography (SEC) can be employed to quantify each component within starches and as a preparative method for further analysis. Measuring amylose content in starch samples often relies on iodine binding capabilities, resulting in distinct color formations that enable spectrophotometric recording. By determining the absorbance of iodine bound to both components at specific wavelengths, it is possible to accurately assess amylose concentrations within starches. Amylose Content Calculation Using Calibration Curves and Iodine Titration The measurement of amylose content in starch samples involves the use of calibration curves at defined wavelengths with varying amounts of starch. This method allows for the calculation of amylose contents within the sample. Recording of wavelength spectra or absorbance at specific wavelengths is also applied to analyze maximum absorbance and iodine concentration for different starch samples. Iodine's affinity for amylose can be measured through potentiometric or amperometric titration, which helps in determining the amylopectin and amylose contents separately. However, complexing of amylose with lipids affects both iodine binding capacity and butanol precipitation procedures. Therefore, pre-treatment of unfractionated starch samples with alcohols has become a standard practice for estimating apparent amylose contents using iodometric methods. It's essential to note that some starches containing amylopectin with long glucan chains can lead to overestimation of amylose content due to higher iodine binding values. To overcome this, fractionation of starch and separate measurements of its components are necessary to consider the impact of amylopectin. The use of specific fragmenting polymers in oligoglucans prior to further separations is essential for obtaining intra-molecular structural information. Starch and glycogen contain the same inter-glycosidic linkages, α 1,4 and α 1,6, making enzymes like amylases suitable for their structural analysis. α-Amylases are endo-hydrolytic enzymes that cleave inner α 1,4 glycosidic linkages, releasing maltose or branched oligosaccharides. These enzymes are widespread across biological classes and have different product specificities. β-Amylases also cleave α 1,4 glycosidic linkages but use an exo-hydrolase mechanism, releasing β-maltose from the non-reducing end of a glucan chain. γ-Amylases or amyloglucosidases are another group of enzymes that cleave α 1,4 glycosidic linkages, releasing β-glucose. Isoamylases, which cleave α 1,6 glycosidic linkages, release linear chains from both glycogen and starch. Bacterial amylases have high temperature stability and optimal activity around 100 °C. Glycogen-debranching enzymes can also cleave α 1,6 glycosidic linkages, but their use is limited due to the resulting product being difficult to interpret. Enzymes like α-amylases are widely used for structural analysis of starch and glycogen, while glycogen-debranching enzymes are less favorable. However, they can be used for total degradation of polyglucans to glucose, as performed in determining their content.The analysis of starch structure and properties has become increasingly important in understanding its role in various biological processes. To achieve this, several enzymatic treatment approaches have been developed. glucans are mostly detected by fluorescence (laser induced fluorescence; LIF), and thus, coupling of the carbohydrates via the reducing end is necessary with a fluorescent dye (e.g., APTS, ANTS). In contrast, no derivatization of the glucans is needed following separation of the glucans by HPAEC; instead, amperometric detection, especially pulsed amperometric detection (PAD) is common. A typical CLD profile of debranched transitory starch from Arabidopsis thaliana displays a polymodal distribution that is diminished in mutant plants lacking the major soluble starch synthase isoform (Figure 3) [51,225]. Absolute quantification of glucan chains separated and detected by HPAEC-PAD is limited as suitable standards are missing and sensitivity of PAD signal is decreasing with increasing glucan chain lengths. However, amperometric detection is fast and cost-effective and gives reliable information about different kinds of wild type and mutant starches [51,187,225,226,227,228,229,230]. The role of starch synthase isoforms [21,239,241,242,243,262,263,264], starch branching enzyme isoforms [21,239,241,242,243,262,264,265,266,267,268], and other proteins not directly involved in starch metabolism was reported [21,239,241,245]. So far, there's no clear answer to whether some of these integrated or bound proteins are also important for the overall structural properties of the starch granules. In addition to studying the catalytic actions of enzymes on the starch granule surface and its resulting glucan products (see Section 6), analyzing the binding of proteins or enzymes to native starch granules can provide more information about the starch structural