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PROSPECÇÃO TECNOLÓGICA DE AMIDOS DE MANDIOCA NATURALMENTE MODIFICADOS

TECHNOLOGICAL PROSPECTION OF NATURALLY MODIFIED CASSAVA STARCHES

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PROSPECÇÃO TECNOLÓGICA E NUTRICIONAL DO AMIDO DE MANDIOCA NATURALMENTE MODIFICADO

Dissertação de Mestrado apresentado em cumprimento parcial às exigências do Programa de Pós-Graduação em Alimentos e Nutrição (PPGAN) da Universidade Federal do Estado do Rio de Janeiro (UNIRIO), para obtenção do grau de Mestre.

Orientadora: Profª: Drª Cristina Yoshie Takeiti

Coorientador: Dr. Carlos Wanderlei Piler de Carvalho

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RESUMO GERAL

O amido de mandioca é uma matéria-prima valorizada para a produção de diversos tipos de amidos (nativos ou modificados) para serem utilizados pela indústria de alimentos e suas propriedades físico-químicas e sua disponibilidade o colocam como um ingrediente interessante e desafiador para a indústria alimentícia. Propriedades do amido de mandioca têm sido muito estudadas tendendo a colocá-lo em patamar superior a outros amidos de raízes e até mesmo de cereais, em especial o milho. Diversos órgãos nacionais e internacionais de pesquisa, investem muitos recursos com o objetivo de entender, identificar e aprimorar características genotípicas que possam indicar a mandioca como um produto de excelência. Sendo assim, esta dissertação está seccionada em dois capítulos: (i) apresentar uma revisão bibliográfica, analisando os dados científicos publicados em bases indexadas sobre o tema entre os anos 2008 - 2023 e (ii) apresentar os resultados de caracterização de amidos naturalmente modificados oriundos do Banco Ativo de Germoplasma da Embrapa (Cruz das Almas/Bahia) e do Centro Internacional de Agricultura Tropical (CIAT/Cali, Colômbia). Os resultados do artigo original demonstraram que as propriedades térmicas e de pasta o tornam apto para aplicação como ingrediente em diversos produtos alimentícios, especialmente os que passam por sucessivos ciclos de congelamento e descongelamento. Estes amidos apresentaram temperatura de pasta (P_T) variando entre 65 – 73 ºC e viscosidade de pico a 95 ºC (PV) oscilando entre 3123 a 5479 cP. De acordo com os resultados obtidos, duas amostras (BGM 7737 e BGM 1403) demonstraram alto potencial de aplicação em alimentos.

Palavras-chave: programa de melhoramento genético, amidos naturalmente modificados, propriedades físico-químicas, ingrediente alimentício.

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PREFÁCIO

O presente trabalho segue as normas da dissertação em formato de artigo definido pelo Programa de Pós-graduação em Alimentos e Nutrição (PPGAN) datado em 14 de Maio de 2019 e foi financiado pelo projeto componente do Sistema Embrapa de Gestão (SEG) denominado "Melhoramento genético de mandioca: desenvolvimento de cultivares para segmentos tradicionais de mercado e amidos naturalmente modificados com base em procedimentos convencionais e biotecnológicos" (Projeto 20.22.01.002.00.00, Chamada 01/2022). Sendo assim, o trabalho de dissertação encontra-se dividido em dois capítulos:

O primeiro capítulo consiste em um artigo de revisão bibliográfica que objetivou analisar dados publicados na literatura científica no período (2008 – 2023) focando em amidos naturalmente e geneticamente modificados de mandioca, subsequentes mudanças em sua estrutura, morfologia, propriedades físicas e digestibilidade, e suas aplicações industriais. O artigo é intitulado "Naturally modified cassava starch: Trends and applications as food ingredients – A review".

O segundo capítulo apresenta um artigo original de dados, intitulado: "Technological and nutritional prospection of different germplasm of cassava starch naturally modified". Neste artigo, o objetivo foi a caracterização e indicação de prospecção tecnológica de dezessete amostras de amido de mandioca oriundos de programas de melhoramento genético. Quinze amostras oriundas do Banco Ativo de Germoplasma (BAG) da Embrapa Mandioca e Fruticultura, localizado em Cruz da Almas (BA) e duas do Centro Internacional de Agricultura Tropical (CIAT), localizado em Cali, na Colômbia. Foram realizadas análises físicas e físico-químicas objetivando a caracterização destes amidos, identificando possíveis diferenças em suas propriedades e sugerir aplicações de uso industrial para produção de alimentos.

INTRODUÇÃO GERAL

Atualmente, a cultura à mandioca tem grande notoriedade na agricultura mundial, destacando-se o Brasil como um dos principais produtores desta cultura. No entanto, apesar de ser um dos principais produtores mundiais, o país ainda perde em competitividade, principalmente para os países asiáticos, que têm expressiva participação no mercado externo e onde a mandioca ganhou notoriedade, inclusive como matéria-prima para biocombustíveis (FELIPE et al., 2010).

Dados da FAOSTAT (2023) colocam o Brasil como quarto maior produtor mundial, contribuindo com cerca de 7,5% da produção mundial de mandioca.

No Brasil, a mandioca tem a característica de ser um produto de subsistência, mas também pode configurar como matéria-prima agroindustrial, pois é produzida em todas as regiões do país. No Norte e Nordeste, esta raíz tuberosa é amplamente utilizada na alimentação humana sendo consumida pós cocção e na alimentação animal *in natura*, além de exercer forte influência sobre a indústria, principalmente a de processamento de farinha. No Centro-Sul, prevalece o destino para a agroindustrialização, para produção da fécula e da sua farinha (FELIPE et al., 2010).

Entretanto, além da produção de farinha a mandioca se destacar pelo seu alto rendimento em amido, mais da metade da produção total de mandioca pode ser destinada a produção de amido (WANG et al., 2022).

O rápido crescimento da demanda pelo amido de mandioca justifica-se por suas características inerentes como baixo potencial alergênico, sabor neutro, altas viscosidade de pasta e de claridade de pasta, boa estabilidade frente ao cisalhamento, congelamento e descongelamento (WANG et al., 2022).

Além disso, um alto potencial de valor pode ser aplicado a este amido quando submetido à modificações de natureza física, química ou enzimática, alterando suas propriedades físico-químicas, colocando o amido de mandioca como um ingrediente desafiador e extremamente interessante para indústria de alimentos (DEMIATE; KOTOVICZ, 2009).

Entretanto, com relação às questões ambientais e de segurança alimentar, métodos físicos de modificação de amidos vêm sendo aperfeiçoados, sendo objeto de estudo e pesquisa (CHEN et al., 2011; MHASHE et al., 2021). Ao mesmo tempo modificações físicas têm sido preteridas frente a técnicas de modificação

química, de mais baixo custo e em consonância com os princípios da química verde, atendendo inclusive à uma demanda de mercado onde o consumidor final, mais consciente escolhe o que irá para sua mesa, dando preferência à produtos cuja lista de ingredientes esteja cada vez menor e com ingredientes na sua forma mais natural possível (SANCHES et al., 2010; CHISENGA et al., 2019; WANG et al., 2022).

Neste contexto, ganha força e visibilidade os programas de melhoramento genético tradicional, não-transgênico como os desenvolvidos pela Empresa Brasileira de Pesquisa Agropecuária (Embrapa), pelo Centro Internacional de Agricultura Tropical (CIAT), entre outros órgãos científicos renomados. Tais programas visam potencializar o uso industrial do amido de mandioca em detrimento ao amido de milho utilizado nos países do Norte Global, conferindo maior valor agregado a mandioca, permitindo o elo entre produtores e a indústria.

Análises com base no germoplasma permitem relacionar características genotípicas com fenotípicas, como propriedades de pasta e de composição química, como maior teor de pró-vitamina A, possibilitando melhora da qualidade nutricional das raízes de mandioca para uso industrial (SANTOS et al., 2022).

Atualmente, o uso de amido de mandioca pela indústria de alimentos é bem diversificado desde sua aplicação como agente espessante em bebidas e sucos vegetais até agente gelificante em produtos refrigerados e congelados, bem como agente retardante da retrogradação em produtos panificados (DEMIATE; KOTOVICZ, 2009).

O presente trabalho tem seu segundo capítulo estruturado como artigo original de dados cujo objetivo foi a caracterização e prospecção de aplicação industrial de dezessete amostras de amido de mandioca, oriundas de programas de melhoramento genético não-trangênicos. Foram realizadas análises físicas e físico-químicas e com base em seus resultados frente ao tratamento estatístico dos dados, seis amostras se destacaram e com base em suas potencialidades foram sinalizadas indicações de aplicações industriais.

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Chapter 1 Naturally modified cassava starch: Trends and applications as food ingredients – A review.

Naturally modified cassava starch: Trends and applications as food ingredients – A review.

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ABSTRACT

Cassava (*Manihot esculenta* Crantz) is an important industrial crop in many tropical and subtropical areas. The cassava root tubers are rich in starch and approximately half of the total root tubers produced are utilized as starch-based products. Furthermore, cassava starch has several applications, especially in the food industry, due to its physicochemical properties related to rheological and thermal quality that result in high-clarity and high-viscosity pastes, while presenting a low gelatinization temperature and low retrogradation trend if compared to cereal starches. The applications of cassava starch in the development of products are guided by the knowledgement of its composition, molecular size, and amylose-amylopectin chain ratio. Nowadays, data on the properties of naturally modified cassava starch are insufficient for effective use in breeding programs, since few studies have used small numbers of genotypes or have often a restricted genetic basis. Most of the previous studies were focused on the physicochemical properties of a limited number of cassava starches or on comparing one or two cassava starches with other botanical sources. This review aims to summarize the literature published in the last fifteen years (2008 – 2023) focusing on the natural and artificial genetic modification of cassava starch and the subsequent changes in its structure, morphology, physical properties, and digestibility.

Keywords: cassava starch, naturally modified starch, physicochemical properties, industrial application

1. INTRODUCTION

Starch is an important agro-based biopolymer used as a raw material in the manufacturing industry for multiple purposes due to widespread availability, versatility, affordability, high energy value, easy of modification, biodegradability and safety that largely contributing to its popularity in food, paper, textile and pharmaceutical industries (ZIA-UD-DIN et al., 2017; MHASKE et al., 2021).

Cassava (*Manihot esculenta* Crantz) is a critical industrial crop in many tropical and subtropical areas. The cassava root tubers are rich in starch and approximately half of the total root tubers produced are utilized as starch-based products (Hsieh et al., 2019; Wang et al., 2022). In 2023, Africa's cassava production has reached more than 60% of the world output, followed by Asia, and South America. Nigeria (20.6% of total cassava production), Thailand (11.2%), Brazil (7.6%), and Indonesia (7.5%) are the main global producers (FAOSTAT, 2023). Although cassava root tubers are primarily consumed as a staple food in Africa, most of them in Asia and South America are processed in order to obtain industrial starches.

Considering corn has been the leading crop for starch production (more than 80% of the world starch production), cassava starch demand is increasing rapidly (over 3% annually) corresponding to approximately 7 % of global starch production (OECD/FAO, 2021). Furthermore, cassava starch has many advantages compared to other crops including higher starch accumulation capacity, year-round availability, low price, resilience to drought and temperature, poor soil factors, pests and diseases, and a relatively simple starch extraction (WANG et al., 2022).

Nowadays, starches are generally rarely used in their native form due to undesirable performance such as low solubility, reduced freeze-thaw stability, low thermal and shear stability, resistance to enzymatic hydrolysis and a high tendency of retrogradation and syneresis. As a consequence, native starches cannot deliver the different demands of distinct industries, and thus chemical and physical modifications are required to create starch suitable for industrial applications. Generally, native starches could be modified using physical, chemical and enzymatic techniques to alter their properties and functionalities (MHASHED et

al., 2021). However, these modifications are often environmentally unfriendly, and may cause additional costs in terms of water and energy footprints. In addition to the growing preference by consumers for natural and unmodified products, there are increasing interest and growing opportunities through conventional breeding and genetic selection and transformation in achieving some of these modifications (SANCHEZ et al., 2010).

Cassava starch has several applications, especially in the food industry, due to physicochemical properties related to thermal quality that result in high-clarity and high-viscosity pastes, while presenting a low gelatinization temperature and low retrogradation tendency if compared to cereal starches (Santos et al., 2021). In fact, the root-tuber starches present lower syneresis and could be considered more stable to freeze-thaw cycles, besides waxy starches produce high viscosity gels and can be used in low concentrations (TAKEITI et al., 2007). Comparisons between native and waxy cassava starches is well documented. Nonetheless, the quality and yield of cassava starch are variable and affected by many factors including starch extraction method, cassava variety growth, and environmental conditions (ZHU et al., 2014; CHISENGA et al., 2019; MHASKE et al., 2021). Thus, it is important to determine the phenotypic diversity of cassava germplasm and your potential to exploit different types of cassava starches (SANCHEZ et al., 2010).

Currently, it is widely believed that cassava's greatest diversity is allocated in Brazilian territory. Cassava's new variations are continually arising spontaneously or with a purpose (COSTA NUNES et al., 2021). Pathways of genetic variability include natural selection or artificial genetic modifications that have been employed to create greater diversity content of cassava starch. By natural selection, a waxy mutant containing low amylose and the absence of granule-bound starch synthase enzyme, which is responsible for amylose biosynthesis in the granules has been reported (HSIEH et al., 2019). Genetically modified cassava genotypes containing low or without amylose content were tested in the field in many countries, showing commercial potential with good root-tuber yield and starch quality, various genetic means were employed to create greater diversity in the amylose content of cassava starch. The amylose content can be suggested as a basis for selecting starches for different applications. The nature of modifications and their influence on starch functional properties and

structure has been studied attending a trend to use green technology to develop novel products in an environmentally friendly way. (ZHU et al., 2014; CHISENGA et al., 2019).

However, databases on the naturally modified cassava starch properties are insufficient for effective use in breeding programs, there are few studies that use a small number of genotypes and have often a restricted genetic basis (SANTOS et al., 2021). Most previous studies focused on the physicochemical properties of a limited number of cassava starches or compare one or two cassava starches with other botanical sources (HE et al., 2020).

In Brazil, the Brazilian Agricultural Research Corporation (Embrapa) is responsible for identifying and catalogue different genotypes of cassava in Germoplasm Active Bank that presents more than 1500 genotypes (CUNHA, L., 2022). In Colombia, Sanches et al. (2009) reported a description of analyses performed in more than 4000 genotypes of starches. In China, He et al. (2020) analysed sixteen cassava germplasms comparing structural characteristics and physicochemical properties. Tappiban et al. (2020) reported analysis of seven different cultivars of cassava starches. A wide diversity in the starch physicochemical properties and molecular fine structures were observed among the seven cultivars. The authors observed positive and negative correlations among parameters of pasting, thermal and retrogradation properties, and the molecular fine structures.

This review aimed to summarize the literature records published in the last fifteen years (2008 – 2023) focusing on the natural and artificial genetic modification of cassava starch and the subsequent changes in its structure, morphology, physical properties, and digestibility. In recent years, several efforts have been made to create a knowledge bases that can promote the processing and use of cassava starch in the food industry around the world, creating new ingredients alternatives and products variants.

2. METHODS FOR CASSAVA STARCH ISOLATION AND YOUR DERIVATES

The most common cassava starch production is by wet milling process. Although wet milling is preferred since it is an easier method of extraction, is an unfriendly environmental option in terms of water footprint. According to Padi; Chiphango (2019) cassava water waste is a major water pollution concern. Starch

extraction using wet milling includes washing and peeling of cassava root tubers, cutting, and rasping, using deionized water addition for starch extraction, filtration, sedimentation/centrifugation, decanting to remove water, drying, milling and packaging (CHIMELA et al., 2011; Zhu et al., 2014; CHISENGA et al., 2019; HE et al., 2020; SANTOS et al., 2021).

On the other hand, glycerol-assisted sedimentation, micro-sieving and differential sedimentation are methods that could be used to separate different types of starches considering different supramolecular structures. However, due to a small range of granule sizes, these methods are rarely applied for cassava starches (ZHANG et al., 2018).

The cassava products are either fermented or unfermented and contribute to the emergent industrial application of cassava. The unfermented by-products include starch and roasted flour. Generally, fresh cassava root tubers can be processed into chips, flour, and bakery products.

The cassava industry generates lignocellulosic residues including cassava stalks from harvesting of the cassava root tubers, rasped pulp, peel termed cassava bagasse and large volumes of wastewater during cassava starch production. The cassava stalks are estimated at 63% of the cassava root tubers mass. Large proportions of cassava stalks are left in the field as waste, whereas, a small proportion, about 10-20% of the total is used as planting materials. The cassava stalks contain 22-39% starch of dry matter that can be recovered for other high-value products as bioproducts or bioenergy (PADI; CHIPHANGO, 2019). The cassava bagasse has 85% moisture content, constituting a major challenge for sustainable storage, handling, and correct disposal (TEIXEIRA et al., 2012). In addition, cassava starch production demands large amounts of water, estimated at approximately 18 m^3 /t, resulting in the generation of wastewater up to 2 m^3/ton of starch. The wastewater contains 0.157 kg/m^3 of starch, which is considered as a loss of the process (CHAVALPARIT; ONGWANDEE, 2009), which contributes to typical organic loadings converting into a major water pollution concern. The high starch contents and high production rates of cassava starch, cassava wastewater and cassava bagasse can motivate biorefinery exploits (Zhu et al., 2015; Zhang et al., 2016), and the co-production of valuable bioproducts and bioenergy from the wastes. The cassava wastes can be converted to biogas,

bioetanol succinic acid and glucose syrup (ZHANG et al., 2016; PADI; CHIPANGO, 2019).

The succinic acid and its derivatives such as tetrahydrofuran, are used extensively in the plastic, polymer, pharmaceutical, surfactants, and detergent industries. The rising demand for glucose syrup is attributed to the growth in the pharmaceutical products and convenience food sectors in which this subproduct serves as a major raw material component. However, the techno-economic feasibility of the combined cassava waste biorefineries for commercial applications has not yet been properly explored (ZHANG et al., 2016).

3. CHEMICAL AND PHYSICAL CHARACTERISTICS OF CASSAVA STARCH

The main characteristics of cassava to drive its industrial exploitation are the dry matter content and cyanogenic potential. The amount of dry matter has a relationship with cassava starch total production whereas amounts over 100 mg/kg of cyanogenic compounds are undesirable (SOUZA et al., 2019).

Typical mature and native root tubers have an average composition ranging from 60 to70% of water, and 30 to 35% of carbohydrate with traces of vitamins and minerals. Mature root tubers presented starch content varying from 15 to 33%. Sanches et al. (2009) found an average of 84.5% of starch content on a dry root-tuber basis. Afoakwa et al. (2012) showed a range from 83.42 to 87.35% of starch content and Costa Nunes et al. (2021) reported an average of 51.75% of starch content in forty genetic accessions of sweet cassava (Table 1). Starch content could reach a maximum value at the end of rainy season. Less mature root tubers will be lower in starch content and higher in water, while overly mature root tubers will have lower recoverable starch content and have a woody texture, making starch processing difficult (BE MILLER; WHISTER, 2009).

