Methods and Protocols
in Food Science

Springer Protocols

Sneh Punia Bangar *Editor*

Standardized Procedures and Protocols for Starch

Humana Press

Methods and Protocols in Food Science

Series Editor
Anderson S. Sant'Ana
University of Campinas, Campinas, Brazil

For further volumes:
http://www.springer.com/series/16556

Methods and Protocols in Food Science series is devoted to the publication of research protocols and methodologies in all fields of food science.

Volumes and chapters will be organized by field and presented in such way that the readers will be able to reproduce the experiments in a step-by-step style. Each protocol will be characterized by a brief introductory section, followed by a short aims section, in which the precise purpose of the protocol will be clarified.

Standardized Procedures and Protocols for Starch

Edited by

Sneh Punia Bangar

Food, Nutrition and Packaging Sciences, Clemson University, Clemson, SC, USA

Humana Press

Editor
Sneh Punia Bangar
Food, Nutrition and Packaging Sciences
Clemson University
Clemson, SC, USA

ISSN 2662-950X ISSN 2662-9518 (electronic)
Methods and Protocols in Food Science
ISBN 978-1-0716-3865-1 ISBN 978-1-0716-3866-8 (eBook)
https://doi.org/10.1007/978-1-0716-3866-8

This Humana imprint is published by the registered company Springer Science+Business Media, LLC, part of Springer Nature.
The registered company address is: 1 New York Plaza, New York, NY 10004, U.S.A.

Paper in this product is recyclable.

Preface

Starch is a polysaccharide consisting of multiple glucose units and is synthesized in the amyloplasts of plant cells. Corn, wheat, rice, potato, and cassava starches are the most widely used starches in the food industry, serving as thickeners, colloid stabilizers, gelling and volume agents, adhesives, moisture retainers, texturizers, and fat substitutes. Starch plays an important role in the appearance, structure, and quality of food products. Each application of starch in the food or non-food industry requires particular functional characteristics. Native starches have unique physicochemical and functional characteristics. These properties include gelatinization temperature, retrogradation, solubility, swelling power, syneresis, and the rheological behavior of pastes and gels. The structural characteristics, shape, and botanical source of the starch influence these properties. Therefore, it is necessary to understand the specific characteristics of starch, experimentation, and protocols to meet new applications and demands in the industrial sector. This book aims to provide the protocols and methodology for understanding starch for its practical applications. Comprising 11 chapters authored by experts, this book is poised to empower researchers, food technology professionals, individuals in the food industry, and academia with a robust knowledge base on starch.

Chapter 1 highlights the significance of starch characterization and analysis and its impact on different industries. Starch characterization involves the assessment of parameters such as amylose and amylopectin content, granule size, molecular weight distribution, pasting properties, gelatinization behavior, and enzymatic digestibility. Chapter 2 introduces the research methods of starch granule morphology characteristics by combining the important methods of particle morphology characteristics research in recent years and provides a methodological reference for starch research. This chapter discusses the methods commonly used to evaluate the morphological characteristics of starch particles (such as optical microscopy, scanning electrical microscope (SEM), transmission electron microscope (TEM), polarizing microscope (PLM), confocal laser scanning microscopy (CLSM), and atomic force microscopy (AFM)). Chapter 3 focuses on an in-depth analysis of amylose and amylopectin required to understand the functional properties of starch. Characterization of amylose and amylopectin include physicochemical properties, thermal properties, rheological properties, and morphological properties. Advanced characterization techniques include different chromatographic methods, which aid in the assessment of molecular mass and chain-length distribution in both fractions. Chapter 4 discusses standardized the protocols of chromatographic techniques, sample preparation methods for different starch sources, and data interpretation of the results obtained. Chapter 5 discusses the paste properties of starch, as well as future perspectives and challenges to optimize its application in various food products. Chapter 6 examines the range of methods, protocols, and standardization of the rheological characterization of starches, an abundant complex carbohydrate biopolymer found in a variety of plants, including corn, tapioca, wheat, potatoes, peas, and rice. Chapter 7 includes an overview of the diffraction theory of X-rays by crystals, structural characterization of different starches using X-ray diffraction, and sample preparation protocols utilized in X-ray diffraction analysis for various starches. Detailed and well-explained protocol for XRD analysis of starch, along with data interpretation, will be helpful for researcher to prepare their research program regarding the structural characterization of

various starches. In Chap. 8, the authors attempt to organize the starch digestibility methods under the headings of in vitro and in vivo methods. These subheadings cover the theory of the method used, materials required, sample preparation, detailed procedure, observations, inferences, and precautions, if applicable. Chapter 9 provides the reader with a basic understanding of the different physical modification methods and their influence on starch properties. Step-by-step procedures for physical modification methods have been added to simplify the understanding. In recent years, various procedures have been formulated to extract the starch with chemical means. Thus, to provide a complete overlay of chemical modification (hydrolysis, cross-linking, esterification, etherification, oxidation, and grafting, etc.) of starch, Chap. 10 has been designed, which also provides a complete overview of improved functional properties of starches after modification. Further, enzymatic modifications of native starch have been extensively explored as a green technology compared to toxic chemical approaches and energy-intensive physical approaches, resulting in modified starches with altered gelation, viscosity, and solubility characteristics. Chapter 11 provides a comprehensive discussion of protocols and methods of enzymatic modifications of starch.

Clemson, SC, USA *Sneh Punia Bangar*

Contents

Contributors

Bilge Nazli Altay • *College of Engineering Technology, Packaging and Graphic Media Science, Rochester Institute of Technology, Rochester, NY, USA*

Sonika Banyal • *Guru Jambheshwar University of Science & Technology, Hisar, Haryana, India*

Vandana Chaudhary • *College of Dairy Science and Technology, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar, India*

Wenjing Chen • *College of Food Science, Fujian Agriculture and Forestry University, Fuzhou, China; Fujian Provincial Key Laboratory of Quality Science and Processing Technology in Special Starch, Fujian Agriculture and Forestry University, Fuzhou, China*

Jeferson Silva Cunha • *Department of Food Technology (DTA), Federal University of Viçosa (UFV), University Campus, Viçosa, MG, Brazil*

Amanda Pereira da Rocha • *Department of Food Engineering, Federal University of Ceará, Fortaleza, Ceará, Brazil*

Luciana Gama de Mendonça • *Department of Food Engineering, Federal University of Ceará, Fortaleza, Ceará, Brazil*

Aastha Dewan • *Department of Food Technology, Guru Jambheshwar University of Science and Technology, Hisar, India*

Fabio Ribeiro dos Santos • *Department of Food Technology (DTA), Federal University of Viçosa (UFV), University Campus, Viçosa, MG, Brazil*

Mukesh Gaur • *Department of Food Technology, Guru Jhambheshwar University of Science & Technology, Hisar, India*

Rakesh Gehlot • *Centre of Food Science and Technology, CCS HAU, Hisar, Haryana, India*