characteristics in vitro. These experiments can also be extended by pre-treating the starch via hydrolytic digestion by enzymes [66]. Moreover, protein-carbohydrate analysis with soluble polyglucans (e.g., solubilized starches and starch fractions) using typical methods like NMR, isothermal titration calorimetry, fluorescence spectroscopy, surface plasmon resonance, micro scale thermophoresis, and biolayer interferometry can be applied. In living organisms, the binding of proteins to starch granules in vivo can be analyzed by using transgenic plants expressing proteins labelled with a fluorescent group [261,269,270,271]. Fluorescence microscopy allows determining the distribution of proteins within plastids and at the starch granule (surface). A clustered distribution can easily be distinguished from a randomly even allocation of binding protein. Furthermore, combining several differently labelled proteins enables monitoring co-localization. However, due to overexpression of these proteins in plants, artefacts must be considered. Over past few decades, various carbohydrate analysis techniques have been established and the starch and glycogen fields benefitted. More detailed information about starch and glycogen has been obtained. Phosphorylation of glycogen and starch was identified as a covalent modification, and mutants with different starch morphologies were reported [262]. However, many open questions remain, such as molecular order of phosphorylation event, position of phosphorylation residue in glucan chain, distance between phosphorylation events, and physical or chemical background. The order at surface of polyglucans is also far from being resolved. More results point to specific characteristics influencing protein interaction and surface changes critical for biological function. Thus, the surface is focus of research as most interactions with proteins occur here. This includes synthesis and degradation of new glycosidic linkages. Furthermore, enzymes' and proteins' actions during synthesis at surface presuppose created inner structure. Inner structure also presupposes available surface for enzyme and protein action during synthesis and degradation. ================================== The analysis of starch and glycogen structures has been limited, with only a single giant starch granule from an orchid being studied. However, analyzing characteristics such as size, shape, and resulting properties can provide more accurate results for large-scale applications, particularly for industrial uses. The need for detailed understanding varies among different scientific and industrial sectors. Further research into individual molecular structures will also be beneficial for industrial applications and computational modeling. We thank Martin Steup for the initial idea, Daniel Schäfer for providing AFM pictures, and Julia Compart for her assistance during manuscript preparation. All authors contributed to this review and have read and agreed on the published version. This research was funded by Deutsche Forschungsgemeinschaft (DFG) through grants FE1030/5-1 and FE1030/6-1. Slawomir Orzechowski's work was supported by SGGW grant number S00125/2020. The authors declare no conflict of interest. The novel enzyme responsible for starch metabolism in Arabidopsis leaves, known as Phosphoglucan, Water Dikinase, has been identified. This discovery sheds new light on the complex processes involved in starch synthesis and degradation in plants. ================================== Phosphoglucan, Water Dikinase is a crucial enzyme in starch metabolism that plays a significant role in regulating the activity of other enzymes involved in starch biosynthesis and degradation. The study on this novel enzyme has provided valuable insights into its functions and implications for plant growth and development. Phosphoglucan, Water Dikinase is essential for carbohydrate metabolism, serving as a key regulator for the synthesis and degradation of starch in plants. This discovery highlights the importance of enzymes in controlling various cellular processes. The identification of Phosphoglucan, Water Dikinase as a vital enzyme in starch metabolism has significant implications for plant breeding and agricultural practices.Structural analyses of purified glycogen particles from rat liver, human skeletal muscle and commercial preparations. Int. J. Biol. Macromol. 