The cyanogenic glucosides are present in all parts of the cassava plant, including the edible root tubers. The two major types of cyanogenic glucosides, namely linamarin and lotaustralin are synthesized from valine and isoleucine, respectively. They are found in cassava in an approximate ratio of 20:1. The toxicity of cassava-based products is due to the release of hydrogen cyanide from these cyanogenic glucosides (Afoakwa et al., 2012). In case of plant tissues damage, cyanogenic glucosides are hydrolyzed by linamarase to liberate glucose

and acid-stable acetone cyanohydrin compounds. Several health disorders and diseases have been reported in cassava-eating populations. Consumption of 50 to 100 mg of cyanide has been associated with acute poisoning and has been reported to be lethal for adults. The cyanohydrin is further decomposed spontaneously under neutral and alkaline conditions (pH ≥ 5) or by enzymes to liberate hydrogen cyanide or free cyanide (AFOAKWA et al., 2012). The content of hydrogen cyanide in cassava root tubers depends on variety, harvest time, environmental conditions of growth and agronomic practices. The cyanide content is highly distributed in the peel portion rather than in the starch parenchyma tissue. Based on the cyanide contents in edible roots, cassava could be classified into three classes: (i) low toxic type (sweet cassava), (ii) medium toxic type, and (iii) high toxic (bitter) type. Sweet cassava is preferred for direct consumption and the bitter type is mostly processed to industrial products such as starch and chips (BE MILLER; WHISTER,2009).

Since 2012, Embrapa has been evaluating and selecting cassava cultivars that stand out in performance (Figure 1) due to erect growth habitat and resistance to insects and diseases (SOUZA; LIMA-PRIMO, 2019).

(a) (b) (c)

Source: https://www.bdpa.cnptia.embrapa.br/consulta/busca

Figure 1. Cassava root tubers, stems and plant architectures in the field of (a) BRS Formosa, (b) BRS Kiriris and (c) BRS Mulatinha.

3.1 Chemical composition

The chemical composition is dependent on several factors such as cultivar, geographical location, maturity stage of the plant and environmental conditions. The cassava root tubers are low in protein and lipid contents, except for starch. The shelf-life stability of fresh cassava is limited due to rapid postharvest and quality deterioration, which may occur immediately after harvest (BE MILLER; WHISTER, 2009).

The average composition of moisture, ash, protein, lipid, phosphorous, and fiber contents for cassava starch ranged from 33.14-44.7 %, 0.03–2.71 %, 1.46-2.85 %, 0.74-1.2 %, 0.0029-0.0095 %, and 0.1-1.9 %, respectively. The fatty acids in the starch granules are mostly palmitic, oleic, linoleic, and linolenic acids (ZHU et al., 2014; CHISENGA et al., 2019).

One of the important aims of breeding programs is to improve nutritional quality in cassava, which could be the focus on bio-fortification with beta-

carotene, constituting a possibility to supply carotenoids in the human diet. Furthermore, compared with other starches, such as potato, corn and wheat, cassava contains a much smaller amount of phosphorus in its composition (ZHU, 2014; CHISENGA et al., 2019). High concentrations of phosphorus lead to enhanced water bounding (BE MILLER; WHISTER, 2009). The phosphorus content resulted in a higher swelling power and have a positive correlation in some pasting properties such as peak and breakdown viscosity, but reduced paste stability and is negatively correlated with starch solubility (WANG et al., 2022).

Sanches et al. (2009) studied cassava starches from 3272 landraces and 772 breeding clones and reported that the average of cyanogenic potential was 327 ppm, and considerably higher in the landraces (340 ppm) than improved clones (267 ppm). Afoakwa et al. (2012) studied six varieties of cassava composed of four genetic breeding varieties (*Ampong, Broni bankye, Sika* and *Otuhia*) with two traditional varieties (*Amakuma* and *Bankye itaa*) in terms of nutritional properties and cyanogenic potential. The authors concluded that all samples demonstrated suitable moisture levels (7.48-9.66%), and thus showed the potential for extended shelf life. Moisture is an important parameter in the storage of cassava flour and levels greater than 12% conduct to microbial growth. Falade et al. (2019) compared flour, native and modified cassava starches from three cassava cultivars, using annealing, heat-moisture treatment (HMT), and citric acid crosslinking (CAC) and observed moisture content of the flours and starches varied significantly with cultivar and modification. The reduction in moisture content, particularly, for HMT and CAC starches could be due to the relative higher temperature applied in the treatments, which may have reduced hydrophilic sorption sites for moisture during storage. Furthermore, the percentages of crude protein ranged between 1.17% (*Broni Bankye*) to 3.48% (*Bankye itaa*), and ash reached between 1.71% (*Broni Bankye*) to 2.34% (*Otuhia*). Phosphorus, which was one of the major minerals identified showed the highest value in Amakuma (2.13 mg/100g) and the lowest in Broni Bankye (1.06 mg/100g). The cyanogenic potential ranged from 0.08 to 0.12 mg HCN/kg dry weight and according to FAO/WHO (2023) desirable levels of HCN for cassava flours must be lower than 10 mg/kg and for sweet and bitter cassava lower than 2 mg/kg.

3.2 Physical characteristics

Zhang et al. (2018) investigated the native structure starches of potato and cassava. The differences may be attributed to different cassava agricultural traits, aggregation of small granules, genetics, plant physiology such as the stage of maturity and different physicochemical properties, especially with regard to swelling properties and water solubility index. The birefringence patterns were also measured, and all the starch granules exhibited the typical "Maltese cross" under polarized light indicating the absence of previous heat treatment. The birefringence phenomenon normally exists in starch granules because of the orderly arrangement of the starch molecules, of the crystalline regions and the disorderly arrangement of the starch molecules of the amorphous regions, and the appearance of birefringence indicates that cassava starch presents spherulite form (ZHANG et al., 2018; HE et al., 2020).

In the present study only three articles evaluated the microstructure of cassava starches, other three articles evaluated starch content. Other data as different types of starch depending on the location within the root, amylopectin chain length distribution, resistant starch content, particle distribution and enthalpy could be the subject of new studies.

3.2.1 Morphological structure

Typical mature root tubers can present different shapes as conical, conical-cylindrical, and cylindrical fusiforms (Figure 2a) and different sizes ranging between 3 to 15 centimeters in diameter (Figure 2 b) depending on variety, age, and growth conditions. The colour of periderm could vary from white to dark brown. The cross-section of cassava root tubers shows two major components (i) the peel and (ii) the central pith (Figure 2 c) (BE MILLER; WHISTER, 2009).

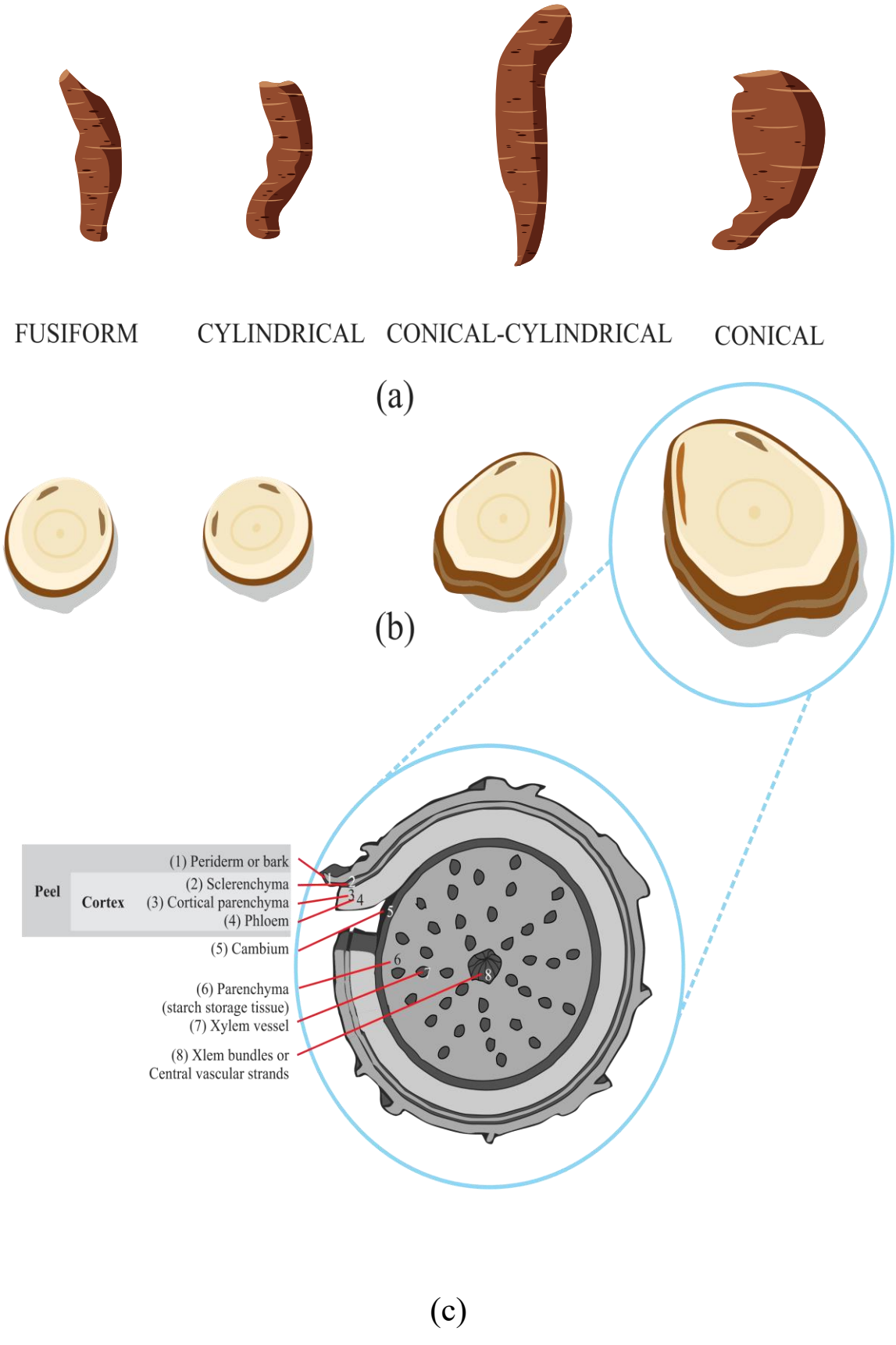

Figure 2. Cassava root tubers and their different shapes: conical, conical-cylindrical, cylindrical, and fusiform (a); cross-section of cassava root tubers (b); drawing of root tubers cross-section containing different structures (c).

The peel comprises the outer layer (denominated as periderm) and the inner layer (cortical region or cortex) that contains sclerenchyma, cortical parenchyma, and phloem tissue. The large central pith of the root tubers is the starch-reserve flesh, comprised of cambium and parenchyma tissue and xylem vessels (CHISENGA et al., 2019).

Starches granules of the same cassava variety could present sizes ranging from 1–100 μm and varied shapes could be spherical, lenticular, polyhedral, and irregular forms (ZHANG et al., 2018).

Chen et al. (2011) studied native and enzymatic breeding varieties of cassava starch and reported an average particle size of 10 μm using scanning electron microscopy (SEM). He et al. (2020) studied sixteen cassava starches using a mixture of water, glycerol, and polarized light. The particle size distribution curve was obtained, and the particle size was defined in terms of the volume-weighted mean diameter D [4,3], 10th percentile [d(0.1)], median [d(0.5)], and 90th percentile [d(0.9)], in which D[4,3] ranged from 13.49 μm to 306.27 μm, and d[0.9] widely ranged from 19.16 μm to 758.42 μm, which was much greater than d[0.5] ranged from 13.66 to 22.45 μm. In this study, d[0.9] was greater than d[0.5], which may be caused by starch not fully dispersing or containing impurities during the test. Falade et al. (2019) observed the flour, native starch and modified starches produced from three pro-vitamin A cassava cultivars, in which there was a wide interval and large variation in mean granule size from 0.24 to 24.18 μm. The authors attributed these findings to the intrinsic genetic differences. Finally, Costa Nunes et al. (2021) studied forty genetic accessions of sweet cassava and observed a particle size ranging from 6.6 to 17.2 μm.

Table 1. Physical and chemical characteristics of different cassava starches data recorded from 2008-2023

Type of starch	Number of accessions	Agricultural traits	Microsctructure	Starch content	Amylose content	Amylopectin chain length distribution	Resistant starch content	Cyanogenic content	Particle distribution	Paste temperature (° C)	ΔH (J/g)	Crystallinity (%)	Reference (year)
Cassava starch	187	3272 landraces and 772 breeding clones	No evaluated	Average of 84.5% (d.b)	Average of 20.7% in landraces and breeding clones	No evaluated		340 ppm in landraces and 267 ppm in breeding clones	No evaluated	Average of 65.3º C	No evaluated	No evaluated	Sanches et al. (2009)
Maize, rice potato and cassava starches	113	Normal and waxy starches from maize, rice, potato and cassava	Oval with surface truncade	Average of 84% (d.b)	16,5 -19,8% Normal cassava starch	No evaluated	No evaluated	No evaluated	No evaluated	Average 67,4 º C waxy cassava starches and 63.3 -64.8 ºC nomal cassava starches	No evaluated	No evaluated	Sanches et al. (2010)
Hydrolyzed and native cassava	148	No evaluated	Truncated form	No evaluated	No evaluated	No evaluated	No evaluated	No evaluated	No evaluated	78.1 ºC (native starch) 67.6 ºC (Hydrolyzed)	11.8 J/g (native) 16.4 J/g (Hydrolyzed)	No evaluated	Chen et al. (2011)
Cassava	109	Four (4) genetic breeding varieties and two (2) traditional varieties	No evaluated	83.42 – 87.35%	No evaluated	No evaluated	No evaluated	0.08-0.12 mg/Kg	No evaluated	No evaluated	No evaluated	No evaluated	Afoakwa et al. (2012)

One hundred (100) F1 hybrid seedlings from a cross between an improved cassava (HB60) and a local cassava cultivar (HN - *Hanatee*)	Not found	No evaluated	No evaluated	No evaluated	No evaluated	No evaluated	No evaluated	No evaluated	Ranged from 6.6 µm -17.2 µm	Location, year Rayong, 2008 HB60 70.65 ºC HN 72.7 ºC F1 Ranged 70.7 – 75.35 ºC Rayong, 2009 HB60 70.85 ºC HN 71.81 ºC F1 Ranged 69.83 – 73.55 ºC Lop Buri, 2009 HB60 69.75 ºC HN 71.5 ºC	No evaluated	No evaluated	Tanyasiriwat, et al. (2013)

										F1 Ranged 68.7 – 72.5 ºC			
Waxy cassava compared with commercial and high amylose cassava starches	59	Five (5) new sources of waxy cassava compared with commercial and high amylose content cassava starch	No evaluated	No evaluated	Average to commercial cassava clones 17.2% (DSC) and 19.9% (IBC) (high amylose content clones) (IBC) 30.2% (DSC) 27.5%	No evaluated	No evaluated	No evaluated	Average 15.9 µm (waxy cassava) compared 14.3 µm (commercial cassava starch)	68.5 ºC (Waxy starch) 64.2 ºC (commercial cassava starch) 64-65 ºC (high amylose cassava starch)	17.4 – 20 J/g (waxy starches) 19.1 -20 J/g (commercial starch)	30-40% (waxy cassava) ~35% for all samples	Morante et al. (2015)
Cassava and potato starch	36	No evaluated	No evaluated	No evaluated	No evaluated	No evaluated	No evaluated	No evaluated	6.3 - 11.7 µm (small-sized cassava starch); 12.3 - 17.8 µm (middle); 15.3 - 22.1 µm (large)	No evaluated	No evaluated	No evaluated	Zhang et al. (2018)
Native and modified starches	32	Produced from three pro-vitamin A cassava cultivars submitted to annealing, heat-moisture treatment, and citric acid crosslinking.	No evaluated	No evaluated	~20% native cassava flour ~30% native cassava starch 29-32% annealed HMT starch ~31 - 33%	No evaluated	No evaluated	No evaluated	0.24 -24.18 µm	75 – 77°C native cassava flour ~75 °C native cassava starch 75 -77 °C Annealed	No evaluated	No evaluated	Falade et al. (2019)

										cassava starch 64 -69 °C Critic Cross-Linking 72 -73 °C HMT			
Cassava starch	25	Seven (7) seven cassava starch	No evaluated	No evaluated	24.8 – 27.6%	3 peaks ranged from 15.9-16.55 DP Indicating short amylopectin chains, ranged from 37.3-37.7 DP indicating long amylopectin chains, ranged from 1295 – 1529 DP indicating amylose chains	Ranged from 0.21 – 1.06%	No evaluated	No evaluated	68,1 ºC – 74,3 º C	11.2 -13,4 J/g	No evaluated	Tappiban et al. (2020)
Cassava Starch	14	Sixteen (16) genetic accessions of cassava	Oval to spherical shapes and bell shapes	No evaluated	No evaluated	No evaluated	No evaluated	No evaluated	2-32 µm	No evaluated	No evaluated	24.6 – 30.8%	He et al. (2020)
Sweet cassava	03	Forty (40) genetic accessions of sweet cassava		51.75%	16.08%				6.6 -17.2 µm	62.6 -70.1 ºC		32.8 - 44.6%	Costa Nunes et al. (2021)

Cassava starch	No evaluated	1031 cassava accessions of the cassava germplam bank, 170 local varieties and 861 breeding varieties	No evaluated	No evaluated	No evaluated	No evaluated	No evaluated	No evaluated	No evaluated	Average from 64.05 – 74.14 ºC	No evaluated	No evaluated	Santos et al. (2021)

3.2.2 Pasting Properties

Pasting and viscosity properties give insights of the rheological behavior of starch materials at controlled temperature and shear (FALADE et al., 2019). Many studies evaluating the relationship between the molecular structure as well as genotypic characteristics of cassava starch and its behaviour in some physicochemical properties suggest that several structural features, such as amylose content, amylopectin chain length distribution and the degree of crystallinity in the granule, are closely related to the gelatinization, retrogradation, granule swelling, amylose and/or amylopectin leaching, granule swelling, loss of radial structure (birrefringence), supra-molecular, molecular order, and recrystallization (DENARDIN; SILVA, 2009).

Sanches et al. (2009) investigated 4.000 landraces of naturally modified cassava starches and observed an average of 65.3ºC for pasting temperature (PT). According to Sanches et al., (2010) the average of PT varied from 63.3 to 64.8ºC for native cassava starches and the average of waxy cassava starches was 67.4ºC (Table 1). Chen et al., (2011) analyzed native and enzymatic-modified cassava starches and reported the transition temperatures in terms of onset (T_0), peak (T_p) and conclusion (T_c) temperatures and the enthalpy (ΔH) of the endotherm. The transition temperatures for native and modified cassava starches were respectively: 68.8° C and 62.5 ºC (T_0), 78,1 ºC and 67.6 ºC (T_p), 87.4 ºC and 73.4 ºC (T_c) and 11.8 e 16.4 J/g (ΔH). The results showed that hydrolysed cassava starches displayed structural surface differences. The authors attributed this finding to the surfaces that were extensively eroded with numerous cracks enabling your application as an adsorbent carrier.