Zebin Guo • *College of Food Science, Fujian Agriculture and Forestry University, Fuzhou, China; Fujian Provincial Key Laboratory of Quality Science and Processing Technology in Special Starch, Fujian Agriculture and Forestry University, Fuzhou, China*

Ru Jia • *College of Food Science, Fujian Agriculture and Forestry University, Fuzhou, China; Fujian Provincial Key Laboratory of Quality Science and Processing Technology in Special Starch, Fujian Agriculture and Forestry University, Fuzhou, China*

Priyanka Kajla • *Department of Food Technology, Guru Jambheshwar University of Science and Technology, Hisar, India*

Ranjan Kaushik • *Centre of Food Science and Technology, CCS HAU, Hisar, Haryana, India*

Ankit Kumar • *Centre of Food Science and Technology, CCS HAU, Hisar, Haryana, India*

Parveen Kumari • *Department of Food Technology, Guru Jambheshwar University of Science & Technology, Hisar, India*

Christopher L. Lewis • *College of Engineering Technology, Manufacturing and Mechanical Engineering Technology, Rochester Institute of Technology, Rochester, NY, USA*

Lu Liu • *College of Food Science, Fujian Agriculture and Forestry University, Fuzhou, China; Fujian Provincial Key Laboratory of Quality Science and Processing Technology in Special Starch, Fujian Agriculture and Forestry University, Fuzhou, China*

Ankur Luthra • *Department of Food Technology, Guru Jambheshwar University of Science & Technology, Hisar, India*

Mitali Madhumita • *Department of Food Technology, School of Health Sciences and Technology, UPES, Dehradun, Uttarakhand, India*
Palak Mahajan • *Department of Biotechnology Engineering and Food Technology, University Institute of Engineering (UIE), Chandigarh University, Mohali, Punjab, India*
Sujosh Nandi • *Department of Agricultural and Food Engineering, Indian Institute of Technology Kharagpur, Kharagpur, West Bengal, India*
Puja Priyadarshini Nayak • *Department of Agricultural and Food Engineering, Indian Institute of Technology Kharagpur, Kharagpur, West Bengal, India*
Ana Flávia Coelho Pacheco • *Cândido Tostes Dairy Institute, Agricultural Company of Minas Gerais (EPAMIG), Juiz de Fora, MG, Brazil*
Flaviana Coelho Pacheco • *Department of Food Technology (DTA), Federal University of Viçosa (UFV), University Campus, Viçosa, MG, Brazil*
Paulo Henrique Costa Paiva • *Cândido Tostes Dairy Institute, Agricultural Company of Minas Gerais (EPAMIG), Juiz de Fora, MG, Brazil*
Rekha Phogat • *Centre of Food Science and Technology, CCS HAU, Hisar, Haryana, India*
Neha Rani • *Centre of Food Science and Technology, CCS HAU, Hisar, Haryana, India*
Prerna Sethi • *Guru Jambheshwar University of Science & Technology, Hisar, Haryana, India*
Kalyani Sharma • *Department of Biotechnology Engineering and Food Technology, University Institute of Engineering (UIE), Chandigarh University, Mohali, Punjab, India*
Shobhit • *Department of Food Technology, Guru Jambheshwar University of Science and Technology, Hisar, India*
Sangeeta Yadav • *Department of Food Technology, Guru Jhambheshwar University of Science & Technology, Hisar, India*
Rafael Audino Zambelli • *Department of Food Engineering, Federal University of Ceará, Fortaleza, Ceará, Brazil*
Baodong Zheng • *College of Food Science, Fujian Agriculture and Forestry University, Fuzhou, China; Fujian Provincial Key Laboratory of Quality Science and Processing Technology in Special Starch, Fujian Agriculture and Forestry University, Fuzhou, China*

Chapter 1

Importance of Starch Characterization

Rafael Audino Zambelli, Amanda Pereira da Rocha, and Luciana Gama de Mendonça

Abstract

Starch, a complex carbohydrate widely found in plants, is crucial in numerous industries such as food, pharmaceuticals, textiles, and paper manufacturing. The characterization and analysis of starch are of utmost importance to gain insights into its structural and functional properties, enabling its optimal utilization in various applications. This abstract highlights the significance of starch characterization and analysis and its impact on different industries. Starch characterization involves the assessment of parameters such as amylose and amylopectin content, granule size, molecular weight distribution, pasting properties, gelatinization behavior, and enzymatic digestibility. These analyses provide valuable information about starch functionality and its behavior under different processing conditions. Starch characterization is vital for formulating and designing food products with desired texture, stability, and sensory attributes in the food industry. It allows for the selection of appropriate starch varieties and modification methods to achieve specific functional properties, such as thickening, gelling, and stabilization. Starch characterization and analysis are essential for understanding the structural and functional properties of starch and its suitability for various industrial applications. The accurate and comprehensive analysis provides valuable information for optimizing processing parameters, improving product quality, and promoting innovation in different industries. The continued advancement in starch characterization techniques contributes to the development of novel starch-based materials and products with enhanced performance, sustainability, and functionality.

Key words Analysis techniques, Functional properties, Industrial applications, Starch characterization, Structural properties

1 Introduction

Starch, composed of glucose monomers, is a significant component in human diets with diverse applications in the food industry and nonfood-related sectors such as paper, clothing, and construction [1]. Starches are derived from a wide range of plant sources, and this diversity plays a significant role in shaping their physical, physicochemical, and structural characteristics. The properties of starches can vary significantly depending on the specific plant from which they are obtained. Various plant species are utilized

Sneh Punia Bangar (ed.), *Standardized Procedures and Protocols for Starch*, Methods and Protocols in Food Science,
https://doi.org/10.1007/978-1-0716-3866-8_1,

for starch production, including corn, wheat, potatoes, rice, tapioca, and cassava, among others. Each of these plants possesses unique qualities that contribute to the distinctive properties of the resulting starch. Its numerous advantages include availability, affordability, ease of manufacturing, low density, high strength-to-weight ratio, inertness to active substances, biodegradability, biocompatibility, nontoxicity, and noninflammatory, making it a desirable choice for various consumer products [2].

The physical and chemical characteristics of starches, such as gelatinization temperature, swelling power, and solubility, are directly influenced by the composition and structure of the starch granules. The properties of these granules, including their size, shape, and arrangement, exhibit variations depending on the plant source [3]. Starch granules can vary in size from a few micrometers to several tens of micrometers, and their shapes can be round, oval, or irregular. These inherent characteristics are crucial in determining how starch behaves when exposed to water, heat, and other components during processing or cooking. Figure 1 shows the starch granules microstructure from different plant species in this context.