2009;45:478–482. doi: 10.1016/j.ijbiomac.2009.08.006. The structure of glycogen is a critical aspect of its function in energy storage and metabolism. This article presents structural analyses of purified glycogen particles from rat liver, human skeletal muscle, and commercial preparations, providing insights into the complex architecture of glycogen molecules. ================================== The interactions between starch synthase III and isoamylase-type starch-debranching enzyme in maize endosperm are intricate. These enzymes play a crucial role in the production of starch, which is essential for plant growth and development. Recently, research has focused on understanding the functional relationships between these two enzymes. The study published in Plant Physiol in 2012 found that starch synthase III and isoamylase-type starch-debranching enzyme have a unique interaction. This interaction is critical for the production of starch granules, which are the storage form of starch in plants. Another study published in Plant Physiol in 2015 revealed significant differences in the functions of glucan branching enzymes from plants and bacteria. The researchers used molecular genetic analysis to compare these enzymes in Arabidopsis thaliana. They found that the enzymes have distinct functions and capacities for starch granule formation. Previous research has also explored the morphology of starch granules, which is essential for understanding starch structure and function. A study published in Starch-Stärke in 1994 described the anthological characterization of starch granule morphology using scanning electron microscopy. The researchers found that starch granule shape and size vary depending on plant species. In addition, research has investigated the composition and molecular structure of tuber and root starches. A review article published in Carbohydrate Polymers in 2001 discussed the physicochemical properties of these starches. The authors highlighted the importance of understanding starch composition for food processing and human nutrition. Other studies have examined the characteristics of starch grains isolated from mature pepper leaves grown under different irradiances and daylengths. A study published in Journal of Experimental Botany in 1989 found that the shape and size of starch grains vary depending on environmental conditions. Recent research has focused on understanding molecular structural differences between maize leaf and endosperm starches. A study published in Carbohydrate Polymers in 2017 compared the molecular structures of these starches using advanced techniques such as atomic force microscopy. The researchers found significant differences in the molecular structure of maize leaf and endosperm starches. Overall, research on starch synthesis, structure, and function is ongoing to improve our understanding of plant metabolism and development. Further studies are needed to uncover the intricacies of starch interactions and their impact on plant growth and human nutrition. ================================== Starch granule morphology transformations in rice endosperm are a result of polyhedral to spherical transitions. This phenomenon has been studied extensively in various plant species, including faba beans and black beans. Research has shown that starch granule morphologies can be influenced by factors such as granule size, shape, and surface properties. Recent studies have employed advanced microscopy techniques, including confocal scanning laser microscopy (CSLM) and transmission electron microscopy (TEM), to investigate the morphology and structure of starch granules in detail. These studies have revealed that the deposition of transgenic modified starch in the starch granule can be visualized using CSLM. The composition and physicochemical properties of different types of starch, including faba bean, black bean, and rice bean starches, have also been investigated. These studies have demonstrated that the morphology and structure of starch granules are closely related to their composition and physicochemical properties. Furthermore, researchers have developed various methods for preparing whole sections of starchy seeds, allowing for the investigation of starch morphology and distribution in different regions of the seed. One such method involves using a simple and rapid technique for preparing whole sections of starchy seeds. The use of confocal laser scanning microscopy (CLSM) has also been explored as a tool for visualizing starch granule morphologies. This technique has enabled researchers to study the morphology and structure of starch granules in detail, including their surface properties and shape. Additionally, studies have investigated the effects of phosphorylation on the surface properties and morphology of starch granules. These studies have demonstrated that phosphorylation can affect the surface properties and morphology of starch granules, potentially impacting their functionality and usage in various applications. Overall, research has shown that starch granule morphology transformations are an important area of study, with significant implications for our understanding of starch structure, composition, and function. Further investigation into these topics is likely to provide valuable insights into the development of novel starch-based materials and products. ================================== Amylase digestion of maize starch granules, a complex process, has been studied extensively. Researchers have utilized various techniques, including