Tanyasiriwat et al. (2013) studied one hundred (100) F1 hybrid seedlings crosses between an breeding cassava (HB60) and a local cassava cultivar (HN - *Hanatee*) (Table 1). The peak viscosity (PV), hot paste viscosity (HPV), cool paste viscosity (final; CPV), pasting temperature (PT), pasting time (PTi) and their derivative parameters, break-down (BD) and setback (SB) were recorded. Parental cultivar HB60 demonstrated mean values of PV and BD higher than the HN sample in all environments (location and year). Furthermore, PTi and PT of HB60 were lower than HN independent of location or year of cultivation. The means of most traits of F1 progeny and their parents evaluated in 2009 showed higher values than those in 2008, except for PTi and PT, and the distribution of

starch pasting properties averaged across the three different environments showed that variations were distributed continuously and normally among F1 progeny. The continuous distribution and transgressive segregations observed in cassava F1 progeny planted at different places and years indicated that traits were quantitative. Morante et al. (2015) studied five new sources of waxy cassava and compared them with commercial and high-amylose content cassava starches. The PT data averages were 68.5º C (waxy starch) and 64.2 º C (commercial cassava starch). Only one sample of cassava starch showed PT similar to commercial cassava starches. This could be related to the larger granule size observed for waxy cassava starches or less crystallinity defects. The PV was higher in waxy cassava starches (1149 cP) than for commercial cassava starches (993 cP). Falade et al. (2019) studied the flour, native starch and modified starches of three pro vitamin A cassava cultivars. Modifications were carried out by annealing, heat-moisture and citric acid crosslinking. The authors observed that native starches had significantly higher PV (6509.5-6564.0 cP) than corresponding flours (1858.5-4648 cP). Annealing significantly increases PV (6288-6876 cP). Citric acid crosslinking severely diminished PV (6509.5 to 88.0- cP) forming starch dimers. Heat-moisture treatment also significantly reduced PV (6509.5 to 5059.5 cP) ($p<0.05$) compared with native starches, possibly due to changes in amorphous-crystalline regions. Tappiban et al. (2020) studied genetic diversity between seven cassava starch samples, and the average for PT ranged from 68.1 ºC to 74.3 ºC. Santos et al. (2021) studied 1031 cassava samples belonging to the Cassava Germplasm Bank (CGB). The average PT value increased from 64.05 ºC to 71.14 ºC, the PV values ranged from 3,279.11 to 6,459.50 cP. For the seven traits analyzed, were observed 13 single-nucleotide polymorphisms (SNPs) located on 4 different chromosomes (3, 8, 17, and 18) and were found a significantly association with the pasting properties of cassava starch. Most of the parameters of RVA (SB, FV, PV, CPV, and HPV) had significant SNPs on chromosome 18, except for PT. The results indicated that these parameters are strongly correlated and possibly under the control of the same gene or even due to closely linked genes. The highest amplitudes were observed for CPV with an average of 2,916.03 cP, and PV with an average of 4,809.67 cP. On the other hand, the amplitude values of SB ranged from 191.01cP to 2,309.42 cP and HPV ranged from 351.41cP to 2,692.87 cP showed moderately high magnitude. Furthermore,

PT ranged from 63.82 ºC to 75.47 ˚C, with an average of 70.05˚C, with low magnitude. They concluded that the variations among cassava roots and their extracted starch qualities are due to genetic and environmental conditions.

3.2.3 Cristallinity and x-ray diffraction patterns (XRD)

Starches are semi-crystalline materials and crystalline types depends on the botanical source of the starch: (i) A-type, which is typically given by cereal starches, (ii) B-type is found in potatoes, amylomaize, retrograded starch, and (iii) C-type contains both A- and B-types crystallite and is found typically in pulses (peas and lentils). The literature values for native cassava starch crystallinity ranged from 15 to 45%, depending on the botanical source. The degree of crystallinity ranged from 24.6 to 30.8% (HE et al., 2020).

Morante et al. (2015) studied five new sources of waxy cassava and observed that all waxy cassava starches exhibit peaks with higher intensity at 2Ɵ 15.02, 16.99, 17.86 19.8, 22.9 demonstrating a mixture of A and B-type crystallites with a majority of A-type (85-95%). They also observed significant statistical differences between the genotypes and 3 different groups, those with higher crystallinity (genotypes 54, 57 and 82) and lower crystallinity (genotypes 9, 46, 21 and 31). The relative crystallinity ranged from 32.8 to 44.6%. He et al. (2020) measured sixteen cassava starch germplasms (50, SC9, SC12, 196, 571, 417, and others) and the results showed peaks at approximately 15°,17°, 18° and 23° that is characteristic of the typical A-type.

As expected, cassava starches from different germplasms showed granule size, relative crystallinity and similar X-ray diffraction patterns with minor differences, indicating that the organization of the semicrystalline structure of the starches was not strongly affected by the cassava germplasm (HE et al., 2020)

4 PHYSICOCHEMICAL PROPERTIES

4.1 Amylose Content

The amylose content is the preferred quality attribute of starch and could determine the diverse properties of starch and eventually the end-use purposes (ZHU et al., 2014). Moreover, the fine structure of starch molecules, molecular size distribution and chain length distribution, are important for understanding starch biosynthesis and genetics structure relations, which play a critical role in the physicochemical properties of starches (TAPPIBAN et al., 2020). Sanchez et al. (2009) found an average 20.7% of amylose content (Table 1). Morante et al. (2015) observed four types of waxy cassava starch and compared with comercial and high amylose content cassava starches finding an average of 19.9% and 30.2%, respectively. Tappiban et al. (2020) studied seven cassava-improved samples and reported values of amylose content ranging between 24.8-27.6% and Costa Nunes et al. (2021) demonstrated in forty clones of sweet cassava, an average of 16.08% of amylose content.

4.2 Solubility and Swelling Power

Swelling power represents evidence of interaction between amorphous and crystalline areas of starch, it is influenced by amylose and amylopectin proportions. Furthermore, starch swelling power depends on the capacity of the starch molecule to hold water through hydrogen bonding during the gelatinization process, and thus the water absorption capacity (WAC) is a consequence of swelling power. It measures the strength of the interaction between water molecules and glucan chains (COSTA NUNES et al., 2021). According to Table 1, Sanches et al. (2009) evaluated 4050 genotypes and reported values ranging from 0.8 to 15.5% to swelling power. Morante et al. (2016) reported that solubility was higher in waxy starches compared with wild types, ranging from 8.4 to 13.8% with an average value of 11.1%. He et al. (2020) measured the WAC of sixteen cassava starches, reported that WAC varied significantly from 193.63% to 226.74% with an average of 211.48%. The solubility of different germplasms of cassava starches was statistically significant ($p<0.05$), with an increase in temperature (0.01–0.07 % at 55°C and 0.06–0.24 % at 95°C), especially at 60°C to 70°C, showed high solubility in a wide temperature range. Costa Nunes et al.

(2021), observed swelling power varied from 6.1 to 12.4%. Differences between data obtained were probably due to differences in germplasms and quantification methods adopted.

5 ADVANTAGES OF NATURALLY MODIFIED CASSAVA STARCH AND POSSIBLE APPLICATIONS

The applications of cassava starch in the development of products and their formulations are guided by the knowledge of its composition physical-chemical and functional properties. Naturally modified cassava starches have been improved through cassava breeding programs and genetic studies have been performed could control properties and their possible applications and effects on food quality.

Nowadays, in Brazil, cassava processing prioritizes the production of flour and starch, the latter also known as *polvilho*. There are also indigenous processes, such as the production of fermented and sun-dried cassava starch or sour cassava starch, known in Brazil as *polvilho azedo.* This fermented and sun-dried starch presents a typical flavour and is used in the production of expanded salty biscuits and also of cheese rolls. In the United States, cassava is the second most important botanical source of industrial starch after corn (DEMIATE; KOTOVICZ, 2011).

In food application, one of the main advantages of cassava starch if compared to corn starch is the absence of the undesired "*cereal flavour*". In this sense, cassava starch could be preferred for application in many processed foods, with particular interest in mild-flavoured products. Genotypes with higher swelling power are suitable for use as thickeners and binding agents for food and non-food use. Cassava starches with lower setbacks are desirable as gelling agents in refrigerated and frozen food products and those with higher amylose content have the potential for use in products that require crispness, resistance and low oil absorption, as required by snacks. Otherwise, products that require staple pastes as soups and sauces need starch with higher amylopectin content (COSTA NUNES et al., 2021).

Glucose and high fructose syrup (HFS) are two other subproducts from cassava with commercial importance with widely used by the food and pharmaceutical industries. Irrespective of the botanical source used (corn, potato, rice, sorghum, wheat or cassava), the process involves two steps such as liquefaction, in which the α-amylase partially hydrolyzes starch to maltodextrins

and saccharification leads to the low dextrose-equivalent (D.E) fractions that is completely converted to glucose by glucoamylase. In the production of HFS, two normal grades 42% HFS and 55% HFS as well as an enriched grade (90% HFS) are commercially available. HFS 42 finds application in beverages and confectionery industry. At the same time, HFS 55 is predominantly used as a sweetener in ice cream, yogurt, processed foods and as feed for honey bees due to its highly soluble and non-crystalline nature, ability to prevent microbial growth and increased shelf life (JOHNSON et al., 2009). Nevertheless, the cost of production is a decisive factor for cassava to gain market share as an agroindustrial ingredient.

The starch dextrinization yielding resistant dextrins (RDs), highly branched dietary fibers with prebiotic activities has gained interest in the food industry. Resistant dextrins (RDs) can derived from various starch sources including cassava starch, and are usually obtained under hard dextrinization conditions using high acid concentrations (≥ 0.1% HCl or H_2SO_4), heating temperatures (≥ 130 ˚C), and prolonged heating times (≥ 60 min). Furthermore, continuing hydrolysys of RDs with α- amylase produces α-limit dextrins resulting in products called resistant maltodextrins (RMDs). Commercial products of RDs and RMDs usually contain ≥ 85% dietary fiber and are considered functional ingredients, showing excellent water solubility, providing low viscosity and tolerate several conditions such as heat and pH with good applications to enhance fiber content or provides prebiotics in food and beverage products (TRITHAVISUP et al., 2022).

In Brazil, cassava is preferred by the meat industry in sausages, ham-like products, *patés* and cooked ham due to the advantage of the jellifying agent, resistance to retrogradation, promoting the formation of a smooth and short gel if compared to cereal starches. Concerning resistance to syneresis at low temperatures, cassava starch can be applied in infant foods, confectionary cream, jellies, ready-to-eat frozen dishes and powder desserts (DEMIATE; KOTOVICZ, 2011).

The failures of native cassava starch in terms of functionality can be overcome by applying chemical modifications or a combination of chemical, physical and enzymatic modifications (WANG et al., 2022).

Currently, most technologies used to produce dietary fibre from starch are chemical-based modifications. However, due to the documented negative consequences on human health of chemically modified food ingredients as well as societal concerns, today's consumers are increasingly avoiding foods containing ingredients produced through chemical reactions. Therefore, novel chemical-free modifications should be investigated to replace traditional methods for producing health-promoting starch based on ingredients with, for example, high resistant starch content and low digestibility (WANG et al., 2022).

The knowledge about the genetic machine of cassava is the future and allows to set up the bridge between the food industry and the farmers (CARVALHO et al., 2022).

6 CONCLUSION

The results of this study contribute to understanding the relationships between physicochemical and functional properties and starch structures in cassava starches, providing new information on starch properties from different cultivars for application in food and non-food industries, as well as helping to identify specific starch properties that can be selected for in cassava breeding programs to select for varieties with improved desired characteristics.

The chemical composition and physical characteristics such as morphology, particle distribution, and crystallinity showed differences that could be related to cassava agricultural traits, genetics, and plant physiology such as the stage of maturity.

The pasting and gelatinization properties of naturally modified cassava starch are important features related to industrial applications. They strongly impact the quality of the final product, since they govern freeze-thaw stability and syneresis notably in the case of cold storage conditions.

The phenotypic aspects of new and spontaneous cassava starches may be used to attend a new demand of consumers seeking clean-label foods, as non-GMO and plant-based products thus increasing the demand for naturally modified cassava.

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CHAPTER 2: Technological and nutritional prospection of different germplasm of cassava starch naturally modified

Technological and nutritional prospection of different germplasm of cassava starch naturally modified

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ABSTRACT

Cassava (*Manihot esculenta* Crantz) is one of the most important sources of starch in the tropics and the current demand for clean-label ingredients has become of primary concern that may lead to focus on the use of non-genetic modified (GMO) starch. Cassava starch has several applications due to physicochemical properties specially related to cook quality that results in pastes of high clarity and viscosity. In the present work, fifteen naturally modified cassava starches of the Embrapa Active Germplasm Bank (AGM) and two arising from the Centro Internacional de Agricultura Tropical (CIAT/Cali, Colômbia) were collected and analyzed for selected physicochemical characteristics and functional properties. Morphological characteristics of cassava starches presented typical round shape with D[4,3] ranged from 12.1 to 17.3 μm. Pasting and thermal properties demonstrated a wide range of applications, particularly in products submitted to freezing-thawing cycles showing P_T ranging from 65 to 73 ºC and peak viscosity from 3123 to 5479 cP. The relative crystallinity ranged from 29.7 to 36.5 % presenting A-crystal pattern. In general, the swelling power abruptly increase at 75 ºC and the freeze-thaw stability revealed a significant increase in maximum force ($p< 0.05$) after the first cycle, except for waxy cassava starches (BGM 7737 and 7788) that showed the lowest values during storage time as well

as syneresis was observed. Regarding amylose content, waxy cassava starch presented the lowest value (6.0 %) as expected and also the lowest gelatinization enthalpy (ΔH_G = 16.4 J/g). BGM 0061 showed 16 % of resistant starch that would be considered a relevant functional ingredient in terms of gut health maintenance making it possible to be used in food ingredients with nutritional appeal.

Keywords: Cassava starch, naturally modified starch, functional properties, food aplications.

1 INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is one of the most important sources of starch in the tropics, despite the limited and contradictory information regarding cassava starch concerning the biochemical characteristics and functional properties (SANCHES et al., 2009). In 2023, Africa's cassava production has reached more than 60% of the world output, followed by Asia, and South America. Nigeria (20.6% of total cassava production), Thailand (11.2%), Brazil (7.6%), and Indonesia (7.5%) represent the four main countries contributing to the majority of global production (FAOSTAT, 2023). As opposed to the extraction of maize starch that requires an adequate steeping step to soften the kernel and easy the separation of protein, germ, hull, and fiber from starch granules, the isolation of starch from cassava root tubers is relatively more feasible and attributable to its low content of protein, fat, and fiber (HSIEH et al., 2019).

Cassava starch has several applications, especially in the food industry, due to its physicochemical properties speciality related to cooking quality that results in high viscosity and transparency pastes, demonstrated a low gelatinization temperature and low retrogradation tendency if compared to cereal starches (SANTOS et al., 2021).

However, native starches are rarely used in their native form due to undesirable behaviours such as low solubility, freeze-thaw, thermal and shear stability, resistance to enzymatic hydrolysis and a high tendency toward retrogradation and syneresis. Thus, there is increasing interest and growing opportunities via conventional breeding and genetic transformation to change some of the modifications and functional properties of starches (SANCHES et al., 2010).

In Brazil, although there is a high production of cassava root tubers, its processing for starch is relatively low if compared to the Brazilian corn starch production. If only the production of modified cassava starch is considered, then our global participation is very limited; there is a growing demand especially for industrial modified starches (DEMIATE; KOTOVICZ, 2011).

In this context, genetic modification provides the potential for tailoring desired functionalities that uniquely fit the end-use in various fields especially as food ingredient applications. In recent years, the demand for clean-label

ingredients has become of primary concern and researchers have focused on the development of non-GMO cassava starch. However, information on the properties of naturally modified cassava starch is insufficient for effective use in breeding programs yet, and few studies have used insignificant numbers of genotypes or have often a restricted genetic basis (SANTOS et al., 2021). In addition, most of the previous studies were focused on the physicochemical properties of a limited number of cassava starches or comparing one or two cassava starches, particularly waxy cassava with other botanical sources (HE et al., 2020).

Among initiatives around the world, Embrapa is responsible for identifying and cataloguing different genotypes of cassava in Germplasm Active Bank (BGM) which stores more than 3000 genotypes. In Colombia, Sanches et al. (2009) reported a description of analyses performed in more than 4000 genotypes of starches. He et al. (2020), in China, analysed sixteen cassava germoplasms comparing structural characteristics and physicochemical properties. Tappiban et al. (2020) reported analysis of seven (7) different cultivars of cassava including pasting properties, gelatinization, molecular size and amylose/amylopectin ratio. Identifying new landraces of cassava starch is highly desirable for the breeding programs notably in times of extreme weather caused by climate changes. It would offer alternatives for breaking undesirable genetic linkages of the mutation locus with other loci controlling traits of agronomy relevance such as dry matter content and pasting properties (MORANTE et al., 2016).

In the present work, fifteen naturally modified cassava starches of Embrapa Active Germplasm Bank (AGB/Cruz das Almas, Brazil) and two others from Centro Internacional de Agricultura Tropical (CIAT/Cali, Colômbia) were collected and analyzed in terms of physicochemical characteristics and functional properties required by food processing industry.

2 MATERIAL AND METHODS

2.1 Materials

A total of fifteen genotypes (BGM 1406, BGM 0011, BGM 0914, BGM 0729, BGM 0953, BGM 0061, BGM 1403, BGM 1044 BGM 0975, BGM 0985 BGM 1586, BGM 0069, BGM 0144 BGM 1103 and BGM 0867) belonging to the Brazilian Active Germplasm Bank (AGB) maintained by Embrapa Mandioca e

Fruticultura in the municipality of Cruz das Almas, in the state of Bahia, Brazil (-12.653 latitude, -39.1219 longitude, 12°39'11"S, 39°7'19"W, at 212 m altitude) were selected. The samples were cultivated between 2022 July and 2023 August, along with two waxy cassava clones (7737-1 and 7788-1), that were provided by International Center for Tropical Agriculture (CIAT, Cali, Colombia), totallizing seventeen different genotypes for characterization and prospection of industrial use Table 1.

Fresh cassava root tubers were washed, peeled, manually cut into small pieces, and placed in a 2 L blender with non-sharp edge blades for 1 min at a ratio of 1:1 (sample: water). Subsequently, the material was filtered using a sieve of 150 mesh aperture (0.105 mm). After, the mixture was left for 30 min to allow starch decantation and then dried in a fan oven at 40 °C until a final moisture content of 8.0 ±2.0 % and stored in plastic bags (SANTOS et al., 2021). The samples were sent to Embrapa Agroindústria de Alimentos, located in the municipality of Rio de Janeiro, in the state of Rio de Janeiro, Brazil.

Table 1 Relation of seventeen genotypes of cassava starch and their respective applications

Clone	Categorization
BGM-0011	Flour
BGM-0061	Industry
BGM-0069	Industry
BGM-0144	Industry
BGM-0729	Industry
BGM-0867	Industry
BGM-0914	Industry
BGM-0953	Industry
BGM-0975	Industry
BGM-0985	Industry
BGM-1044	Flour
BGM-1103	Flour
BGM-1403	Flour and Industry
BGM-1406	Industry
BGM-1586	Industry
7737	Industry
7788	Industry

2.2 Methods

2.2.1 Scanning electron microscopy (SEM)

The starch granules was previously kept in a desiccator with saturated $CaCO_3$ solution at least 15 days until reached the equilibrium moisture. The starch granules were accurately glued on aluminum stubs utilizing a double-sided conductive metal tape and then observed at low vacuum benchtop scanning electron microscope (SEM) TM 3000 (Hitachi, Tokyo, Japan) at an accelerating voltage of 15 kV and magnitude of 1000 x following Mishra; Rai (2006).