Starch granules can exist in two different compositions: simple or compound. The granules are present individually in the simple composition, while in the compound composition, they form

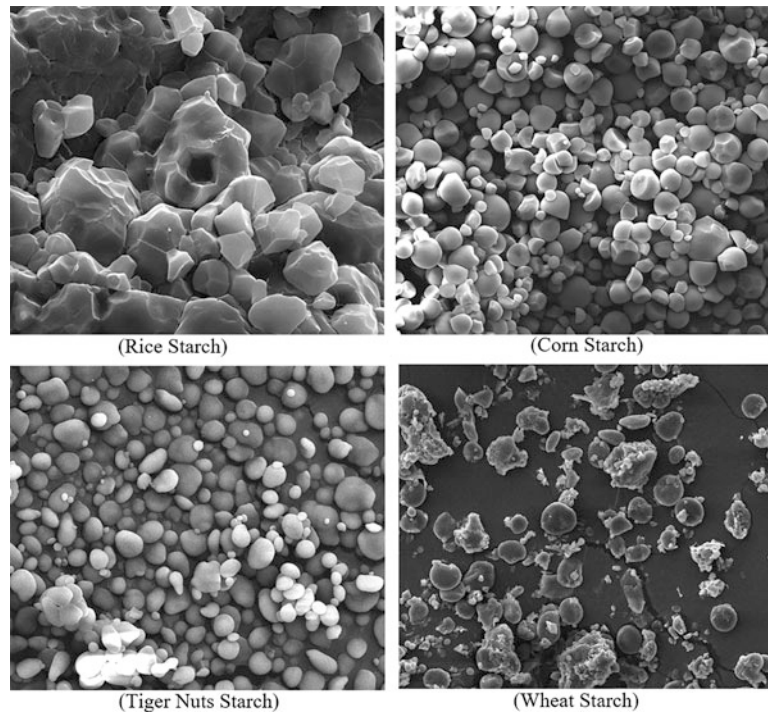

Fig. 1 Starch granules microstructure by scanning electron microscopy (SEM)

clusters. These granules exhibit a distinctive structure characterized by growth rings of amorphous and crystalline domains. Within the granule, a complex network of amylose and amylopectin molecules is responsible for the formation of these growth rings. The amylose and amylopectin are organized in alternating concentric shell-like structures, giving the granule its unique appearance. These shell-like structures have a thickness ranging from 120 to 400 nm [4].

The composition and organization of these growth rings influence the functional properties of starch granules. The crystalline regions provide the granules with structural integrity and play a role in determining their gelatinization temperature. The amorphous regions, on the other hand, contribute to the granules' solubility and swelling properties [5]. Starch has garnered significant attention in research and applications due to its exceptional film-forming ability, favorable biocompatibility, wide availability from diverse sources, low cost, renewable nature, and biodegradability. For several decades, starch has found applications in many industries, both in the food and nonfood sectors [6].

Understanding the variations in physical, physicochemical, and structural properties of starches obtained from different plant sources is essential for tailoring their use in various applications. Manufacturers can optimize their performance in food products, pharmaceuticals, textiles, paper, and many other industries by harnessing the unique characteristics of specific starches. Starch characterization is important in various fields, including food science, pharmaceuticals, materials science, and biofuels. Starch, a polysaccharide composed of glucose units, serves as a major energy source for plants and an essential component of our diet [7]. There are several compelling reasons that highlight the significance of starch characterization, which are listed below:

1. *Food science and nutrition:* Starch plays a pivotal role as an essential component in numerous food products, wherein its properties exert a profound influence on the quality, texture, and functional attributes of such foods. The process of starch characterization enables the determination of key factors, including starch composition, granule size, amylose–amylopectin ratio, gelatinization properties, retrogradation tendencies, and digestibility [8].
2. *Pharmaceutical industry:* Starch is widely employed as an excipient in pharmaceutical formulations, assuming various roles as a binder, disintegrant, and filler in tablets, capsules, and other dosage forms. The characterization process facilitates the evaluation of the physicochemical attributes of starch, including particle size, morphology, viscosity, and swelling behavior. These properties significantly impact drug release, formulation stability, and the overall performance of the pharmaceutical product [9].

3. *Materials science:* Starch has emerged as a prominent renewable and biodegradable polymer for the development of bioplastics, films, coatings, and other environmentally sustainable materials. Characterization methodologies offer valuable insights into the structural attributes, thermal behavior, mechanical strength, and barrier properties of starch-based materials [10].
4. *Biofuels and biorefineries:* Starch-based feedstocks, including corn and cassava, are frequently employed to produce biofuels, particularly ethanol. Characterization techniques are vital in assessing crucial parameters such as starch content, enzymatic digestibility, fermentation efficiency, and yield potential [11].
5. *Plant breeding and agriculture:* Starch characterization assumes a critical role within plant breeding programs focused on the development of crop varieties with desired starch properties. The analysis of starch characteristics aids plant breeders in selecting and developing cultivars featuring enhanced yield, improved nutritional composition, favorable processing properties, and increased resistance to diseases or environmental stressors [12].

2 Materials

2.1 Importance of Starch in the Food Industry

Starch is widely used in the food industry for its functional and nutritional properties. Some key contributions of starch in the food industry include the following:

2.1.1 Starch as Thickening

In general, the thickening agents used in the food industry consist of substances that can modify texture and rheological properties to improve sensory quality attributes. These substances act on the water-binding capacity, modify the structure, and change the flow properties [13]. By increasing the viscosity, they contribute to the desired thickness, smoothness, and stability of sauces, dressings, soups, and other liquid-based products. Thickeners can impart a pleasant mouthfeel, preventing unwanted separation or syneresis and ensuring a uniform texture throughout the product [14]; these thickeners are derived from diverse sources, including plants, microorganisms, and animal connective tissues.

In this context, starch is the most used hydrocolloid thickener due to the characteristics of being abundant in nature, cheap, not conferring flavor at low concentrations, and not interfering with color—the potential of potato starch as a thickener and its interaction with other components, such as pectin and starch. Potato starch is able to increase the viscosity in solution without swelling and disintegration of the starch, making it an alternative for use in the food industry, mainly because it is a clean label [15].

The starch extracted from the jackfruit seed was evaluated as a thickening agent in pepper sauce [16]. Starch had a higher amylose content, and its granules were smaller than traditional corn and potato starches. In this way, the jackfruit seed starch obtained greater thermal and mechanical shear resistance during the cooking process, suitable for use as a thickening agent. An increase in viscosity and flow resistance when adding chemically modified starch was verified. Differences in viscosity values and consistency indices (viscosity for non-Newtonian fluids) were small but significant [17].

The influence of time on the viscosity of two types of starch-based thickeners at a temperature of 20 °C was significant. The thickeners were produced with pure corn starch, maltodextrin, and nonionic polysaccharides. Viscosity was evaluated for 17 h at 30-min intervals after shearing. It was found that the thickeners showed a reduction in viscosity in the first 4 h. In addition, the thickener produced from maltodextrin was more stable compared to corn starch. This result may be related to the lower complexity of the structure of maltodextrin since it is derived from the enzymatic action of starch [18].