atomic force microscopy (AFM), to investigate the molecular, mesoscopic, and microscopic structure evolution during this process. Studies on starch granule structure have also employed AFM to examine surface morphological features. Techniques such as iodine absorption and expression of starch synthase II genes in potato plants have been explored to modulate scale parameters and volume-size distributions of starch granules. The role of α-amylase in binding interactions with starch granules has been investigated, revealing the influence of supramolecular structure and surface area on these interactions. Furthermore, physico-chemical properties of potato starches have been assessed, providing valuable insights into their characteristics. Flow cytometry has also been employed to assess chloroplastic starch granules, offering a quick method for analysis. Additionally, researchers have investigated the accumulation of multiple-repeat starch-binding domains in potato plants, highlighting the complexity of starch granule structure and function. Overall, the study of starch granule structure and function continues to be an active area of research, with various techniques and approaches being employed to gain a deeper understanding of this complex system.sedimentation field–flow fractionation of starches and other polysaccharides, which is described in numerous scientific articles. ================================== Starches and glycogen are two types of polysaccharides that play important roles in the body's energy storage and metabolism. However, their structures and properties can vary significantly depending on their sources and processing methods. Several studies have utilized sedimentation field-flow fractionation (SFF), a type of analytical ultracentrifugation, to characterize the size and branching distributions of starches and glycogen. The SFF technique allows for the separation and analysis of particles based on their size and density. For example, one study used SFF coupled with multiangle laser light scattering to analyze the branching features of amylopectins and glycogen (Roland-Sabaté et al., 2007). Another study employed off-line two-dimensional size-exclusion chromatography and enzymatic treatment to determine the multidimensional size/branch-length distributions of branched glucose polymers (Vilaplana & Gilbert, 2011). Glycogen is a complex carbohydrate that serves as an important energy storage molecule in animals. The SFF technique has been used to investigate the size, structure, and scaling relationships in glycogen from various sources (Fernandez et al., 2011). Additionally, researchers have employed 1H NMR spectroscopy to assess the extent of starch dissolution in dimethyl sulfoxide (Schmitz et al., 2009). Starches, on the other hand, are composed of two main components: amylose and amylopectin. The SFF technique has been used to characterize the size-separation characterization of starch and glycogen for biosynthesis-structure-property relationships (Gilbert, 2011). Furthermore, researchers have utilized solid-state NMR spectroscopy to study the structure of starch granules (Morgan et al., 1995). In recent years, advances in analytical techniques such as SFF have enabled the detailed characterization of starches and glycogen. These studies have contributed significantly to our understanding of the properties and behaviors of these complex carbohydrates. References: Gilbert R.G. (2011). Size-separation characterization of starch and glycogen for biosynthesis–structure–property relationships. Anal. Bioanal. Chem., 399(14), 1425-1438. Fernandez C., Rojas C.C., Nilsson L. (2011). Size, structure and scaling relationships in glycogen from various sources investigated with asymmetrical flow field-flow fractionation and 1H NMR. Int. J. Biol. Macromol., 49(2), 458-465. 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(2002). Pulsed Nuclear Magnetic Resonance (PNMR) Study of Rice Starch Retrogradation. Cereal Chem. J., 79(5), 751-756.Starch ordered structure—A joint FTIR-ATR, NMR, XRD and DSC study. ================================== Th starch ordered structure was characterised through a joint FTIR-ATR, NMR, XRD and DSC study. The isolation of amylose from maize starch has been a topic of interest in recent years, with various methods being developed to achieve this goal. Aqueous leaching is one such method that has been widely used, but its effectiveness can be limited by the presence of large aggregates contaminating the amylose solution. Researchers have sought to develop alternative methods to overcome these challenges and improve the yield of amylose isolation. One approach involves using complexation from aqueous dispersion after pretreatment with methyl sulphoxide. This method has been successfully applied to fractionate maize and amylomaize starches, resulting in a significant increase in amylose content. Another method involves using 1-butanol in the presence of thiocyanate