2.2.2 Light Microscopy (LM)

Starch granules were sprinkled onto a glass slide and deionized water was dropped over. The suspended starch granules were covered with a glass cover slip and then observed and photographed using a Leitz Laborlux S optical microscope (Leica, Wetzlar, Germany) equipped with a polarized lens to observe starch birefringence in a digital câmera (MShot, Guangzhou, China), model MD30 according to Mishra; Rai (2006) method.

2.2.3 Particle size distribution

The particle size of starch granule was determined by a laser particle size analyzer, S3500 (Microtrac, Montgomery, EUA), according to the method ISO13320-1 and 55-40.01 (AACC, 1989). Isopropyl alcohol was used as a solvent to minimize starch swelling. All determinations were done in duplicate.

2.2.4 Apparent density

The apparent density, ρ_{ap} was obtained in triplicate according to Vargas-Solórzano (2019) and as the non-compacted sample mass, freely poured in a graduated cylinder with the top levelled to 50 cm3. The bed of particles was obtained by placing the graduated cylinder under the outlet of a single-screw volumetric feeder (Brabender, Duisburg, Germany) and then dropping 50 g of sample at 4 kg. The feeding rate (kg/h) was calculated by interpolating rotational speed curves (rpm) as a function of particulate mass, collected in 1 min.

2.2.5 Pasting Properties

Pasting properties of starches samples were determined using a Rapid Visco Analyzer (RVA) (RVA-4, Newport Scientific PTY Ltd., Warriedwood, Australia) coupled with Thermocline software for Windows according to the method 61-02 (AACC, 2000), with modifications. Approximately 3.0 g at 14% of moisture content (wet basis) was weighed and 25 mL of deionized water was added. Each sample was heated at 25 °C, stirred for 10 s for through dispersion, and kept at 160 rpm until the end. The slurry was held at 50 °C for up to 1 min, and then heated to 95 °C for 3.7 min and held at 95 °C for 2.5 min. Finally cooled at 50 °C for 3.8 min and then kept at 50 °C for 2 min. All determinations were plicates and results were expressed in mPa.s. The evaluated characteristics were Ti (Initial paste temperature), PV (Peak viscosity), MV (Minimum viscosity), BD (Breakdown viscosity - difference between maximum viscosity and minimum viscosity at 95 °C), FV (Final viscosity) and SB (Setback viscosity - difference between lower and viscosity at 95 ° C and final viscosity).

2.2.6 Thermal properties

Thermal properties were evaluated using the Q200 Differential Scanning Calorimeter (TA Instruments, New Castle, DE, USA). The degree of gelatinization was measured with the Universal Analysis 2000, version 4.5A, software (TA Instruments, New Castle, DE, USA). The heating curve profile was used to calculate the onset (To), peak (Tp) and conclusion (Tc) temperatures, as well as calorimetric enthalpy (ΔH_G) according to Bernardo et al., (2018) method.

2.2.7 X-ray Diffraction

The crystalline structure of dried oven cassava starch granules (10 ± 1% w.b.) was analyzed using an X-ray diffractometer D2 Phaser (Bruker, Karlsruhe, Germany), operating at 20 mA, 30 kV and wavelength with 0,154 nm. Samples were scanned from 2 to 32° (2θ) at the rate of 0.15°/ min, with a step size of 0.02°, a divergence slit width of 0.6 mm, a scatter slit width of 0.6 mm and a receiving slit width of 0.2 mm. The area under the main peaks from 2 to 32 ° (angle 2θ) corresponded to the crystalline region, whereas the amorphous area was characterized as the difference of the total area (baseline from 2 to 32°) minus the

crystalline region using the Diffract Evaluation Eva v.3.0 software (Bruker, Rheinfelden, Germany) following Bernardo et al., (2018) method.

2.2.8 Swelling power and solubility

Water absorption and swelling power patterns were determined according to Tsi et al. (1997). Dry starch (1g) was mixed with 10 ml deionized water in a previously tared 50 ml centrifuge tube. Each sample was homogenized using a Vortex Genie 2 (Scientific Industries Inc, Bohemia, NY, EUA) for 10 seconds. The tubes were placed in a water bath (Dubnoff double-boiler, modelo, City, Country) for 30 min under agitation at temperatures of 55ºC, 65ºC, 75ºC, 85ºC and 95°C. All determinations in each temperature range were carried out in quadruplicate. The heated samples were cooled rapidly to room temperature in an ice-water bath for 10 min. After that, each suspension was centrifuged in a Universal/320R (Hettich, Tuttingen, Alemanha) at 9000 rpm for 10 min. The supernatant was dried at 105ºC overnight until dry matter was obtained at constant weight (Ms).

The Solubility Index (SI) is expressed as the ratio of dry matter supernatant (Ms) and the initial starch weight (Mi):

$$SI = Ms/Mi$$

Swelling power (SP) is described as the ratio of the sedimented starch weight (Ma) and the initial starch weight (Mi) multiplied by (1 – SI)

$$SP = Ma / [Mi \times (1-SI)]$$

2.2.9 Gel texture after freezing-thawing cycles

Each starch gel was prepared using suspensions at 10% (g/mL) using a Brabender Viscoamylograph (Brabender OHG, Duisburg, Germany) operating at 115 rpm and heated from 25 ºC to 90 ºC according to Deffenbaugh; Walker (1989) method with some modifications. The gel (56g) was distributed in 20 units of aluminium sample holder. Samples were cooled at room temperature for 1 hour and frozen for 24 hours in a vertical freezer at – 18ºC. After that, the samples were thawed at room temperature for 4 hours and returned to the freezer simulating the

freezing-thawing cycle according to Takeiti et al. (2007). The texture of the starch gels was measured in a TA-XT2 Stable Micro Systems texture analyzer (Surrey, England) using a P/10 cylinder probe at a penetration distance of 6.0 mm, 5.0 mm/s speed, 1.0 mm/s pre-test speed, 10.0 mm/s post-test speed, 20 g contact force, 8.0 mm distance and 5 g test sensitivity according to Bouvier et al (1997) with some modifications. These measurements were performed after each freezing-thawing cycle, totalizing 4 cycles per week, in five replicates per day for each starch. The results were expressed using Force and Area.

2.2.10 Syneresis

The determination of syneresis followed the methods described by Takeiti et al. (2007) and Kaveh et al. (2020) with modifications. Each starch gel was prepared using suspensions at 10% (g/ml) using a Brabender Viscoamylograph (Brabender OHG, Duisburg, Germany) operating at 115 rpm and heated from 25 ºC to 90 ºC. Aliquots of 8-10 ml of each starch gel were placed in six Falcon tubes. Afte 20 min samples were frozen for 24 hours in a vertical freezer at -18 ºC and thawed at room temperature for 4 h. These measurements were performed solely after four freezing-thawing cycles, simulating 4 freezing-thawing cycles.

2.2.11 Total starch, amylose and resistant starch content

The total starch content was determined using a kit K-TSTA-50 (Megazyme®, Bray, Ireland) according to 996.11 (AOAC, 2005) and 76-13.01 (AACC, 2015) methods.

The amylose content was determined according to Yun; Matheson (1990) by using an enzymatic kit Megazyme® K-AMIL 06/18 (Bray, Ireland), in which activity is based on Con-A enzyme activity. The Con A enzyme reacts with amylopectin, forming a precipitated removal by centrifugation in a Universal/320R (Hettich, Tuttingen, Alemanha) at 14000 x g for 10 min, leaving amylose free to react in the next step with a peroxidase enzyme.

Finally, the resistant starch content was determined using K-RSTAR kit (Megazyme®, Bray, Ireland) according to 2002.02 (AOAC, 2002) e 32-40.01 (AACC, 2000) methods.

3 STATISTICAL ANALYSIS

Statistical analyses were performed by Tukey's test ($p < 0.05$) using StatSoft Statistica Program, version 12 (Chicago, USA).

Principal Component Analysis (PCA) was performed using XLStat Pro version 7.5 (Addinsoft, New York, USA). PCA was used to describe the data obtained in the present study and to group similar variables and samples. PCA was applied after standardization, using only the variables that presented statistical differences.

4 RESULTS AND DISCUSSION

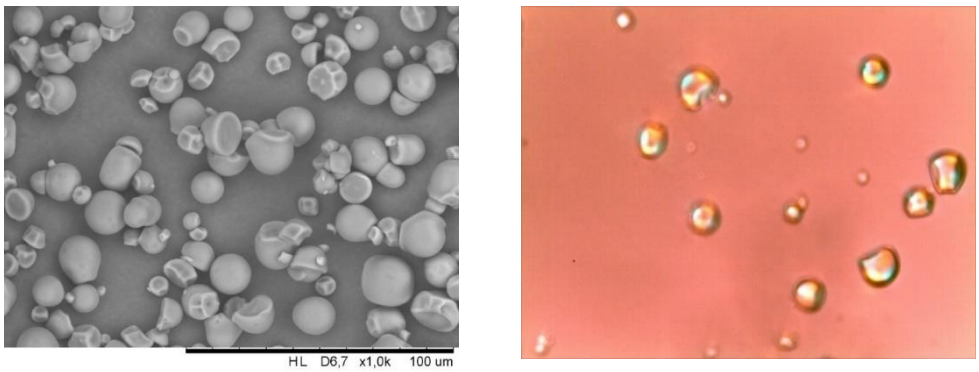
4.1 Physical Properties

4.1.1 Morphological structure, size particle distribution and apparent density

Granule morphology and size particle distribution of the starches are shown in Figure 1 and Figure 2. The SEM images showed that size of the granules was heterogeneous for all starches and no pores were observed at the surfaces. The morphological characteristics of cassava starches presented oval to spherical shapes, truncated and rounded forms similar as reported by Gomand et al. (2010); Sanches et al. 2010; Chen et al. (2011) and He et al. (2020). PLM images showed the birefringence pattern unveiling the maltese cross and centric hilum located at the large granules (Figure 1), indicating the integrity of granules starches.

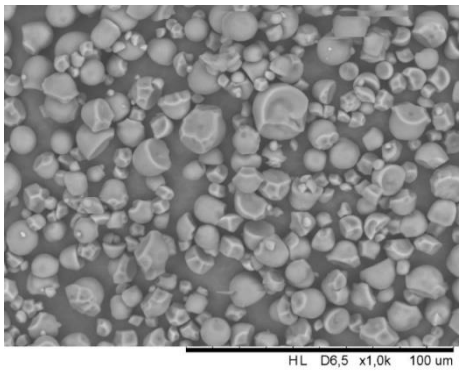
BGM 1406

(a) (b)

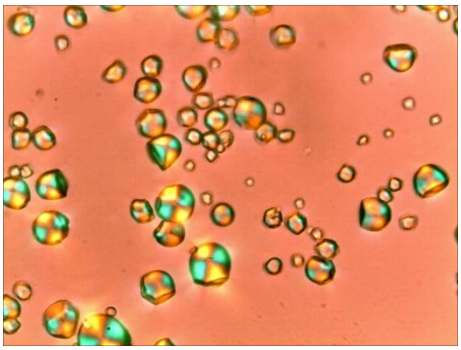


BGM 1103

(a)

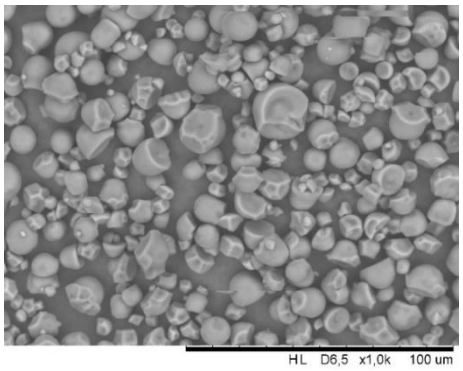


(b)

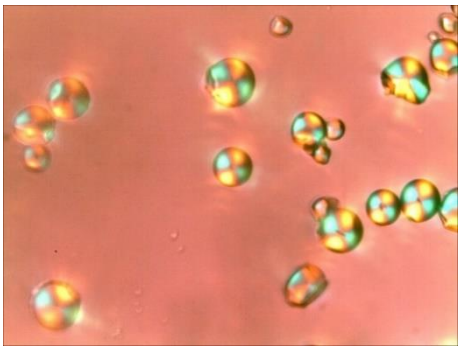


BGM 0011

(a)



(b)



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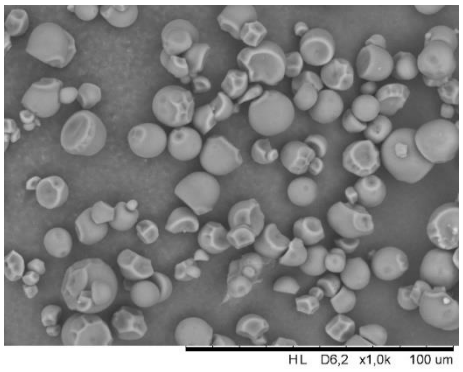

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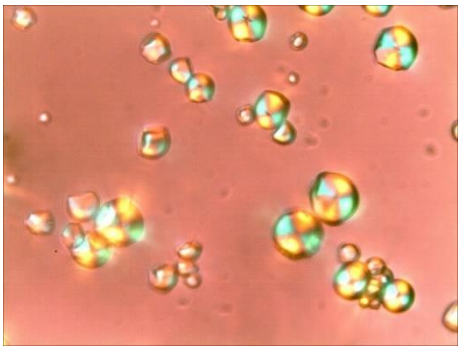

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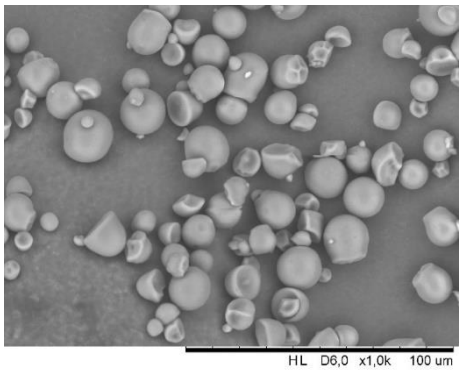

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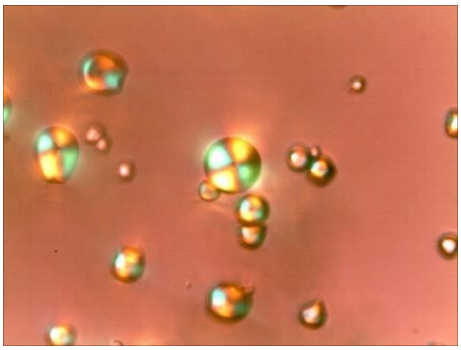

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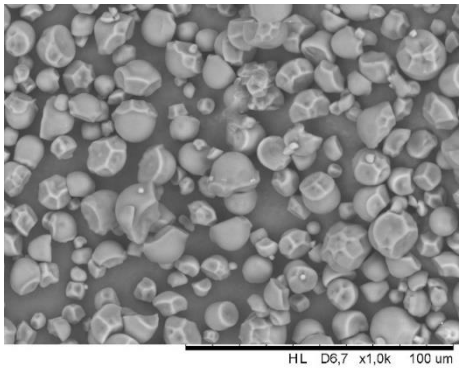

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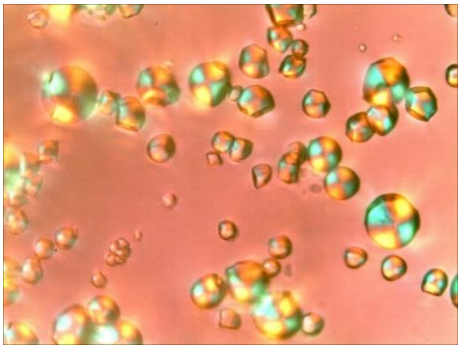

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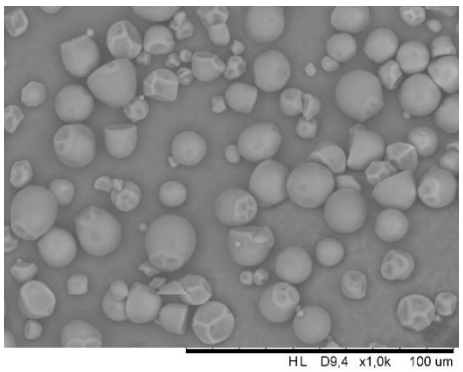

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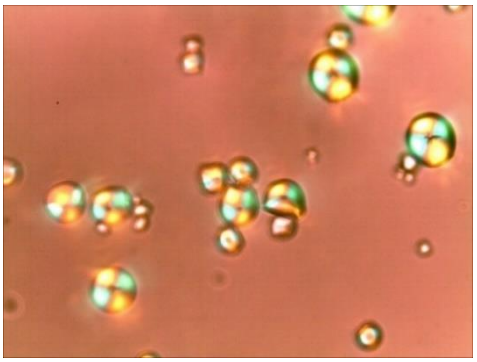

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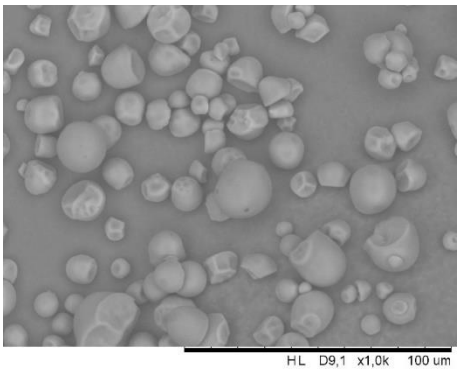

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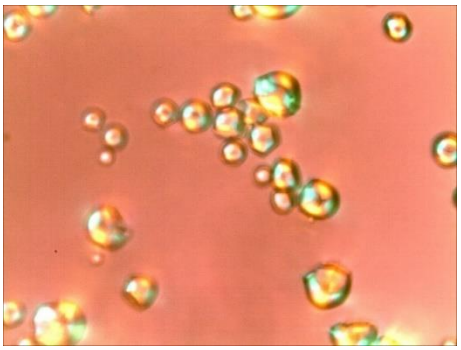

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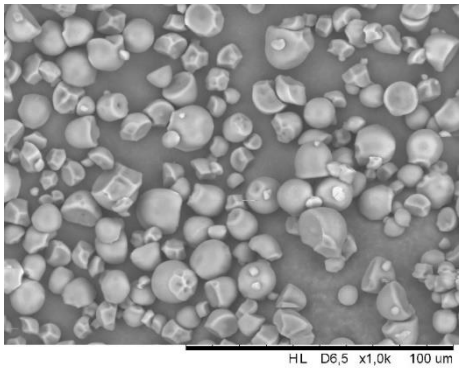

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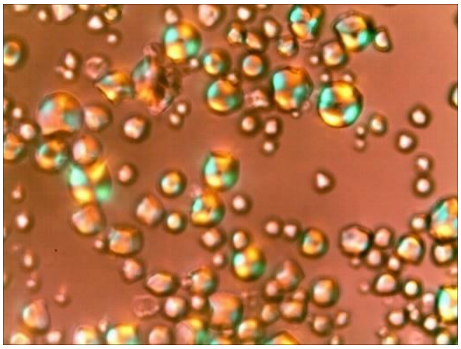