The application of cassava starch as a thickening agent in yogurt to avoid the phenomenon of syneresis, that is, the expulsion of liquid from the gel phase during product storage, was verified. The starches were added in the proportions of 2.5%, 5.0%, and 7.5% and caused an increase in the consistency of the yogurt, without any sensory damage, in addition to preventing the phenomenon of syneresis during 14 days of refrigerated storage of the yogurt samples [19]. Similar results were obtained in the application of thickening starches in the production of whipped yogurt. Starch inclusion was from 1.05% to 2.35%. The increase in starch concentration in yogurt formulations promoted an increase in viscosity and modulus of elasticity, as the starch granules absorbed a greater amount of water and resulted in thickening. Sensorially, the starches increased the sensory firmness and improved the cuteness of the yogurt. Higher starch inclusion values provided a cohesive texture feel [20].

2.1.2 Starch as a Binding and Gelling Agent

Starches are also recognized for their ability to act as binding and gelling agents in various industrial applications. They exhibit structure and molecular properties that provide excellent binding and gelling capabilities, making them indispensable ingredients in the food industry [13], and can bind ingredients together, providing cohesion and stability to various food formulations, improving the texture, consistency, and appearance of the final product. One of the functions of the starch binding agent can be expressed in baking, as it is used to bind the ingredients of the dough, helping to create a cohesive structure and a defined shape [21]. Its binding

properties also contribute to its functionality in baked goods. In bread-making, for instance, starch can interact with gluten proteins to form a network that traps gases produced during fermentation. This binding action enhances the volume and structure of the bread, resulting in a softer texture and improved crumb quality [22].

Naturally, starch and proteins can interact with each other. Thus, mixed gel systems with different proportions of starch and protein extracted from fava beans were studied to understand the binding capacity between these two polysaccharides. It was found that the affinity for iodine was lower for samples with high protein content. In gel samples with a higher proportion of starch, there was a higher gelling capacity and viscosity. The starch granules were compacted, and the protein structures filled in the empty spaces between the starch granules. Finally, the protein content reduced the viscosity of the gels and provided them with greater softness [23].

The gelled structure formed by starch provides desirable textural attributes to food products. Starch gels can contribute to the smoothness, firmness, and stability of various food formulations. For example, in desserts such as custards, puddings, or pie fillings, starch gels can provide the desired consistency and mouthfeel. In fruit preserves or jams, starch gels can help achieve the desired gel-like texture and enhance the suspension of fruit particles [24]. In meat products such as sausages or burger patties, starch can help bind the meat particles and other ingredients, improving texture, juiciness, and overall product integrity [25]. The addition of pea protein improves the syneresis of starch solutions and stability during storage. This is probably due to the interaction of protein-starch hydrophilic groups and between the starch molecules themselves [26].

Improvement in the stability of starch gels was also identified by providing a mixture of isolated soy protein with different amounts of native corn starch and waxy corn starch [27]. The authors observed that gel strength, water-holding capacity, hydrophobic interactions, thermal stability, and gel strength increased with the inclusion of the protein. Corn starch exhibits excellent binding properties with phenolic compounds, and this behavior improves the physicochemical properties of starch, in addition to adding biological activities to phenolic compounds [28], which may have direct applications in the manufacturing of packaging films, food production, vehicles of biologically active substances, etc. The binding and gelling properties of starch can be further enhanced through modifications such as physical, chemical, or enzymatic treatments. These modifications can alter the molecular structure of starch, resulting in modified starches with specific binding and gelling properties tailored to applications [29].

2.1.3 Starch as Fat Replacement

The increasing consumer demand for low-calorie food products has led to a surge in research and development efforts by food manufacturers. This is driven by the growing awareness of the negative impact of high-energy diets on human health [30]. Starch serves as a promising fat-replacement ingredient in various food applications. Its ability to mimic the texture, mouthfeel, and functionality of fats makes it an attractive option for reducing the fat content in food formulations without compromising sensory attributes [31]. It can be used as a fat replacement in certain food products to reduce the fat content and create a lower calorie alternative and can help provide a similar texture and mouthfeel to that of fat in certain applications. It can contribute to the creamy and smooth texture desired in products such as dressings, sauces, and desserts [32].

One of the primary reasons for using starch as a fat replacement is its ability to contribute to the desired texture and creaminess in food products. Starch can create a perception of richness and smoothness in products such as sauces, dressings, and dairy alternatives. By incorporating starch, manufacturers can achieve a similar mouthfeel and sensory experience to full-fat products [33]. Modified arrowroot starch as a fat substitute in mayonnaise was studied. The starch was modified through octenyl succinic anhydride, citric acid hydrolysis, annealing, heat and moisture treatment, and acetylation. The aim was to produce different starch pastes with 30–50% fat replacement in mayonnaise formulations. Fat-reduced mayonnaise had greater emulsion stability, especially those with starches modified by octenyl succinic anhydride, annealing, and citric acid hydrolysis [34].

The effects of including waxy maize starch as a fat substitute in beef sausage formulations were studied. In general, corn starch promoted an increase in water retention and cooking loss of sausages, in addition to increasing sensory acceptance and reducing the total caloric content, reducing fat by up to 57.4% and 34.9% in the calorific value [35]. Reducing the fat content in bakery product formulations has become an important issue, especially in sugar cookies. In this sense, they developed a hybrid gel formed by mixing canola oil and candelilla oil gel to replace fat in biscuits. It was found that the inclusion of the hybrid gel promoted greater hydration and disorder in the secondary structure of starch and proteins, in addition to obtaining a 50% reduction in fat content [36]. Mungbean starches were modified by substituting functional groups with acetyl succinic as a fat substitute in cakes. It was observed that the treatment with octenyl succinic increased the viscosity of the paste and starch and increased the specific volume of the cakes without any sensory damage [37]. It is worth mentioning that various fat replacement strategies exist, and starch is just one option. Other ingredients and techniques, such as gums, fibers, emulsifiers, and innovative food processing methods, are also utilized to reduce fat content in food products [38].

Fig. 2 Amylose and amylopectin structure

2.2 Composition and Structure of Starch

Starch, a complex polysaccharide composed of two main components, namely, amylose and amylopectin, exhibits a unique structural arrangement. Its multiscale structure is complex because of the spatial alignment of amylose and amylopectin. Figure 2 shows the structures of amylose and amylopectin.

Amylose possesses a linear structure comprising glucopyranose units that are connected by α-D-(1–4) glycosidic bonds. On the other hand, amylopectin exhibits a branched structure in which the linear branches are linked by α-D-(1–4) glycosidic bonds, with periodic connections formed by α-D-(1–6) glycosidic bonds [39]. Amylose exhibits a unique property known as iodine affinity. This property allows amylose to interact with iodine molecules, forming an inclusion complex with a distinct bluish color. The formation of this complex is dependent on the size and structure of amylose. The iodine affinity of amylose can be quantified by measuring the intensity of the bluish color, which provides information about the amount of amylose present and its structural characteristics. Higher iodine affinity indicates a larger proportion of amylose and a more organized and compact structure [40, 41].