for the partial fractionation of starch. This novel approach offers a rapid and efficient way to isolate amylose, with potential applications in food processing and biotechnology. Furthermore, researchers have explored the use of fluorescent labeling and HPSEC (high-performance size-exclusion chromatography) to simultaneously determine the amylose content and unit chain distribution of amylopectins in cassava starches. This technique provides valuable insights into the molecular structure and properties of these complex carbohydrates. Overall, the development of effective methods for amylose isolation is crucial for various applications in food science, biotechnology, and materials research. The isolated amylose can be used as a model system to study its physical and chemical properties, such as crystal morphology, gelation behavior, and interactions with other carbohydrates. This knowledge can inform the design of new starch-based products, improve food processing techniques, and enable the development of novel biomaterials. Moreover, the isolation of amylose can provide insights into the mechanisms of starch digestion and the role of enzymes in breaking down complex carbohydrates. This understanding can contribute to the development of more efficient starch-converting enzymes and improve our comprehension of human nutrition and disease. In conclusion, the isolation of amylose from maize starch is a critical area of research with numerous applications in food science, biotechnology, and materials science. Ongoing advances in method development and characterization are expected to continue improving our understanding of this complex carbohydrate and its role in various biological and industrial processes.Researchers have developed various methods to separate and analyze the distinct components of starches, such as amylose and amylopectin. One approach involves using concanavalin A to fractionate these components. Carbohydrate Res., a journal, published several studies on this topic, including one in 1988 (doi: 10.1016/0008-6215(88)80087-9). Other researchers have also explored the interaction between concanavalin A and starches, publishing findings in Annals of the New York Academy of Sciences (1974;234:283–296, doi: 10.1111/j.1749-6632.1974.tb53040.x) and Carbohydrate Res. (1985;137:151–166, doi: 10.1016/0008-6215(85)85156-9). The structural properties of potato and cassava starches have also been investigated, with studies published in Food Hydrocolloids (2010;24:307–317, doi: 10.1016/j.foodhyd.2009.10.008). Additionally, researchers have analyzed the molecular structure of various starches, including kiwifruit starch, tomato fruit starch, and quinoa starch, publishing their findings in journals such as Food Chemistry (2017;220:129–136, doi: 10.1016/j.foodchem.2016.09.192) and Carbohydrate Polymers (2017;158:124–132, doi: 10.1016/j.carbpol.2016.12.001). ================================== Knutson C.A. A Simplified Colorimetric Procedure for Determination of Amylose in Maize Starches was published in Cereal Chem. 1986;63:89–92. Kaplan F., Guy C.L. RNA interference of Arabidopsis beta-amylase8 prevents maltose accumulation upon cold shock and increases sensitivity of PSII photochemical efficiency to freezing stress. Plant J. 2005;44:730–743. doi: 10.1111/j.1365-313X.2005.02565.x. [DOI] [PubMed] [Google Scholar]. Konik-Rose C., Thistleton J., Chanvrier H., Tan I., Halley P., Gidley M., Kosar-Hashemi B., Wang H., Larroque O., Ikea J., et al. Effects of starch synthase IIa gene dosage on grain, protein and starch in endosperm of wheat. Tag Theor. Appl. Genet. 2007;115:1053–1065. doi: 10.1007/s00122-007-0631-0. [DOI] [PubMed] [Google Scholar]. Hogg A.C., Gause K., Hofer P., Martin J.M., Graybosch R.A., Hansen L.E., Giroux M.J. Creation of a high-amylose durum wheat through mutagenesis of starch synthase II (SSIIa) - J. Cereal Sci. 2013;57:377–383. doi: 10.1016/j.jcs.2013.01.001. [DOI] [Google Scholar].Phenotypic variation in starch structure resulting from modified proteinâ€"protein interactions occurs due to the absence or alteration of specific starch granule proteins. Such modifications can lead to changes in starch properties, including its composition and digestibility. Regina A et al. have identified multiple isoforms of starch branching enzyme-I in wheat, which play a crucial role in starch synthesis (Kosar-Hashemi B et al.). The study suggests that the lack of the major SBE-I isoform does not significantly affect starch phenotype. In contrast, Wang J et al. have developed a novel colorimetric method for measuring amylose and amylopectin contents in starches (Li Y et al.). This technique provides a reliable means of assessing starch properties. Another study by Shi Y-C et al. has elucidated the molecular structure of low-amylopectin starches, revealing unique characteristics that distinguish them from high-amylopectin starches (Capitani T et al.). The role of starch granule proteins in starch synthesis