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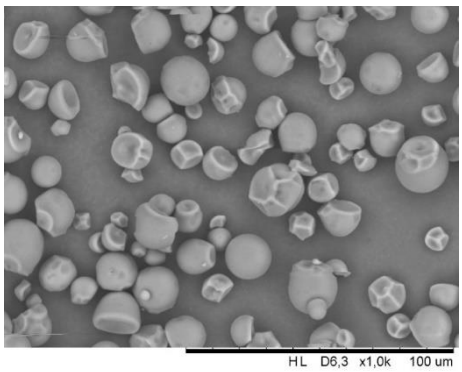

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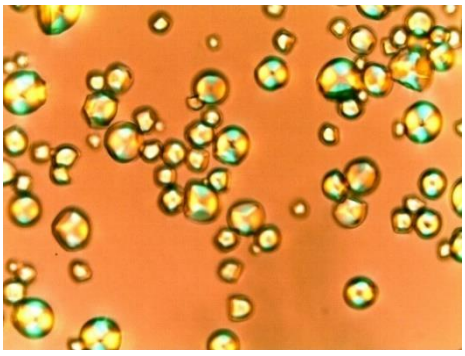

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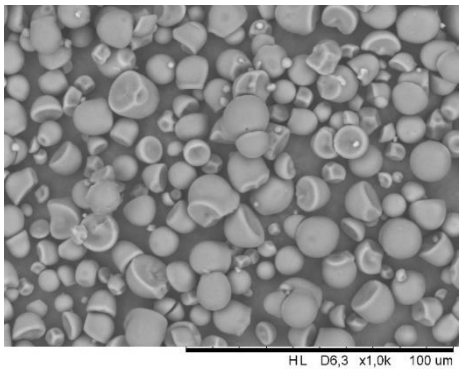

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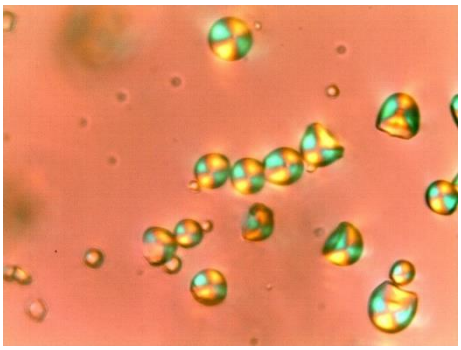

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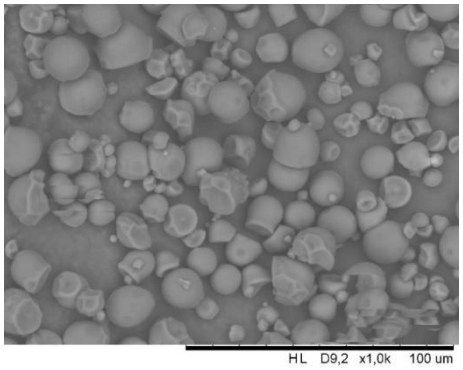

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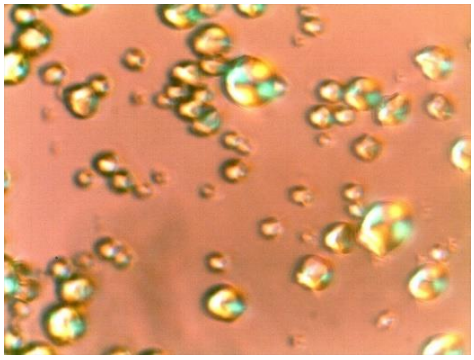

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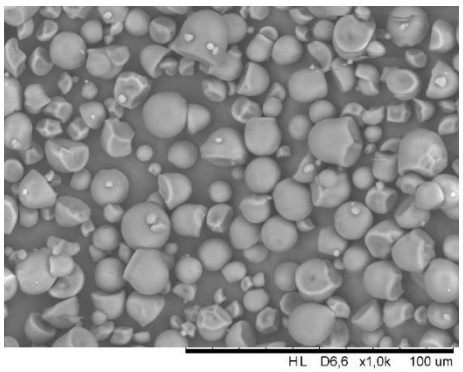

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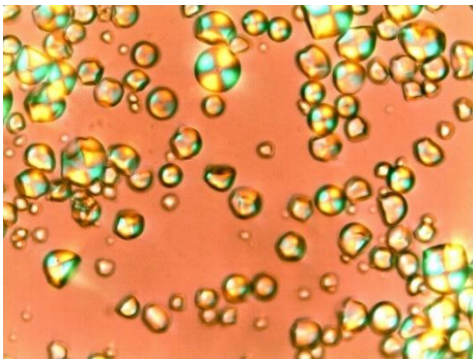

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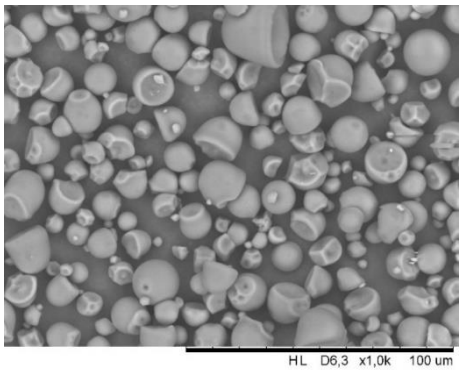

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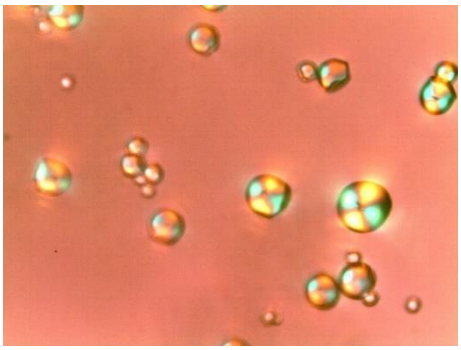

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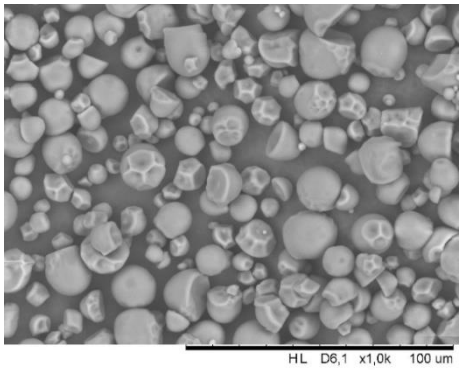

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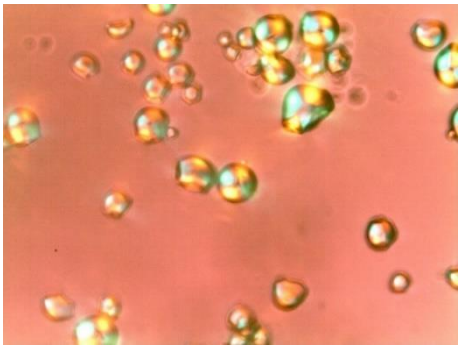
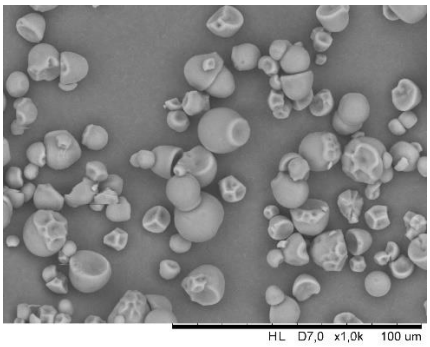


7737

(a)



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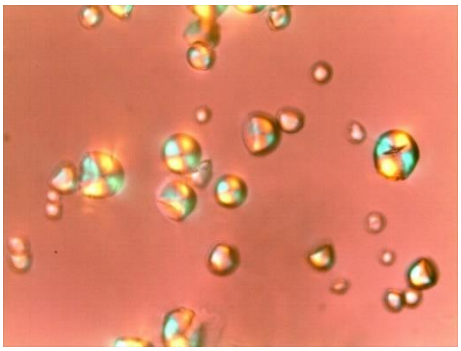
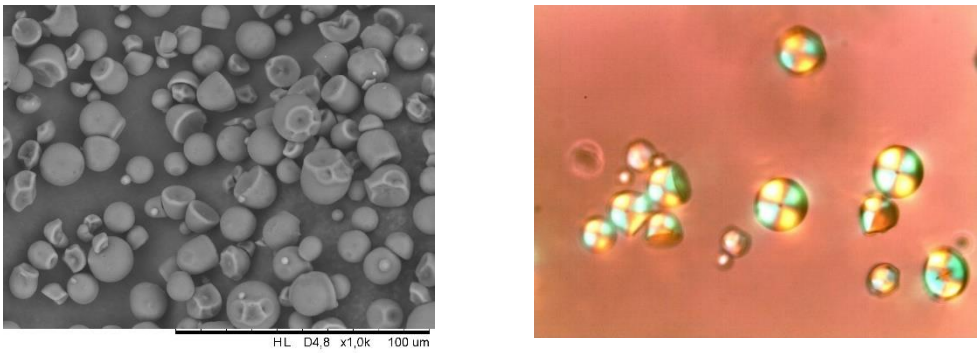


7788

(a) (b)



Credits: Palheta, S.

Figure 1. Scanning electronic microscopy (SEM) and polarized light microscopy (PLM) images of seventeen cassava starches (a) SEM 1000X and (b) PLM 400X.

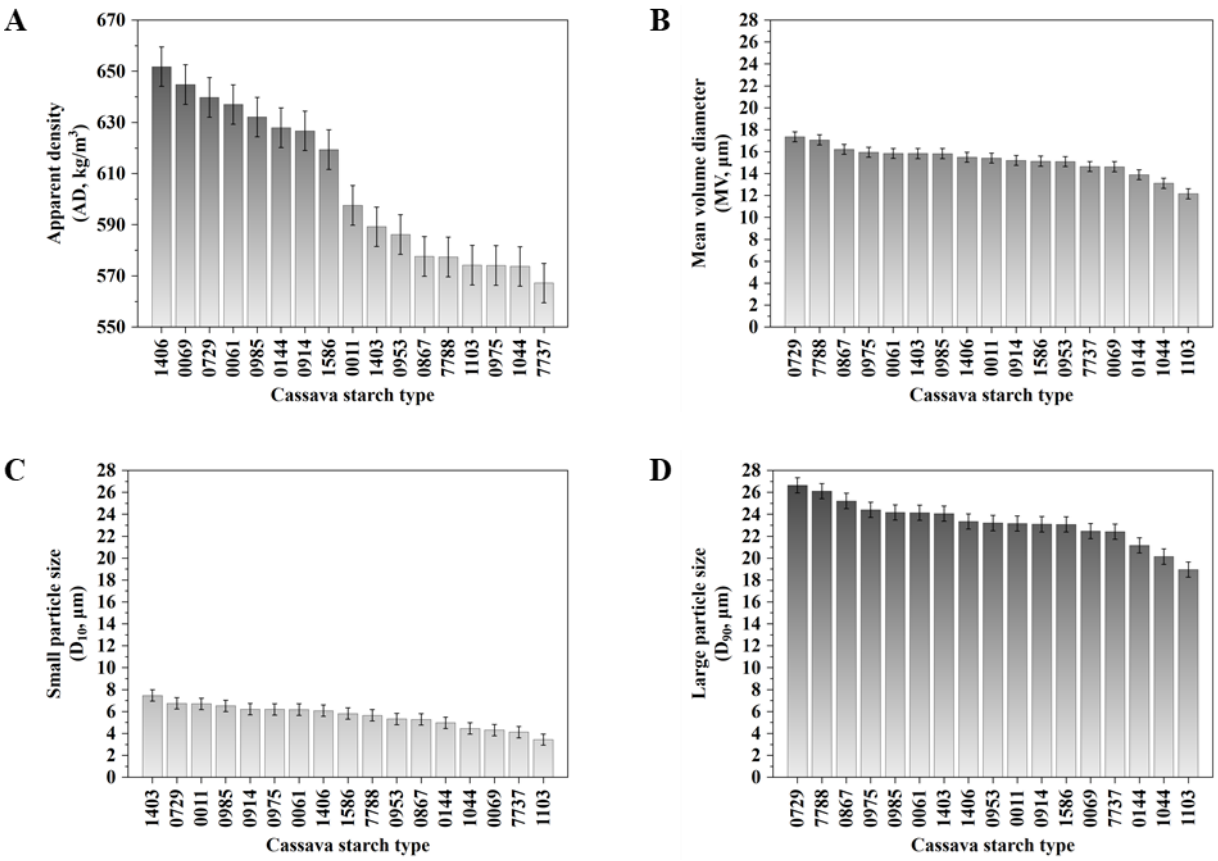


Figure 2. Particle parameters of cassava starches from seventeen genotypes: (A) apparent density, (B) Mean volume diameter (D[4,3]), (C) small particle size and (D) large particle size.

In this study, the mean average granule size ranged from 13 to 18 μm (Figure 2B) similar to that reported by Gomand et al., (2010) (mean value of 18 μm for native cassava starch and 16.9 μm for waxy cassava starch); Tanyasiriwat, et al. (2013) (6.6 μm to 17.2 μm); Morante et al. (2015) (14.3 μm for native cassava starch and an average of 15.9 μm for waxy cassava starch) and Costa Nunes et al. (2021) (6.6 to 17.2 μm). The D [0.9] ranged from 19 to 27 μm and D [0.1] ranged from 4.0 to 7.0 μm (Figure 2 C, D). The largest average particle sizes were observed by BGM 0729 and 7788 (Figure 2 D) and the lowest average sizes by the samples BGM 1044 and BGM 1103 corroborating with SEM images in which BGM 0729 demonstrate a great number of large particles (Figure 1). The differences in size distribution may be attributed to the differences in the cassava growing environment, genetic diversity, and plant physiology as the stage of maturity suggesting different physicochemical properties, such as the swelling power and water solubility index. Concerning apparent density, the highest values were showed by BGM 1406 and BGM 0069 although these samples did not show the largest particle sizes (Figure 2D).

4.1.2 Pasting Properties

The pasting properties of seventeen cassava starches are shown in Figure 3 and Table 1(Appendix) and the parameters recorded were pasting temperature (PT), peak viscosity (PV), through viscosity (TV), breakdown viscosity (BV), maximum cooling viscosity (MCV), setback viscosity (SV) and final viscosity (FV). The starch suspensions exhibited a wide variation of pasting properties. PT ranged from 65.88 ºC (BGM 1403) to 73.0 ˚C (BGM 0011), showing a variation of 10% among samples. PV ranged from 3123.0 (BGM 0729) to 5479.0 cP (BGM 1403); MCV values varied from 1879.5 (7737) to 5333.0 cP (BGM 1044); BV ranged between 2119.0 (7737) and 3932.0 cP (BGM 1403); SV ranged from 757.0 (7737) to 3777.5 Cp (BGM 1044) and FV ranged from 1787.0 (BGM 7737) to 4528.0 Cp (BGM 1103), demonstrating that cassava starch has potential for different application as food ingredient.

The PV of BGM 1403 presented the highest value exhibiting (5479,0 cP) 28,61% greater than the mean value of all samples. PV had a significantly positive correlation with BV demonstrating that the water-binding capacity of starch at the highest viscosity is significantly related to the disruption of starch granule during the cooling suggesting that the use of this starch should be avoided in frozen

products. MCV showed a significant and positive correlation with SB, which is similar to non-waxy cassava properties indicating that the cooling temperature affects the reassociated form and the retrogradation of starch (Tappiban et al., 2020).

In this study, BGM 1044 showed the highest values of SB and FV considered a useful characteristic to apply in the refrigerated desserts such as a flan or custards. On the other hand, 7737 (from CIAT) presented the lowest values of these parameters and can be used in frozen products due to low retrogradation.

Santos et al. (2021) studied 881 pre-selected genotypes due to their industrial potential and compared with waxy cassava and waxy maize clones. They concluded that two starches from the Brazilian germplasm bank showed a paste viscosity reading close to that of the starches of the 7745-5WX waxy cassava and WX-Maize waxy maize (*Zea mays*) clones, indicating their potential usues both to the industry due to this specific viscosity characteristic and to an alternative use for different foods that require stability during freezing.

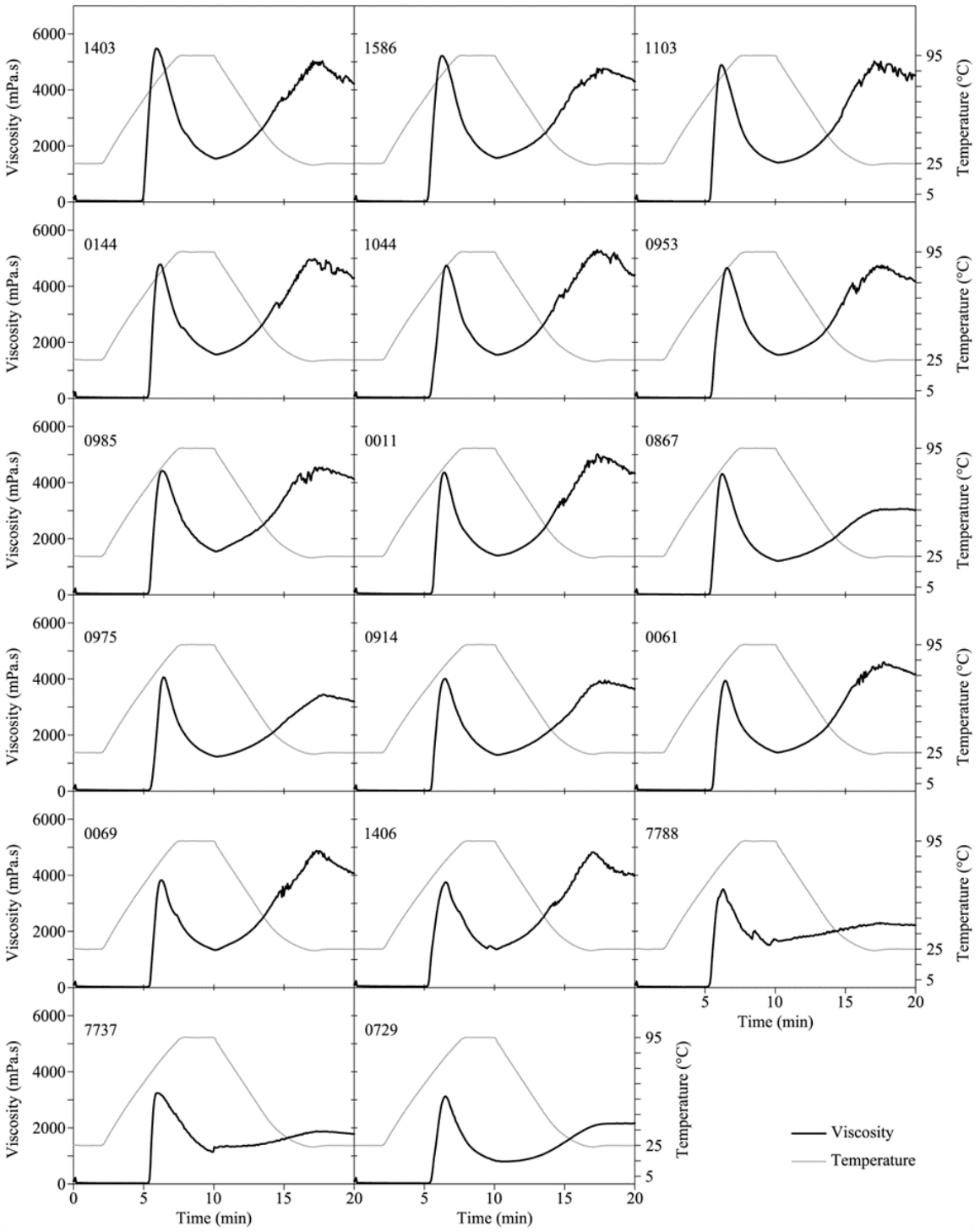


Figure 3. Viscosity curves of seventeen genotypes of cassava starch suspensions by RVA

4.1.3 Thermal Properties

The onset temperature (T_o), peak temperature (T_p), conclusion temperature (T_c), and gelatinization enthalpy (ΔH_G) exhibited a wide variation of all parameters among the seventeen cassava cultivars (Figure 4 and Table 2, Appendix). The values of T_o ranged from 57.37 ° C (BGM 1403) to 65.94 ºC

(BGM 7737), T_p ranged from 62.42 °C (BGM 1103) to 72.45 ºC (BGM 0975), T_c ranged from 81.03 ºC (BGM 0953) to 89.01 ºC (BGM 0069) and ΔH_G ranged from 16.41 (7737) to 20.16 J/g (BGM 0914).