This iodine affinity test is commonly used to determine the relative amounts of amylose and amylopectin in starch samples. By measuring the intensity of the bluish color formed during the reaction with iodine, the amylose content can be estimated. Starches with high amylose content will show a more intense blue coloration, while those with high amylopectin content will have a weaker or no color change [42]. In addition to providing insights into starch composition, the iodine affinity test can also be used to study the structural variations of amylose. Different sources of starch and variations in processing conditions can lead to variations in amylose structure, affecting its interaction with iodine and resulting in differences in color intensity [39]. Amylopectin, which accounts for most starch composition, consists of external and internal chains [43]. The external chains, known as A-chains, play

a significant role in the crystalline regions of starch. These A-chains are primarily responsible for the formation of tightly packed crystalline flakes within the starch granule. The crystalline regions contribute to the granule's stability and resistance to enzymatic degradation [44].

The internal chains, referred to as B-chains, form an amorphous structure within the starch granule. These B-chains are mainly present in the amorphous flakes dispersed between the crystalline regions. The amorphous flakes provide flexibility and contribute to the swelling and solubility properties of starch [45]. The differential distribution of the A- and B-chains within the granule affects the overall functionality and behavior of starch. The crystalline flakes formed by the A-chains contribute to the granule's rigidity and influence parameters such as gelatinization temperature and retrogradation. In contrast, the amorphous structure of the B-chains influences properties such as viscosity, pasting behavior, and digestibility [46].

However, it is important to point out that starch composition is variable and depends on several factors, the botanical origin being the main one. It provides different ratios between amylose and amylopectin contents, which directly impacts the physical, physicochemical, and structural properties of starch. For example, the separation between rice amylopectin and amylose occurs at a degree of polymerization (DP) of around 100, with a noticeable transition (commonly observed at DP ~ 100). Amylose is characterized by DP $>$ 100, while amylopectin consists of chains with DP $\leq$ 100 [47].

Although high-amylose maize, barley, and potato are already accessible in the market, the development of high-amylose variants for other major crops such as wheat and rice is a more recent endeavor. These variants are expected to become commercially available in the coming years. Corn starch is known for its relatively high amylopectin content, which imparts a greater viscosity to its gel-like structures than other starches. On the other hand, potato starch contains a higher proportion of amylose, resulting in a more rigid gel network. Table 1 shows the amylose and amylopectin composition for different starch types.

The amylose and amylopectin contents of starch can vary significantly depending on factors such as the specific variety of the crop, the growing conditions it is subjected to, and the methods used for processing. It's important to acknowledge that these values are approximate and can differ between different plant varieties, cultivation practices, and processing techniques. Factors such as soil composition, climate, crop maturity stage at harvest, and post-harvest processing methods can all influence starch composition in terms of its amylose and amylopectin proportions. The amylose and amylopectin ratios in starches significantly influence their functional and physicochemical properties, including

Table 1
Composition of amylose and amylopectin for different types of starch

	Botanical origin of starch	Amylose (%)	Amylopectin (%)	References
Cereals	Wheat	25–28	72–75	[48]
	Corn	25–30	70–75	[49]
	Rice	15–30	70–85	[50]
	Oat	25–29	70–74	[51]
Tubers	Potato	20–25	75–80	[52]
	Tapioca	15–20	80–85	[7]
	Yam	20–25	75–80	[53]
	Cassava	15–25	75–85	[54]
	Tiger nuts	11.5–19.1	80.9–88.5	[55]
	Taro	18.1–22.2	75–85	[56]
Legumes	Faba	30–35	60–70	[57]
	Chickpea	30–40	60–70	[58]
	Lentil	23–32	65–75	[59]
Others	Lotus seeds	35–45	50–60	[60]
	Jackfruit	22.1–38.3	70–80	[61]

gelatinization, paste viscosity, gel stability, and solubility. It is worth noting that the amylose content can vary depending on the extraction method used for obtaining the starch. Starch extracted using water extraction methods tends to have a slightly higher amylose content. This higher amylose content enhances the film-forming ability of the starch, making it suitable for applications where film formation is desirable, such as in the production of edible films or coatings [56, 62].

The affinities of the starch components, namely, amylose and amylopectin, exhibit distinct behaviors when interacting with lipids. Amylose displays a pronounced ability to interact with lipids, forming a single-helix V-type complex [63]. Amylopectin exhibits a limited capacity to interact with lipids. This limitation can be attributed to the complex structure of its short-branched chains and the significant steric effects, which impede sufficient contact between amylopectin and lipids. Starch, being a natural polymer with a high molecular weight, possesses numerous free hydroxyl groups along its molecular chain. These hydroxyl groups facilitate the formation of hydrogen bonds between adjacent starch molecules, resulting in the development of a microcrystalline structure. This complex phenomenon gives rise to alternating regions of crystalline and noncrystalline zones within the starch particles [56].

3 Methods

3.1 Methods and Techniques for Starch Characterization

Starch, being a complex polysaccharide, requires a comprehensive set of methods and techniques to unravel its intricate characteristics. This section provides an overview of the commonly employed approaches for starch characterization, encompassing various analytical tools and methodologies. By delving into imaging techniques, spectroscopic analysis, thermal analysis, and other relevant methods, this chapter aims to provide researchers and scientists with a comprehensive toolkit for investigating the physical, chemical, and structural properties of starch. These characterization techniques offer valuable insights into starch functionality, processing, and its diverse applications in fields such as food science, pharmaceuticals, materials engineering, and more. The multiscale structure of starch granules can be analyzed and characterized using various technologies. These techniques provide valuable insights into the hierarchical organization and properties of starch at different levels [64].

In recent years, advanced imaging techniques have also emerged as powerful tools in the food structure. Techniques such as confocal microscopy, atomic force microscopy (AFM), and magnetic resonance imaging (MRI) allow for the visualization and analysis of microstructure and deformation within food samples. These imaging methods offer valuable insights into the structural changes occurring during processing and storage and their impact on the structure properties of food [65]. At the macroscopic level, imaging techniques such as scanning electron microscopy (SEM) and optical microscopy allow for the visualization of the overall size, shape, and surface morphology of starch granules. SEM provides high-resolution images, revealing details of the granule surface and any structural irregularities. Optical microscopy, on the other hand, enables the examination of granules in a larger field of view, aiding in the assessment of granule distribution and aggregation [66]. Moving to a finer scale, techniques such as transmission electron microscopy (TEM) and atomic force microscopy (AFM) provide detailed information on the internal structure and organization of starch granules. TEM allows for the visualization of the internal substructures, such as lamellae and crystalline regions, within the granule. It also enables the measurement of granule size, shape, and degree of crystallinity. On the other hand, AFM offers high-resolution imaging of the granule surface at the nanoscale, providing information about surface topography and molecular interactions [67, 68].

X-ray diffraction (XRD) is another powerful tool used to study the crystalline structure of starch granules. By analyzing the scattering patterns of X-rays passing through the granules, XRD provides information about the arrangement and packing of starch

molecules within the crystalline regions. This technique can determine parameters such as crystallinity index, crystal size, and unit cell dimensions, contributing to a comprehensive understanding of the granule's structural properties [69].