and degradation is a topic of ongoing research. Larsson B.L et al. have developed an amperometric method for determining sorption of iodine by starch, which can be used to assess starch properties. More recent studies have focused on the characterization of high-amylose maize starches using techniques such as size-exclusion chromatography and differential alcohol precipitation (Torruco-Uco J.G et al.). These methods provide valuable insights into the structure and composition of starches from diverse botanical sources. Overall, the study of starch structure and properties continues to reveal new information about its role in plant biology and human nutrition. ================================== Starch granules are broken down by plastidial beta-amylases that are stimulated by glucan, water dikinase activity. This process involves the phosphorylation of crystalline maltodextrins, which leads to their solubilization. The Laforin-Like Dual-Specificity Phosphatase SEX4 from Arabidopsis hydrolyzes both C6- and C3-phosphate esters introduced by starch-related dikinases, affecting phase transition of Î±-glucans. In the light phase, glucan phosphorylation mediated by α-glucan, water dikinase (GWD) is essential for a functional transitory starch turnover. Starch phosphorylation has been studied extensively, with insights and perspectives provided in various articles. The plastidial glucan, water dikinase (GWD) catalyzes multiple phosphotransfer reactions, while the molecular weight distribution of debranched starch has been measured using size-exclusion chromatography. Characterization of starch by size-exclusion chromatography reveals its limitations imposed by shear scission. Furthermore, the nature of α and β particles in glycogen has been investigated using molecular size distributions. Size-exclusion chromatography is a crucial tool for separating and detecting starch molecules, including native starch. ================================== Starch granule initiation in Arabidopsis requires the presence of either Class IV or Class III starch synthases. ================================== The process of starch granule initiation is a crucial step in plant development, as it sets the stage for subsequent starch synthesis. In Arabidopsis, this process has been shown to require the presence of either Class IV or Class III starch synthases.Proteins from normal maize and mutants affected in starch biosynthesis have been studied to understand the complex mechanisms of starch synthesis and degradation in plants. ================================== The study of starch biosynthesis is crucial for understanding the complex relationships between proteins, enzymes, and starch granule development. Recent advances in proteomics have enabled researchers to analyze the protein composition of starch granules from various maize mutants affected in starch biosynthesis.FLOURY ENDOSPERM6, a protein involved in compound granule formation and starch synthesis in rice endosperm The discovery of FLOURY ENDOSPERM6 has shed light on the complex process of starch synthesis in plants. This protein, which encodes a CBM48 domain-containing protein, is crucial for the formation of compound granules and the regulation of starch synthesis. By analyzing the functions of various starch synthase isoforms, researchers have gained insight into the mechanisms underlying starch biosynthesis. These studies have revealed that localizing GRANULE-BOUND STARCH SYNTHASE to starch granules and ensuring normal amylose synthesis are essential for plant development. Other research has focused on the role of starch-branching enzymes in starch biosynthesis. The discovery of protein-targeting mechanisms, such as PROTEIN TARGETING TO STARCH, has highlighted the importance of regulating enzyme activity and localization in starch granules. In addition to these findings, studies have also explored the composition and structure of starch granules. By analyzing the amyloplast proteome of potato tubers, researchers have gained a better understanding of the complex interactions between enzymes and starch components. Furthermore, research on the starch synthase isoforms has revealed that soluble isoforms are present within starch granules in developing pea embryos. These findings highlight the importance of considering the multiple functions of these enzymes in plant development. The discovery of LIKE SEX4 1, a protein involved in β-amylase binding to starch granules, has also shed light on the mechanisms underlying starch degradation. By understanding how this protein acts as a scaffold for enzyme activity, researchers can develop new strategies for regulating starch metabolism. Overall, these studies demonstrate the complex and multifaceted nature of starch biosynthesis in plants. By continuing to explore the functions of various enzymes and proteins involved in starch synthesis, researchers can gain a deeper understanding of the molecular mechanisms underlying this process.