BGM 7737 presented the highest T_o (65.94 ºC) that could be attributed to perfect crystallites and stronger bonding between internal molecules, but unexpectedly showed the lowest gelatinization enthalpy value (ΔH_G = 16.41J/g) that corroborating with minimum RVA values after starch melting, i.e BGM 7737 showed low values of MCV, BV, SV and FV.

Gomand et al. (2010) compared the structure properties and gelatinisation characteristics of potato and cassava starches and reported an average T_p of 60 ºC and ΔH ranged from 19.1 to 21.5 J/g in case of cassava starches. Chen et al. (2011) compared hydrolyzed and native cassava starches and found T_o of 68.8 ºC, T_p of 78.1ºC, T_c of 87.4 ºC and ΔH_G 11.8 J/g for native cassava starch. Morante et al. (2016) comparing waxy starches and native cassava starch samples, presented an average T_p 66.3 ºC (waxy) and 62.1 ºC (native) and ΔH 17.4 – 20 J/g (waxy) and ΔH 19.1-20 J/g. Tappiban et al. (2020) studied the physicochemical properties of seven cassava starches reported values of T_o, T_p and T_c ranged from 60.7 ºC to 67.9 °C, 66.8 to 72.9 °C and 75.2 ºC to 78.6 °C with an average of 63.7 ºC, 68.9 ºC and 77.1°C, respectively and ΔH_G ranged from 11.8 to 13.4 J/g. Our results are in agreement with these authors.

In fact, the gelatinization enthalpy (ΔH) is correlated with the crystalline organization and stability among the granules, therefore highest values of enthalpy suggest an increase in crystalline structure (CHEN et al., 2011; MORANTE et al., 2016) that impacts the energy required for processing, an important key factor in the industrial adoption of the material.

According to Tappiban et al. (2020), the pasting time (P_T) is positively correlated with both T_p and T_c implying starches with high gelatinization temperatures demand a longer time to disruption and gelatinization. Moreover, the pasting temperature (P_T) showed highly significant and positive correlations with T_o and T_p which are similar to results reported by Singh et al. (2008), Srichuwong et al. (2005) and Kaur et al. (2007) suggesting that higher transition temperatures produces in high stability of starch granules.

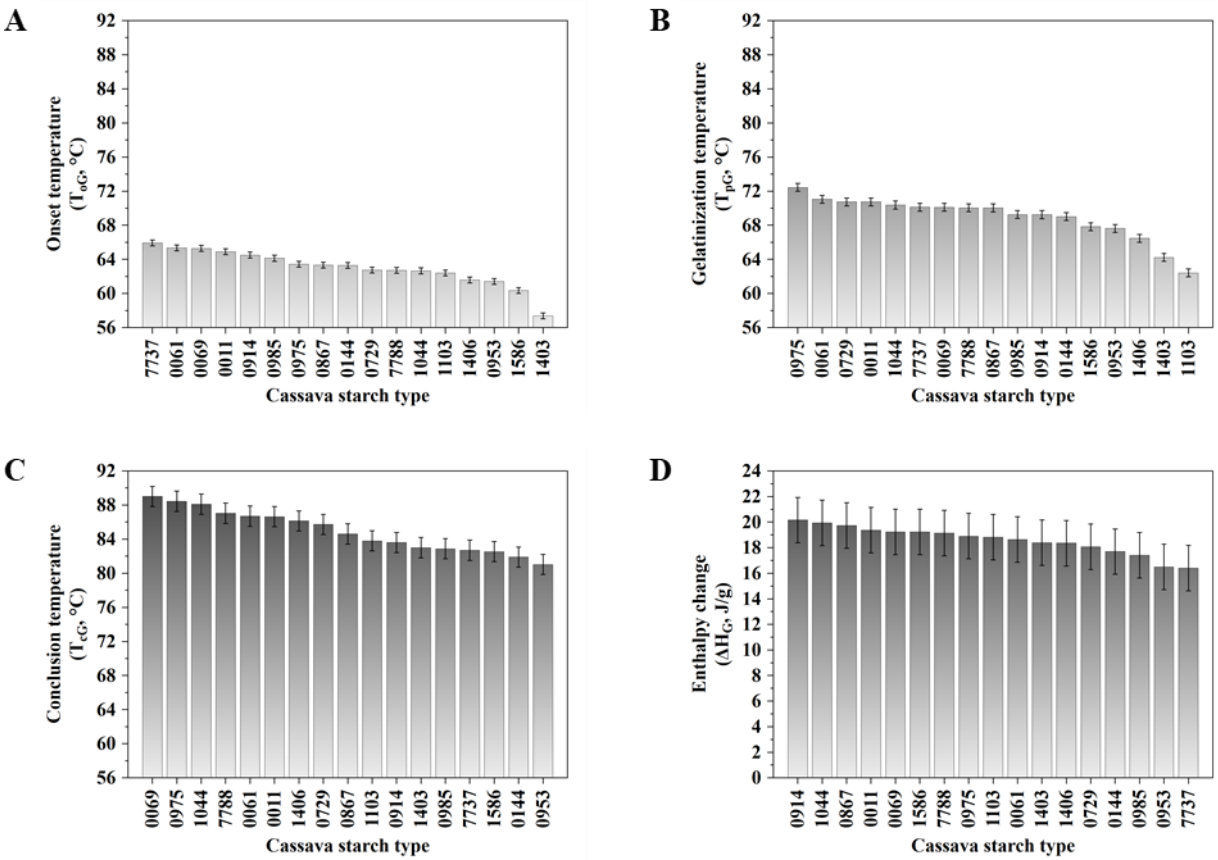


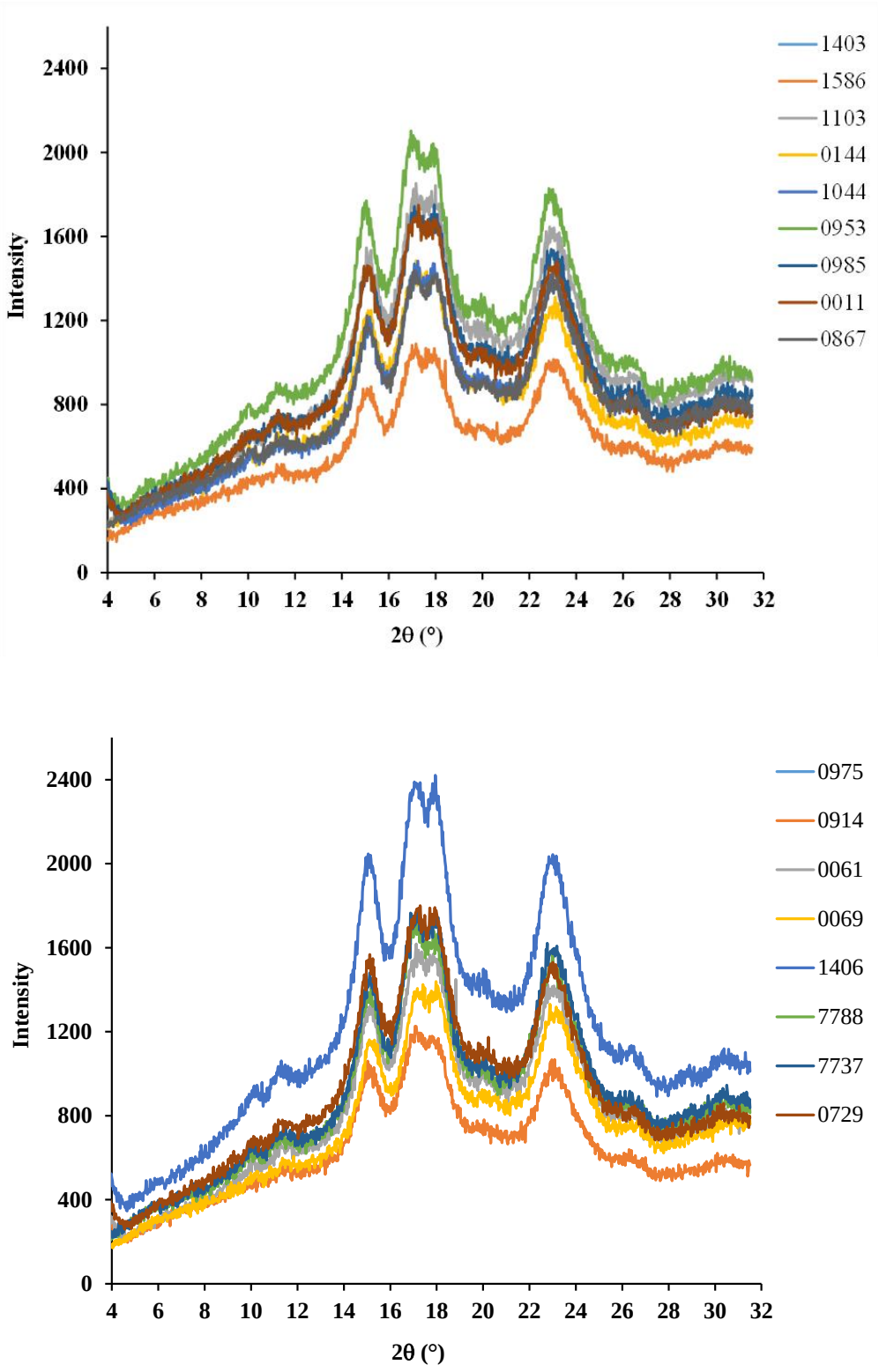
Figure 4. Differential scanning calorimeter (DSC) parameters from seventeen genotypes of cassava starches

4.1.4 Relative crystallinity by X-ray diffraction (XRD)

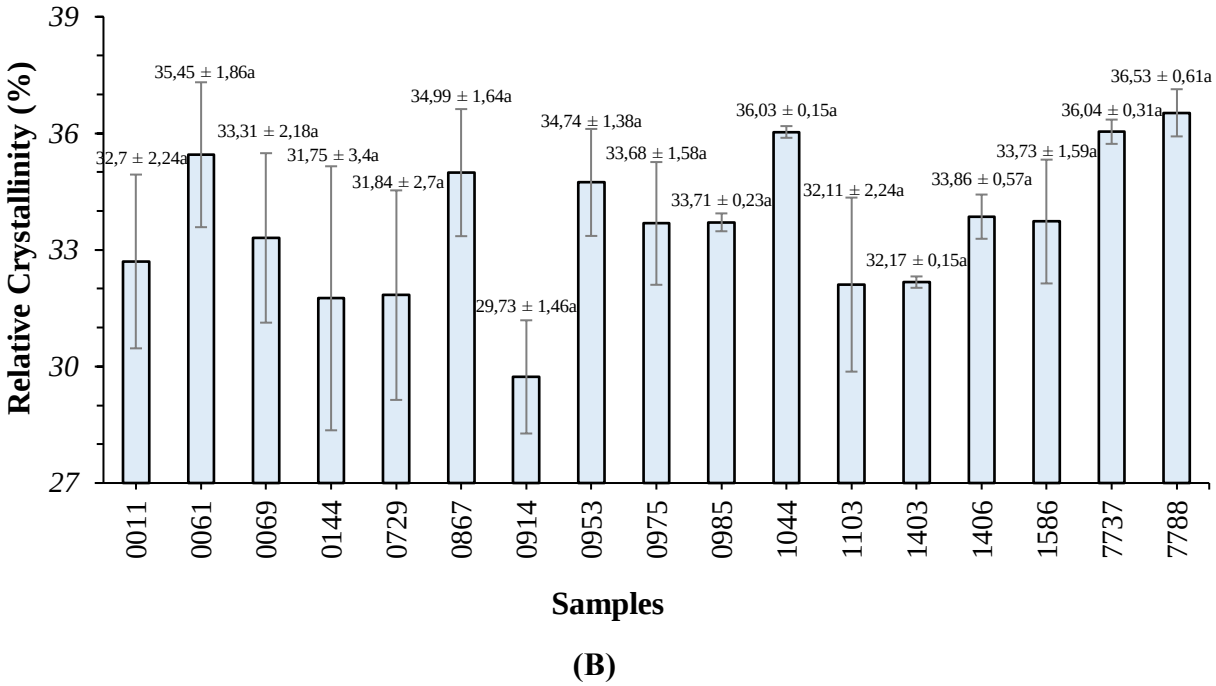
Granular starch is semi-crystalline consisting of amylose and amylopectin chains and can be classified into A-, B and C-type polymorphs. Literature data revealed cassava starches present an A- or C-type pattern (GOMAND et al., 2010; HE et al., 2020).

The X-Ray diffractograms are presented in Figure 5 A and the relative crystallinity are shown in Figure 5 B. The occurrences of peaks at 2θ were 15º, 17º, 18º and 24º in agreement with reported by Huang et al. (2007), Chen et al. (2010) and He tal al. (2020), relating that the crystallinity structure of cassava starch is an A-pattern. The degree of relative crystallinity was calculated from XRD patterns ranged from 29.73% (BGM 0914) to 36.52% (7788, a waxy cassava starch). The crystallinities of the cassava starches of this study were similar as

described by Morante et al. (2016) (35 %), and He et al. (2020) (25-40 %), but different from Gomand et al. (2010) (40-49%).



(A)



(B)

Figure 5. X-ray diffractograms (A) and relative crystallinity (B) of seventeen genotypes of cassava starches

According to He et al. (2020), cassava starches from different germplasms had similar XRD diffraction patterns with minor differences, indicating that the organization of the semicrystalline structure of the starches was not affected by the germplasms. The different crystallinities of different cassava starch germplasms depended on the source, moisture content of the starch, and if or not has been subjected to modification physical, chemical or enzymatic.

According to Srichuwong et al. (2005), PV demonstrated a positive correlation with chain length distributions and particle size. In fact, BGM 0914 showed the lowest relative crystallinity (29.73%), but presented the highest values of PV (4012.5 cP), SV (2673.0 cP), BV (2725.0 cP), and intermediate value of D[4,3] (15.21 µm). Contrary, the waxy cassava starch7788 presented the highest percentage of crystallinity (36.53%) however, demonstrated low values of PV (3507.5 cP), SV (837.5 cP), BV (2023.0 cP), and high value of D[4,3] (17.08 µm), indicating that low amylose content led to larger particle size.

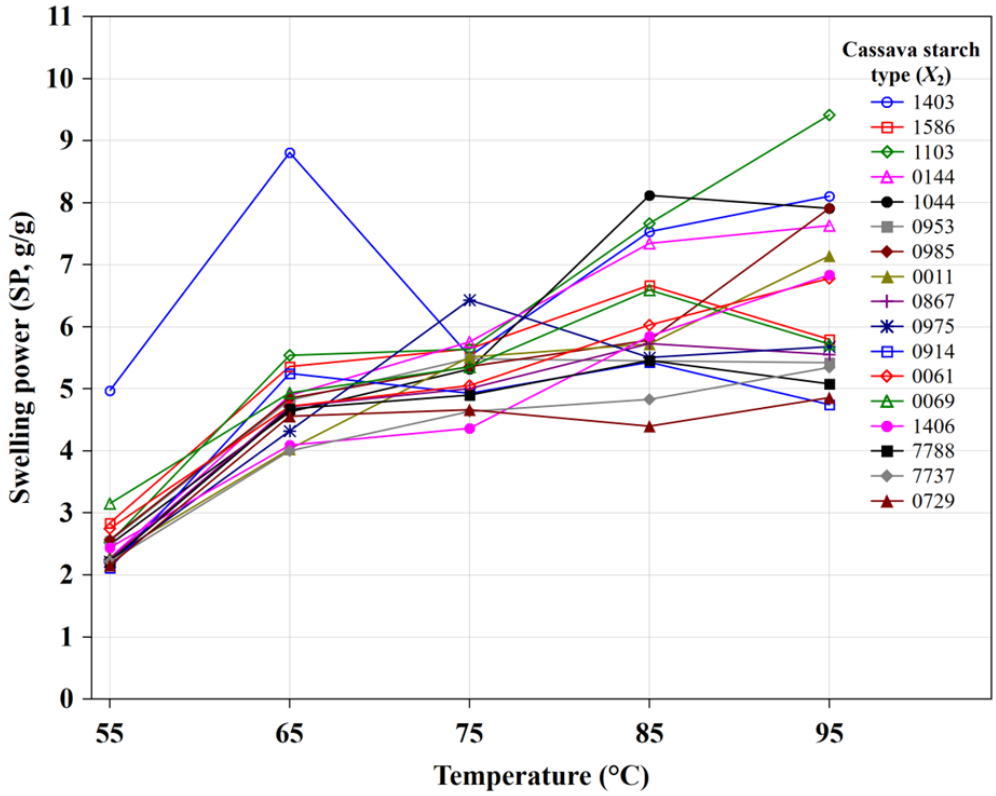
5 PHYSICOCHEMICAL PROPERTIES

5.1 Swelling Power and Solubility

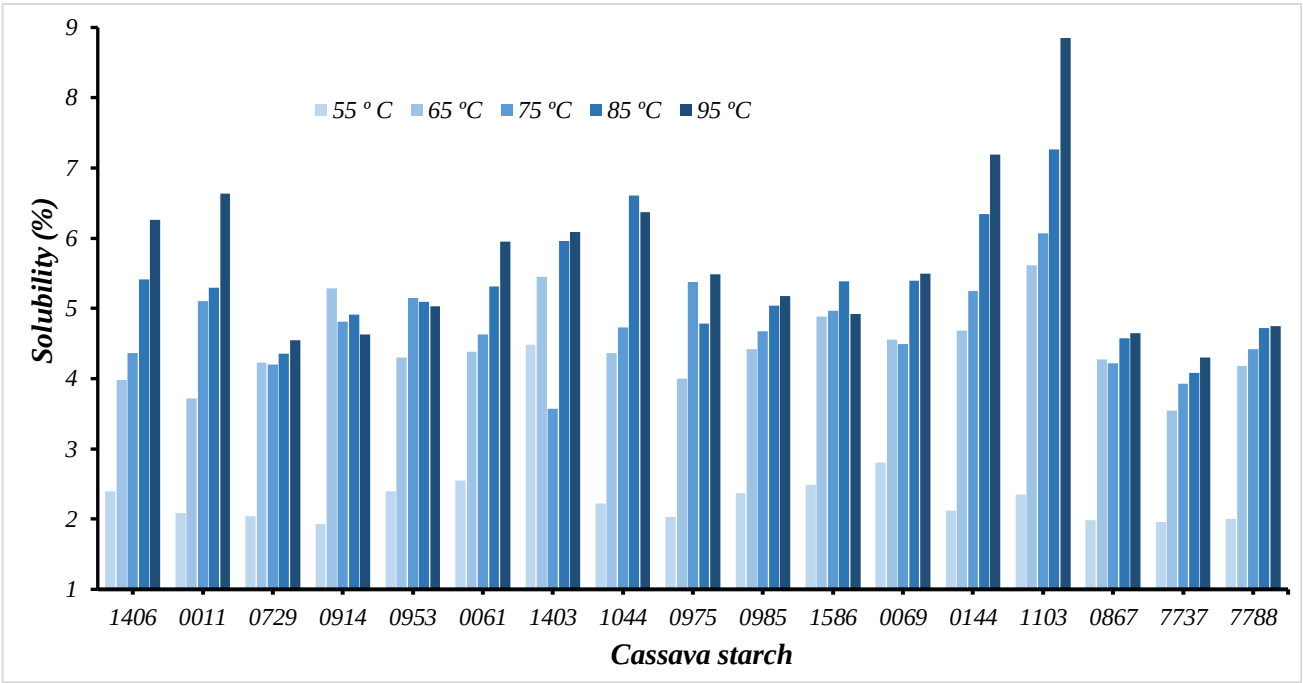
The swelling power and solubility index of cassava samples are shown in Figure 6, Figure 7 and Table 3 (Appendix). In general, the swelling power of all starches presented an abrupt increase at 75 ºC and decreasing as of 95 °C which is in agreement with the T_p and T_c determined by DSC (72 and 85 °C, respectively). During the gelatinization process, the maximum swelling occurs at T_p and decrease due to disruption and melting of the granule under agitation and heating. The amylose content of starches has been suggested to influence their ability to absorb water and differences among the samples could be explained by variations in starch granule size and an increase in relative crystallinity (KAYODE et al., 2022). BGM 1403 and BGM 0729 showed the maximum and minimum swelling performance at all temperatures, respectively. Concerning these samples, no influence was observed since the particle size presented the largest values (16 and 17 µm, Figure 2) and similar RC (32.2 and 31.8 %, Figure 5B).