In addition to imaging and diffraction techniques, spectroscopic methods Fourier-transform infrared spectroscopy (FTIR) and nuclear magnetic resonance (NMR) spectroscopy are employed to study the chemical composition and molecular interactions within starch granules. FTIR provides information about functional groups and chemical bonds present in the granule, aiding in the identification of different starch components. On the other hand, NMR spectroscopy can reveal insights into the molecular dynamics and mobility of starch molecules within the granule [70].

3.2 Characterization Methods

Characterization methods play a crucial role in understanding the properties and behavior of starch. These methods involve the analysis of various physical, chemical, and physicochemical aspects of starch samples, allowing researchers to obtain valuable insights into their structure, morphology, thermal properties, and rheological behavior. This section will discuss some commonly employed physical characterization methods for starch.

3.2.1 Differential Scanning Calorimetry

Differential scanning calorimetry (DSC) is a widely used technique for analyzing the thermal behavior of materials, including starch. It provides valuable insights into the changes in heat flow that occur during heating or cooling processes, allowing researchers to investigate various thermal transitions, such as gelatinization, melting, crystallization, and chemical reactions. By measuring the heat flow associated with these processes, DSC enables the characterization of the thermodynamic properties and stability of starch samples and is a valuable analytical technique that provides both qualitative and quantitative information about the thermal properties of solid materials. By subjecting a sample to controlled temperature changes, DSC allows for the measurement of key parameters such as melting temperature, degradation temperature, glass transition temperature, enthalpy of melting and crystallization, specific heat, latent heat, polymorphism, and material purity [71]. DSC enables the determination of melting temperatures, which indicates the point at which a solid material transitions into a liquid state. It also provides insights into the degradation temperature, which indicates the temperature at which the material starts to break down or undergo chemical decomposition. These temperature parameters are essential in understanding the stability and behavior of materials under different heating conditions [72]. The variation in heat flow is plotted as a function of temperature or time to generate a typical thermogram, as depicted in Fig. 3.

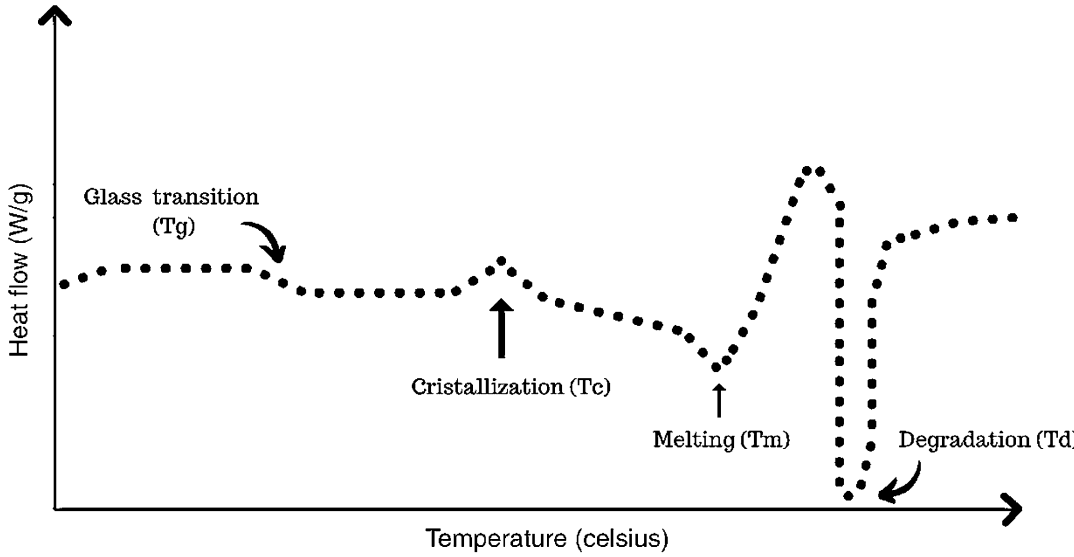

Fig. 3 Schematic representation of thermal transitions in a semicrystalline material observed in a DSC thermogram

This graphical representation provides a visual depiction of the changes in heat flow that occur during the heating or cooling process of a material. By analyzing the thermogram, researchers can gain insights into the thermal behavior, transitions, and energy changes associated with the material under investigation. The thermogram obtained through this method serves as a valuable tool for the interpretation and characterization of the material's thermal properties. It is worth noting that first-order phase transitions manifest as well-defined peaks, while second-order transitions result in variations in the heat flux curve. The initial slope of the curve corresponds to the glass transition, which represents the physical transformation that certain materials undergo when cooled below their glass transition temperature. This transition is reversible in amorphous materials, as they can shift from a rigid, glassy state to a more flexible state. During the glass transition, the molecular mobility of the material increases, leading to significant changes in its physical properties. The glass transition temperature is influenced by various factors, including the presence of plasticizers or additives, molecular weight, and chain flexibility [73].

As the temperature increases during the analysis, the material absorbs energy and undergoes microstructural rearrangement, resulting in an exothermic peak known as the crystallization temperature. This temperature marks the transition from a liquid or amorphous state to a crystalline state. During crystallization, the molecules within the material align themselves into an ordered, crystalline structure. The crystallization temperature is an

important parameter for understanding the behavior and properties of materials, providing insights into the energy requirements and kinetics of the crystallization process.

The material absorbs sufficient energy at higher temperature levels to break intermolecular interactions, causing the molecules to separate. As a result, the material's viscosity decreases and melts at the melting temperature. The melting temperature is the temperature at which a solid material transitions from a crystalline state to a liquid state. At this point, the ordered arrangement of molecules in the crystalline lattice is lost, leading to a loss of structural integrity. The melting temperature is significant in assessing the thermal stability, crystalline structure, and purity of the material [74]. Finally, as more energy is imparted to the material through temperature elevation, processes such as decomposition and oxidative degradation occur, as observed during the Td period. Measuring the degradation period of a material using DSC provides valuable information about its decomposition characteristics and thermal stability. It helps in understanding how the material behaves under elevated temperature conditions and the potential for chemical breakdown or degradation [75]; therefore, it is a technique that can provide diverse information about the different types of native and modified starches.

Native starch, despite its wide availability, faces limitations in meeting the requirements of various engineering applications due to its inherent water sensitivity and subpar mechanical properties. Moreover, the high melting point of starch often surpasses its thermal degradation temperature, further constraining its potential for use in engineering applications [15]. Starch granules exhibit a complex internal structure comprising both crystalline and amorphous components. The amorphous regions lack a regular arrangement, while the crystalline regions consist of ordered chains of glucose molecules. The proportion and organization of these structures within the granules significantly impact the physicochemical properties and thermal behavior of starch [76].