Native cassava starches exhibit high swelling power and dispersed volume fraction compared with starches from other tuber crops. Comparisons between native and waxy cassava starch demonstrated that the latter increased the clarity and stability of gels and the granule melting temperature by almost 2 ºC, without affecting particle size or phosphorus contents (SANCHES et al., 2009).

The solubility between the samples at different temperatures were statistically significant ($p<0.05$). The solubility increased with temperature continually, especially at 75 °C to 95 ºC. The samples BGM 1103 showed high solubility in a wide temperature range. The general trend for solubility as affected by raising temperature agreed with previous studies on cassava starch. The solubility appeared similar to Sanchez et al. (2009), but lower than that of Ceballos et al. (2008). This is probably due to the difference in germplasms and quantification methods such as supernatant sampling method, centrifugation speed, etc. In this study, the difference in solubility of different germplasms under the same conditions may be related to the bond strength and structure in starch granules.



(A)



(B)

Figure 6. Curves of swelling power (A) and solubility index (B) from seventeen genotypes of cassava starches as a function of temperature

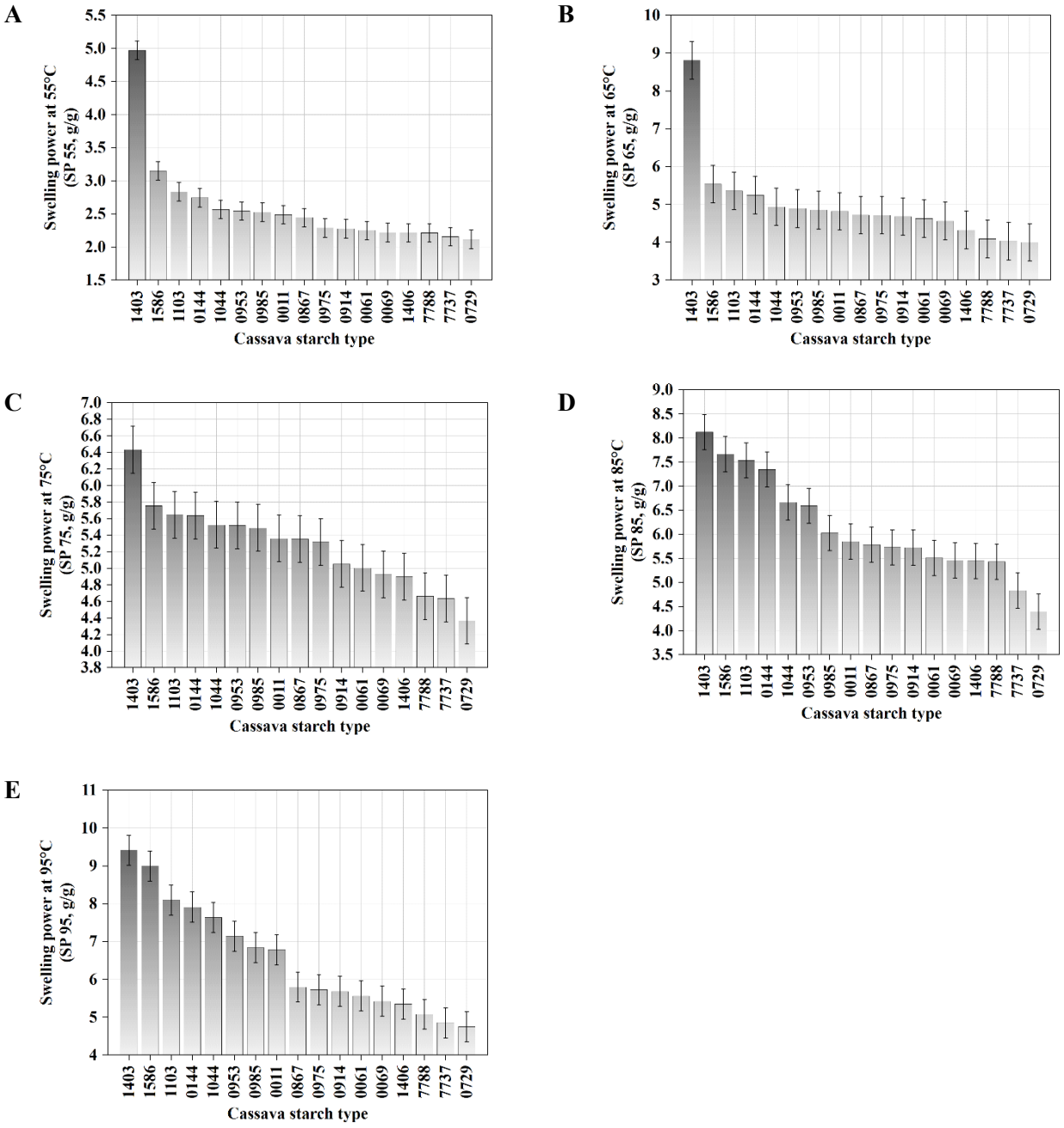


Figure 7. Swelling power at different temperatures (A-E) from seventeen genotypes of cassava starches

5.2 Freeze-thaw stability

The texture of cassava starch gels submitted to four consecutive freeze-thaw cycles and syneresis are presented in Figure 8 and Table 3 (Appendix). The visual aspect of cassava starch gels is shown in Figure 9.

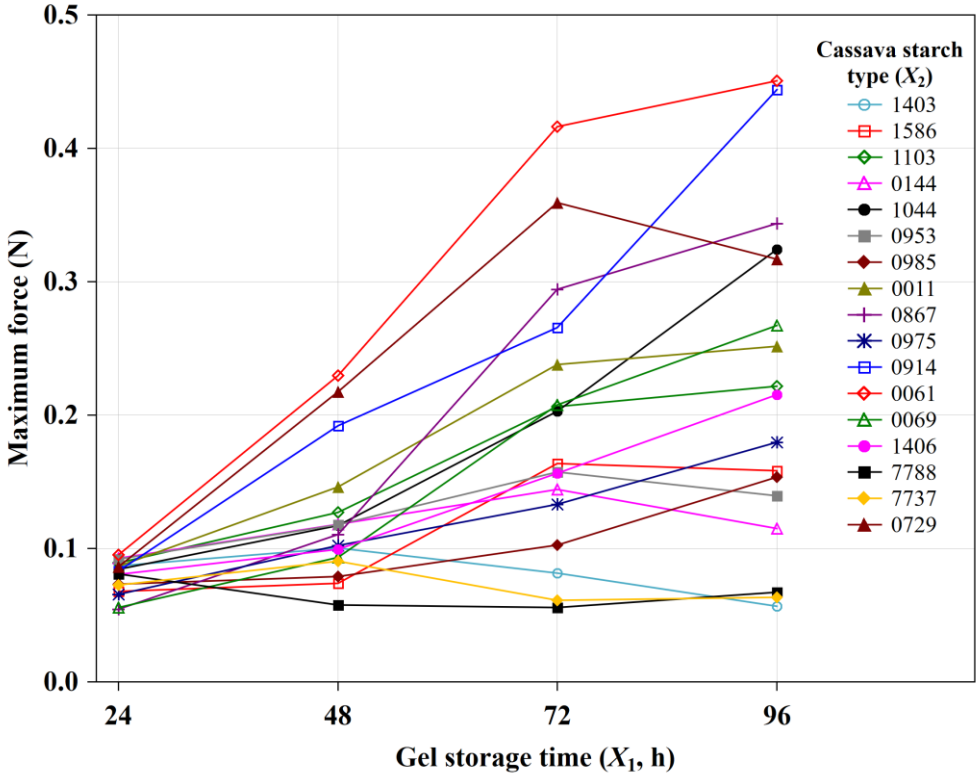
In general, after the first cycle a significant ($p < 0.05$) increase in maximum force was observed, except for waxy cassava starches (7737 and 7788 from CIAT) that remain continuously showing the lowest values of maximum force. After the fourth cycle, BGM 0061 (a non-waxy starch) presented the highest maximum force ($p < 0.05$) and BGM 7737 showed the lowest value. In fact, Takeiti et al. (2007) reported an increase in gel hardness from the first cycle specially for root tuber starch.

The high amylose content in the starch granule leads to form harder gels and a reduction in gel adhesiveness and hardness may be associated with the reduction in amylose content of the starch samples and an increase in the relative crystallinity. Starches with higher amylose content showed higher force (hardness) than starches with low amylose (KAYODE et al., 2022).

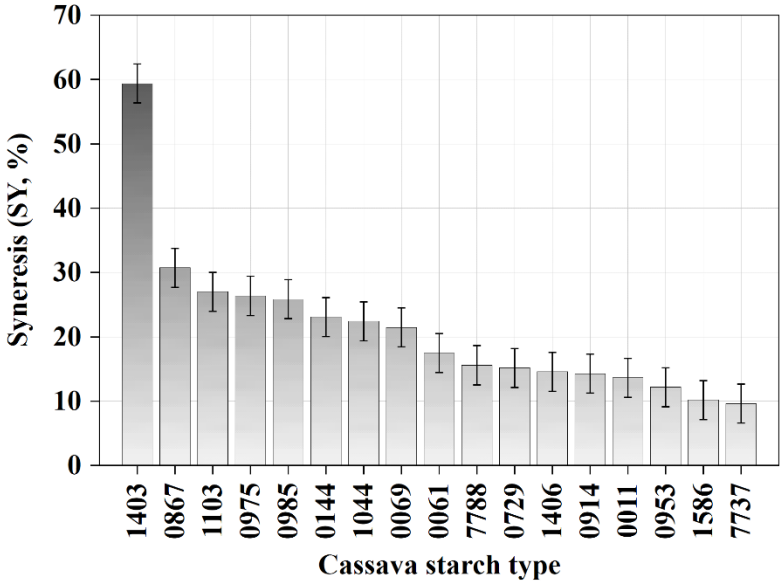
Therefore, rapid freezing has also been found to reduce the hardness and adhesiveness of starch during storage. Starch gel textural features are very important parameters that can be used to determine the potential use of starches in various food applications.

Relating syneresis and maximum force (Figure 8A; B), BGM 1403 showed higher syneresis (59.4%) and lower maximum force than 7737 (waxy) which demonstrated low values of both after the fourth freeze-thaw cycle. This result corroborates with the RVA parameters in which 7737 presented high final viscosity value during the cooling step (Figure 3, 4223.5 cP).

Visual aspects of cassava starch gels are presented in Figure 9. In T_0 all the samples showed bright and transparent gels, varying in gel force, fundamentally. In T24 hs all the samples lose in bright and force, taking on an opaque appearance, since T 48hs it can be observed syneresis, and surface changes (wrinkle). Finally, in T 96 hs was observed maximum degree of syneresis and opacity, with differences among the samples.



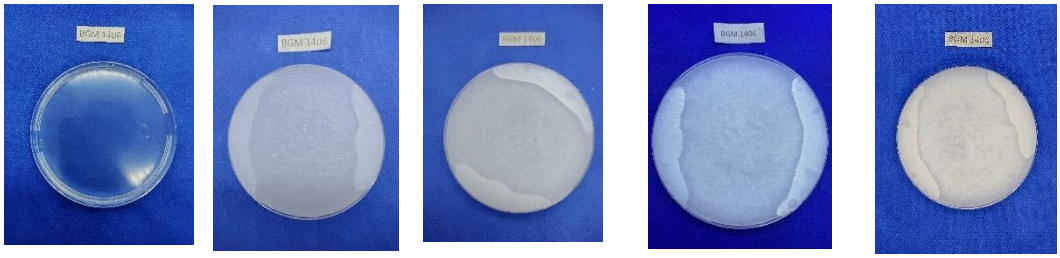
(A)



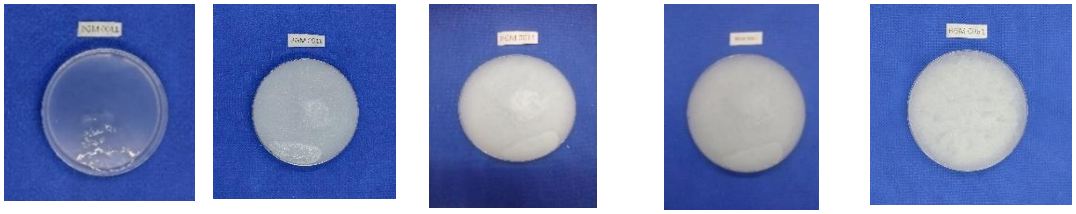
(B)

Figure 8. Curves of maximum force (A) and syneresis (B) from seventeen genotypes of cassava starch gels as a function of storage time

1406 T0 **T 24 hs** **T 48 hs** **T 72 hs** **T 96 hs**

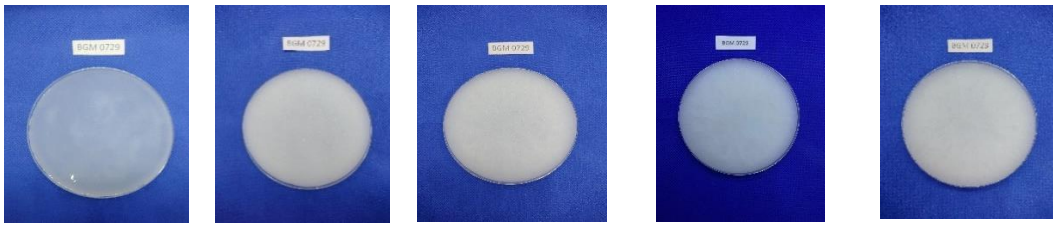


0011 T0 **T 24 hs** **T 48 hs** **T 72 hs** **T 96 hs**

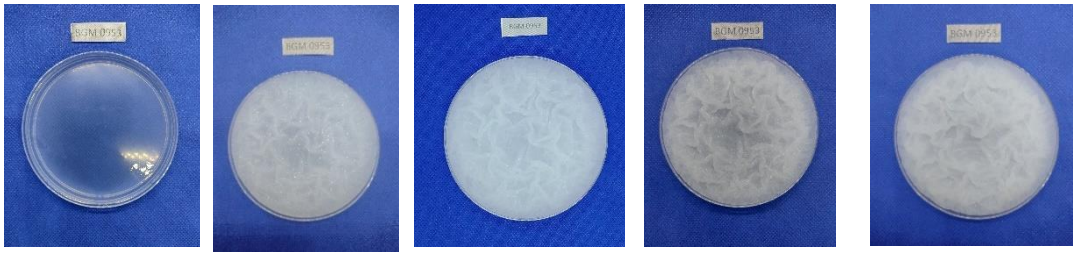


0914 T0 **T 24 hs** **T 48 hs** **T 72 hs** **T 96 hs**

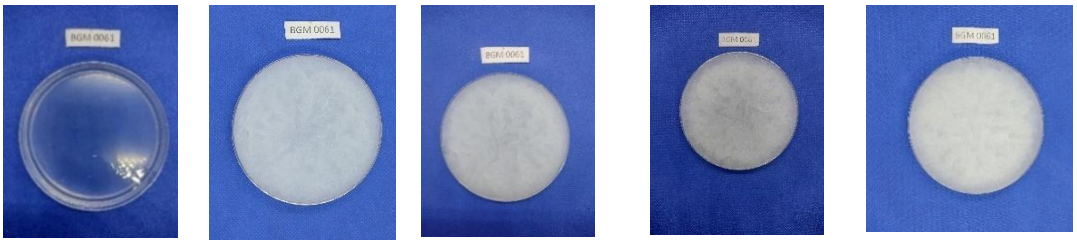
0729 T0 **T 24 hs** **T 48 hs** **T72 hs** **T96 hs**



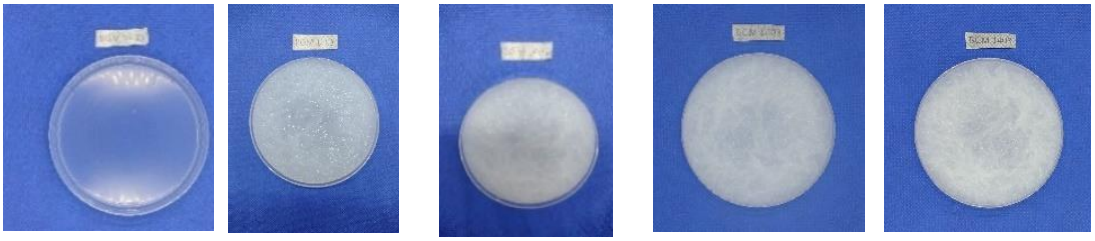
0953 T0 **T 24 hs** **T 48 hs** **T 72 hs** **T 96 hs**



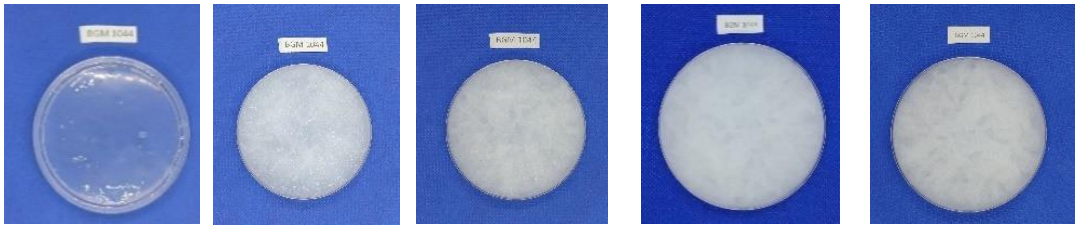
0061 T0 **T 24 hs** **T48 hs** **T 72 hs** **T 96 hs**