The amorphous/crystalline ratio in starch granules affects their solubility, digestibility, and functional properties. Higher amorphous content generally leads to increased water absorption capacity, swelling power, and improved gel-forming ability. On the other hand, a higher proportion of crystalline regions tends to enhance the granules' resistance to enzymatic degradation and retrogradation [77]. The presence of crystalline regions contributes to the granules' melting and gelatinization temperatures, while the amorphous regions play a role in their glass transition temperature. These thermal properties are crucial for various food processing applications, such as determining the optimal heating conditions for achieving desired textural and sensory attributes in starch-based products [78, 79].

DSC is a widely used technique for investigating the thermal behavior of isolated starches. It allows for the determination of transition temperatures and corresponding enthalpies, providing valuable insights into the properties of starch. Gelatinization has been extensively studied due to its significance in starch processing for food and nonfood applications [80]. During the process of gelatinization, starch granules undergo a transformation when heated in water, resulting in the formation of a thick starch paste. Gelatinization involves the swelling of the granules and the disruption of their molecular structure, allowing them to absorb water and form a viscous paste. This phenomenon is crucial in various food preparation processes, as it contributes to desirable texture and consistency [81]. The gelatinization process of starch is characterized by a nonsharp transition, primarily due to the polydispersity of starch granules. Although gelatinization typically occurs within a temperature range of 60–80 °C, it does not occur abruptly [82]. Several factors influence the starch gelatinization process, including the starch source, amylose content, moisture content, and heating profile. These factors play a significant role in determining the extent and ease of gelatinization. For instance, high-amylose starch tends to have a lower susceptibility to gelatinization than regular starch. Moreover, higher moisture content and elevated heating temperatures typically result in more pronounced starch gelatinization [41, 82, 83].

Apart from the inherent structure of starch, various external factors can significantly influence its properties and interactions with water. These factors include temperature, pressure, water content, and small molecules such as salt, sugar, acids, and other macromolecules like hydrocolloids. These conditions can profoundly impact starch, altering its behavior, functionality, and physical characteristics when exposed to water. Understanding and controlling these external factors are essential for manipulating starch properties and optimizing its performance in different applications [84].

The determination of starch gelatinization is commonly carried out using the differential scanning calorimetry (DSC) technique. In this method, starch is subjected to controlled heating in the presence of water within a sealed container. The temperature at which gelatinization begins (onset), the peak temperature (Tp), the temperature at which gelatinization is completed (conclusion), and the associated enthalpy change (ΔH) is measured and recorded. It is worth noting that the DSC parameters can be influenced by various factors, including the rate at which heating occurs, the ratio of starch to water, and the specific experimental conditions employed. These variables can affect the observed values of To, Tp, Tc, and ΔH, potentially influencing the interpretation of the gelatinization process [85].

This information is vital for understanding the functionality and processing characteristics of starch in food formulations and the development of starch-based materials for nonfood applications. By subjecting starch samples to controlled heating or cooling cycles, DSC measures the heat flow associated with phase transitions, such as gelatinization, melting, and retrogradation. This technique provides valuable information about the temperature ranges at which these transitions occur and the corresponding enthalpy changes [86, 87]. DSC measures the heat flow and enthalpy changes that occur in a sample as a function of temperature or time, providing valuable information about phase transitions, thermal stability, and energy changes associated with the studied material [88].

The sample is typically placed in a hermetically sealed container and subjected to a controlled heating or cooling program. A reference material, which ideally does not undergo any phase transitions in the temperature range of interest, is also included for comparison. As the temperature changes, the DSC instrument measures the heat flow, which consists of a sample and reference thermocouple connected to separate temperature-controlled cells [89]. From the DSC analysis, it is possible to identify the hydrogen bonds of starches because of the elongation of the O–H bond and the vibration of the hydrogen bond and glycosidic bonds in the polysaccharide and is evidenced by peaks in known regions [90].

DSC can be used to measure the presence of exothermic or endothermic changes. In the case of starch, endothermic peaks can be observed, which are associated with its thermal degradation and subsequent carbonization at temperatures close to 300 °C [91]. This technique allows for the identification and characterization of the thermal properties of starch, providing valuable insights into its behavior and stability under different temperature conditions. Studies in the literature have reported modifications in the thermal properties of modified starches, particularly using branching enzymes and transglucosidase. In these cases, the modified starches exhibit lower gelatinization enthalpy than native starches, which can be attributed to the reduced presence of double helices in the modified starches. Consequently, the reduction in the amount of double helices in the starch structure lowers the energy required to disrupt the crystalline structure. These findings highlight the impact of enzymatic modifications on the thermal behavior of starches and provide valuable insights into the relationship between starch structure and its thermal properties [92, 93]. DSC can confirm the formation of complexes of other substances with starch since the dissociation of the complex formed during the heating promoted by the analysis results in one more endothermic peak, which was observed and confirmed by [76, 80].

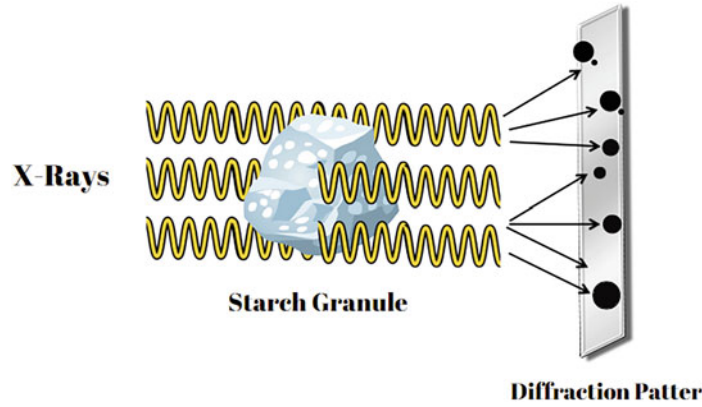

Fig. 4 Operation of X-ray analysis to obtain the diffraction pattern for starch samples

3.2.2 X-Ray Diffraction

X-rays, which are also known as X-ray radiations, are a type of high-energy electromagnetic radiation capable of penetrating various materials. They possess frequencies ranging from 30 × 1015 Hz to 30×10^{18} Hz, energies spanning from 145 eV to 124 keV, and wavelengths falling between 1×10^{-12} m and 1×10^{-9} m. As a result, X-rays have wavelengths shorter than those of ultraviolet rays but longer than those of gamma rays [62]. Figure 4 demonstrates how the X-ray analysis works to obtain the diffraction pattern for starch samples.

Initially, X-rays are propagated toward the starch sample, which, due to part of its structure being crystalline, deviates the X-rays according to the degree of crystallinity, forming a characteristic diffraction pattern for each type of starch. It is a widely used technique for analyzing the structural properties of starch. XRD involves passing X-rays through a sample and measuring the resulting diffraction pattern. This pattern provides information about the arrangement of atoms or molecules within the sample, including the crystalline structure of starch [94].

When applied to starch, XRD can reveal important details about its crystalline regions, as starch granules contain both amorphous and crystalline components. By analyzing the diffraction pattern, XRD can determine the degree of crystallinity, crystal size, and crystalline polymorphs present in the starch sample [95]. Starch typically exhibits a characteristic XRD pattern with distinct diffraction peaks corresponding to its crystalline structure. The positions and intensities of these peaks can be used to identify specific crystalline forms of starch, such as A-type, B-type, V-type, or C-type. The relative intensities of these peaks can provide insights into the organization and packing of starch molecules within the granules [96]. Starch granules display a semi-crystalline structure characterized by alternating crystalline and amorphous lamellae. Starch granules can adopt three crystalline forms

depending on the degree of hydrolysis and the arrangement of amylose double helices. The A-type form produces square-shaped nanocrystals composed of left-handed double helices tightly packed in a monoclinic unit cell. The B-type form generates round nanocrystals with a high amylose content, formed by six double helices in a loosely packed hexagonal unit cell. Lastly, the C-type form consists of a B-type crystalline core structure surrounded by A-type crystalline starch granule crystals [65].

The arrangement of helices and the presence of water molecules between them facilitate the formation of starch crystallites through hydrogen bonding. This phenomenon enables crystal unit assembly, contributing to starch structure development at a long-range scale [40]. XRD analysis is particularly valuable for studying the effects of various factors on the crystalline structure of starch. For example, changes in processing conditions, such as temperature, moisture content, or additives, can alter the degree of crystallinity and the crystalline polymorphs present in starch. XRD can be used to assess these structural changes and understand their impact on the functional properties of starch, such as digestibility, gelatinization behavior, and textural characteristics [97, 98].

This analysis can provide information on crystallinity and pattern of botanical origin; it is a nondestructive and robust analysis. However, it is limited to essentially crystalline materials, is time-consuming, requires large amounts of sample, and may have low diffracted X-ray intensity depending on the material to be analyzed [99]. It also provides valuable information about the crystalline and amorphous regions within starch. Larger peaks in the patterns correspond to the crystalline regions, which are primarily associated with the presence of amylopectin. On the other hand, smaller peaks represent the amorphous regions characterized by amylose [100].

X-ray diffraction provides a means to investigate alterations in the crystalline fraction of starch caused by hydrothermal effects, such as cooking. These effects disrupt the type A polymorphs and induce changes in the molecular-level hydrogen bonding. Consequently, the previously disordered starch chains undergo reassembly into helical structures, leading to the emergence of B-type polymorphs [101]. Therefore, it is widely employed as a primary method for examining the crystalline nature and interatomic distances of specimens. In a particular investigation, native arrowhead starch samples exhibited a crystalline pattern consistent with the A-type structure, characterized by diffraction peaks at reflection angles of 15°, 17°, 18°, and 23° [102].

Starch derived from cereal grains typically exhibits diffraction peaks at diffraction angles of approximately 15°, 17°, 18°, and 23°. These diffraction patterns confirm that the starch possesses the typical characteristics of A-type cereal starch. This finding provides valuable insights into the structural properties of cereal grain starch

and further contributes to our understanding of its composition and behavior [103]. The gelatinization process of starch can cause the double helical structure to break down, resulting in the loss of characteristic peaks associated with starch [104]. XRD analysis revealed that the formation of a starch-polyphenol complex follows a similar pattern to the formation of a starch-lipid complex, forming a V-type lens structure [105]. This observation suggests that the interaction between starch and polyphenols is similar to that between starch and lipids. The V-type lens structure formed in both complexes indicates a specific arrangement of the molecules, which is critical for their functional properties and potential applications [106]. It is possible to visualize the phenomenon of retrogradation of starches through XRD analysis since the starch molecules reassemble to form recrystallization through hydrogen bonds. The formation of crystals at peak $2\theta = 17°$ in potato starch is due to recrystallized amylopectin [107].

3.2.3 Scanning Electron Microscopy

Starch has a unique hierarchical structure that affects its digestion and subsequent nutritional impact. At the microscopic level, starch granules are composed of layers known as lamellae, consisting of alternating amylose and amylopectin regions [108]. SEM is a powerful technique used to examine the microstructure and surface characteristics of starch. It allows for detailed observations of starch granules at high magnification, providing valuable insights into their morphology, size, shape, and surface features [109]. When applied to the starch analysis, SEM can provide the following information:

1. *Granule size and shape:* SEM enables the measurement and characterization of starch granules in size and shape. Starch granules can vary significantly in size, ranging from a few micrometers to several tens of micrometers. SEM images allow researchers to determine the size distribution of starch granules in a sample. The shape of starch granules can also vary, such as spherical, ellipsoidal, oval, or irregular, and SEM can capture these variations [110].
2. *Surface characteristics:* SEM provides a detailed view of the surface features of starch granules. Starch granules can have a smooth or rough surface, and SEM images can reveal the presence of surface irregularities, pores, cracks, or even fine structural details. These surface characteristics can influence the functional properties of starch, such as its water absorption capacity, gelatinization behavior, and interactions with other food components during processing [111].
3. *Structural organization:* SEM allows for the examination of starch granules at a microscale level, providing insights into their internal structure and organization. It can reveal the

presence of lamellae within starch granules, which are composed of alternating amylose and amylopectin regions. The organization and arrangement of these lamellae can affect the digestibility and functional properties of starch [15].

4. *Starch modifications:* SEM can also be used to study starch modifications, such as physical, chemical, or enzymatic treatments. Researchers can assess the impact of various treatments on the granule structure and surface morphology by comparing SEM images of modified starches with unmodified ones. This information is crucial for understanding the effects of modifications on starch functionality, such as improved stability, enhanced thickening, or gelling properties, or altered digestibility [112].

From SEM image analysis, several structural parameters of starch can be defined. For example, in the work developed by [113], corn starch granules showed a polygonal shape in their native state. However, from the process of gelatinization and retrogradation, the starch molecules showed crosslinking to form a dense and uniform network structure. In addition, SEM images allowed an understanding of the interaction between starch and phenolic compounds, where the complexity of the structure of the phenolic compound causes damage to the surface structure of the starch through the appearance of holes and cracks. From these results, it is possible to mold the starch for several applications and understand the interaction phenomena. SEM images provided the identification of slight corrosion and variation of shapes and sizes in the structure of taro starch modified with octenyl succinic anhydride; in addition, ball milling caused the development of smaller starch fragments that presented a rougher surface than native taro starch [114]. Figure 5 shows an example of a micrograph obtained from a scanning electron microscope.

The digestion and breakdown of starch are strongly dependent on its structure [115]. A porous honeycomb structure with a "cell wall" shape in potato starch through scanning electron microscopy was observed [116]. Furthermore, they verified the inclusion of cations and their effect on the microstructure of potato starch. The size and shape of the cavities varied according to the inclusion of cations, promoting marked changes in the potato starch microstructure. The morphology of potato and pea starch granules was visualized through SEM and observed that the potato starch granules were large and round, while the pea starch presented a similar shape to a bean. The identified variation in granule sizes by SEM was consistent with the particle size distribution of the granules [117]. The gelatinization process of potato starch was followed using SEM. After heating the starch solution for 20 min, the gel still has a granular structure, and the borders between the granules can be associated with the borders between the ruptured and intact