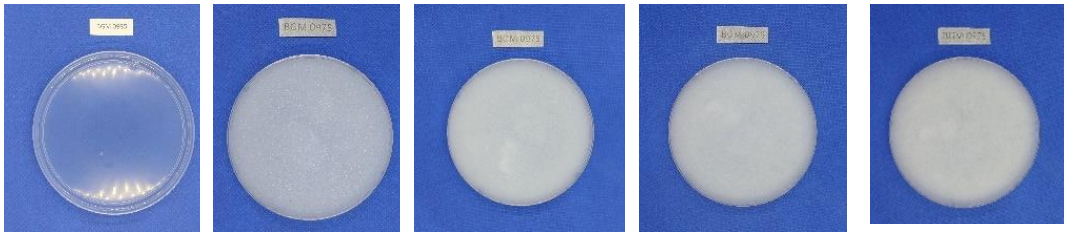
1403 T0 **T24 hs** **T 48 hs** **T 72 hs** **T 96 hs**



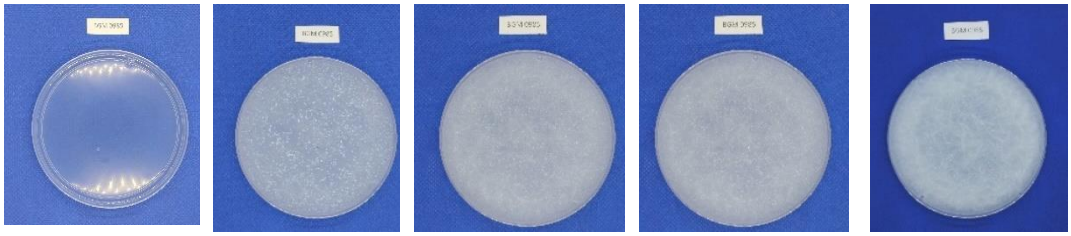
1044 T0 **T 24hs** **T48 hs** **T 72 hs** **T 96 hs**

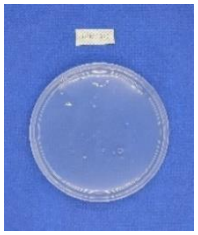
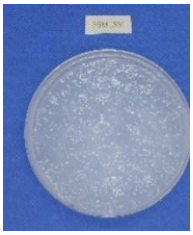
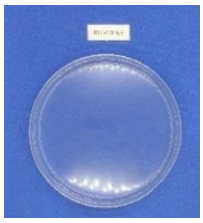
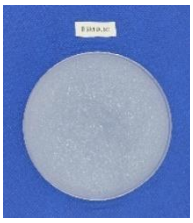
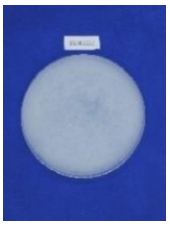
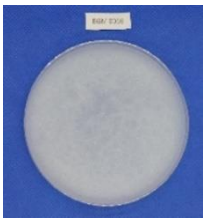
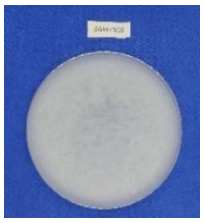
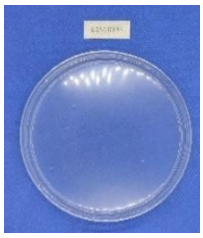
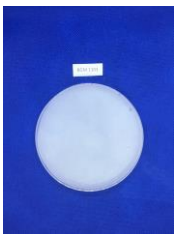
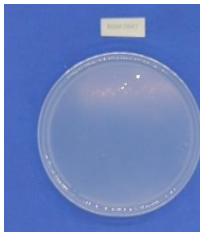


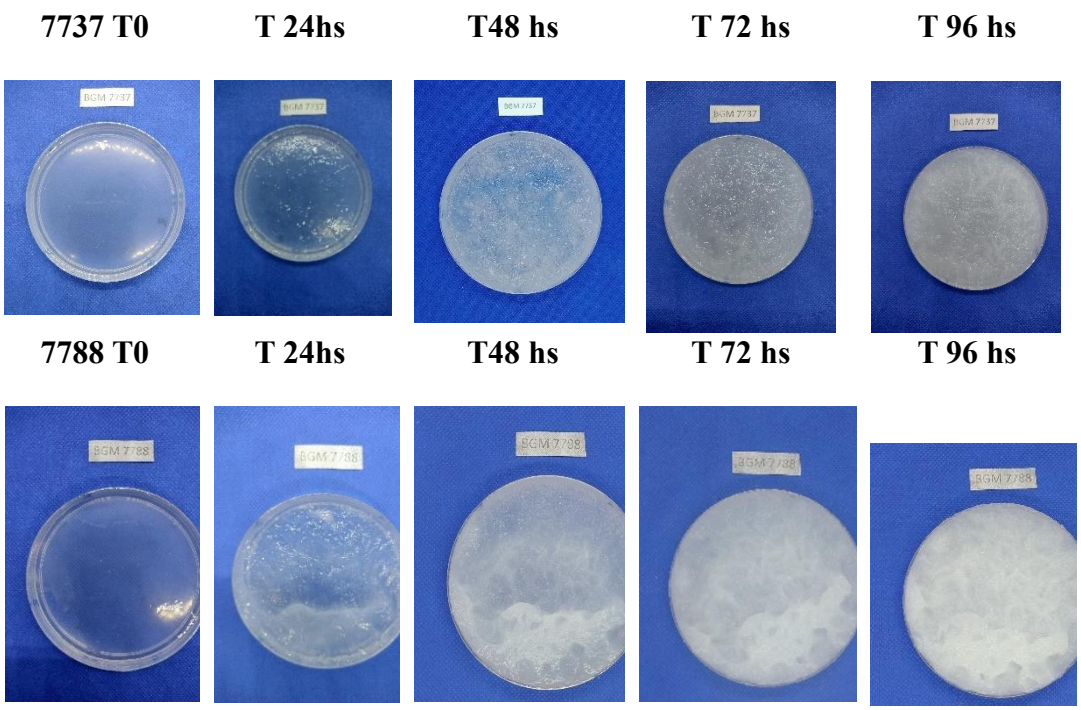
0975 T0 **T 24hs** **T48 hs** **T 72 hs** **T 96 hs**



0985 T0 **T 24hs** **T48 hs** **T 72 hs** **T 96 hs**



1586 T0	T 24hs	T48 hs	T 72 hs	T 96 hs
				
0069 T0	**T 24hs**	**T48 hs**	**T 72 hs**	**T 96 hs**
				
0144 T0	**T 24hs**	**T48 hs**	**T 72 hs**	**T 96 hs**
				
1103 T0	**T 24hs**	**T48 hs**	**T 72 hs**	**T 96 hs**
				
0867 T0	**T 24hs**	**T48 hs**	**T 72 hs**	**T 96 hs**
				



Credits: Palheta, S.

Figure 9. Images of cassava starch gels as a function of storage time

6 PAIRWISE CORRELATION COEFFICIENT (r) AND PRINCIPAL COMPONENT ANALYSIS (PCA) OF DIFFRENT CASSAVA STARCH PARAMETERS

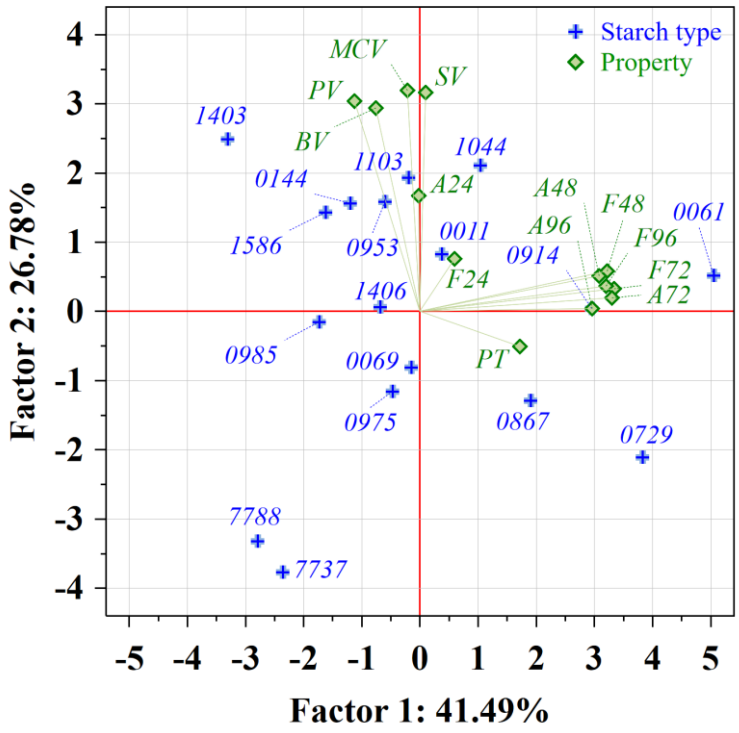
A correlation-focused was undertaken to understand the relationship between RVA and texture parameters of cassava gels (Figure 10). Variability explained by Dim 1 and Dim 2 was 41.49 % and 26.78 %, respectively, thus accounting for 68.27 % of total variability. Based on the results of Principal Component Analysis (PCA), four samples were elected in order to determine the amylose and resistant starch contents (BGM 1403,7737, BGM 0061, and BGM 0729) since they showed the highest vectors. Dim 1 is described by only P_T. In contrast, the most variables are described by Dim 2: PV, MCV, BV, SV, F and A. The parameters of the close vectors are positive and correlated.

In Figure 10 B, another four samples were elected, considering To, PV, PT and ΔH (BGM 1403, BGM 1586, 7737 and BGM 0069). Variability explained by Dim 1 and Dim 2 was 53.98 % and 27.75 %, respectively, thus accounting for 81.73 % of total

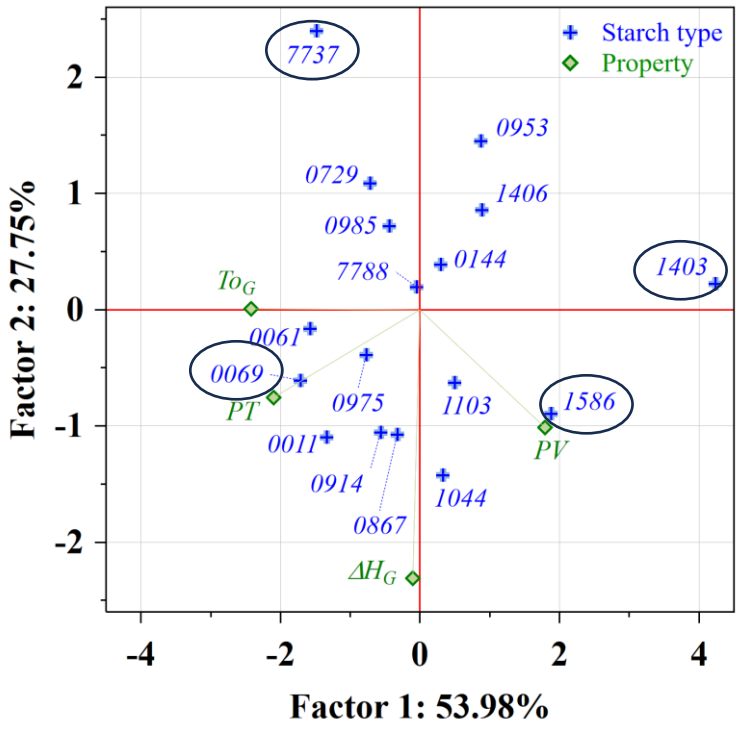
variability. Dim 1 is described by only PV. The other variabless are described by Dim To, PT and ΔH. The parameters of the close vectors are positive and correlated.

Finally, in Figure 10 C, another four samples were elected, considering MCV, SV, F 96 and Syneresis (BGM 0729, 7737, BGM 1044 and BGM 1403). Variability explained by Dim 1 and Dim 2 was 53.17 % and 29.84 %, respectively, thus accounting for 83.01 % of total variability. Dim 2 is described by MCV, SV, F 96 and Syneresis. The parameters of the close vectors are positive and correlated.

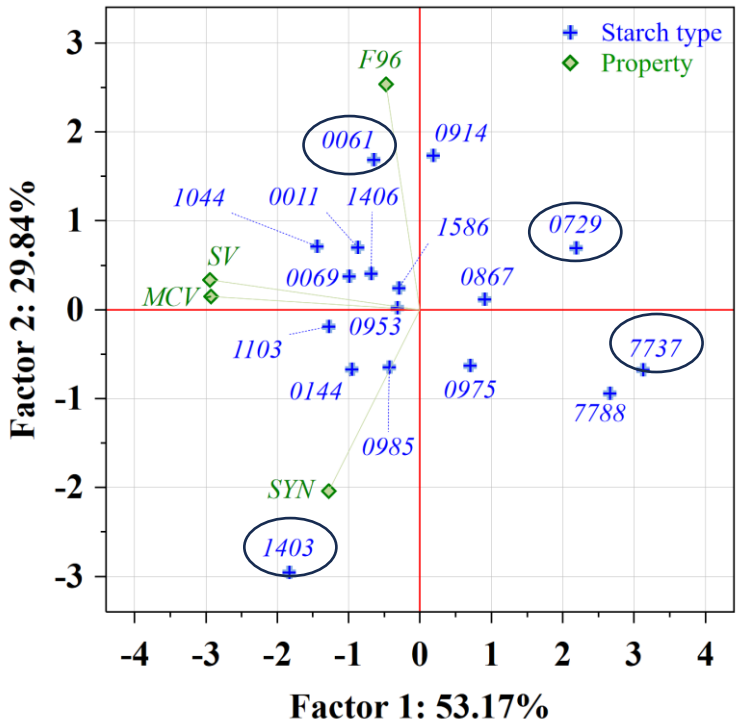
The results highlight BGM 1403 and 7737 they present themselves as complementary opposites in physical and physico-chemical properties.



(A)



(B)



(C)

Figure 10. **(A)** Principal component analysis (PCA) for RVA and texture parameters of cassava starch samples from 17 different germplasm accessions. (PT: pasting temperature; PV: peak viscosity; BV: breakdown viscosity; MCV: maximum cooling viscosity; SV: setback viscosity. F24, F48, F72, F96: maximum force of the starch gels after 24, 48, 72, and 96 h, respectively. A24, A48, A72, A96: area under the force curve

of the starch gels after 24, 48, 72, and 96 h, respectively. **(B)** PCA for some pasting and thermal properties (heating period) (To: onset temperature, ΔHG: gelatinization enthalpy). **(C)** PCA for some pasting and thermal properties (cooling period). (SYN: Syneresis)

7. AMYLOSE AND RESISTANT STARCH CONTENT

Regarding amylose and resistant starch (RS) contents (Table 1), values ranged between 6.02 % (7737) to 16.01 % (BGM 1403) and 1.5% (BGM 1403) to 16.03 % (BGM 0061), respectively. Pasting Properties are controlled by a significant proportion of amylose and amylopectin. Therefore, the performance of BGM 1403 corroborated with the RVA results, exhibiting the highest values for PV, BV, and syneresis and low values for MCV and T_0 (onset). The sample 7737 is a waxy starch and the performance is characterized by the lowest values of MCV, SV, % syneresis, but the highest T_0 value. The samples BGM 1403 and BGM 0061 showed highest values for SB indicating are not recommended to frozen storage due to the tendency to syneresis.

The samples BGM 0061 and BGM 0729 presented significantly resistant starch content that is known as healthy starch with proven health benefits including decreasing meal-associated hyperglycemia in diabetics and prevention of colonic câncer (WANG et al., 2022).

Table 1. Comparison between 4 samples of cassava starch

Parameter	**Unit**	**Cassava starch**			
		1403	**0061**	**0729**	**7737**
Setback	(cP)	3543.0	3230,0	1373.5	757.0
Amilose	(g/100 g)	16.01	10,47	14.67	6.02
AR	(g/100 g)	1.5	16,03	5.59	1.97
D [4,3]	(um)	15.44	15,85	17.35	14.64
Entalpia	(J/g)	18.39	18,65	18.07	16.41

6 CONCLUSION

This study investigated the structural, functional and technological features of naturally modified cassava starches. It was found that breeding did not affected the morphological characteristics of seventeen germplasm accessions of cassava starches studied since they presented round shape and particle size in agreement with the literature. The pasting properties revealed that naturally modified starches presented different

aptitudes for using as food ingredients, especially in refrigerated and frozen formulations. Gelatinization peak temperature (Tp) occurred between 62.4 and 72.4 °C showing A-crystal pattern with maximum relative crystallinity of 36.5 % (7788, a waxy starch). Overall, the swelling power presented an abrupt increase at 75 ºC and reduction at 95 °C. Concerning freeze-thaw stability, a significant increase in maximum force ($p < 0.05$) was observed after the first cycle, except for waxy cassava starches (7737 and 7788) that showed the lowest values during storage time as well as syneresis. In relation to amylose content, 7737 presented the lowest value (6.0 %) leading to the lowest gelatinization enthalpy observed (ΔH_G = 16.4 J/g). BGM 0061 showed 16% of resistant starch that would be considered a relevant functional ingredient in terms of gut health maintenance. The application of those starches in food products would be part of further studies thinking on refrigerated and frozen products, bioplastic packaging and as thickeners or stabilizers in food systems.

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CONCLUSÃO GERAL

A aplicação do amido de mandioca no desenvolvimento das formulações de produtos alimentícios é guiada pelo entendimento de que suas propriedades físico-químicas e funcionais apresentam características desejáveis à industrialização de produtos. Amidos de mandioca naturalmente modificados têm despertado o olhar de programas de melhoramento genético possibilitando a modificação de suas propriedades de acordo com as aplicações de interesse devido à alta claridade e viscosidade de pasta, além da baixa tendência à retrogradação se comparada ao amido dos cereais. Dados da literatura científica sobre amidos naturalmente modificados oriundos dos programas de melhoramento genético são insuficientes pelo baixo número de amostras estudadas. Deste modo, esta dissertação de mestrado teve por objetivo estudar o estado da arte entre os anos de 2008 a 2023 sobre amidos naturalmente modificados por técnicas de bases convencionais e biotecnológicas. Os dados da literatura científica (sete) reportaram que os cruzamentos genéticos resultavam em modificações das temperaturas de pasta e de viscosidade de géis dos seus amidos e que não afetou as características morfológicas e de tamanho dos grânulos de amido. Estes resultados reforçam os achados obtidos no estudo experimental da dissertação onde as propriedades térmicas e de pasta sugerem sua utilização em produtos refrigerados e congelados dada sua baixa tendência à retrogradação e sinérese. De modo geral, o poder de inchamento apresentou uma elevação abrupta aos 75 ºC com redução significativa a partir dos 95 ºC. Quanto à estabilidade aos ciclos de congelamento e descongelamento, observou-se um aumento significativo ($p<0.05$) logo após o primeiro ciclo, exceto para as amostras 7737 e 7788, que são caracterizados como amidos cerosos. Com relação às determinações da química de carboidratos complexos, a amostra 7737 apresentou o menor valor do conteúdo de amilose (6 %) o que levou à baixa entalpia de gelatinização observada dentre as amostras estudadas ($\Delta H_G = 16.4$ J/g). A amostra BGM 0061 demonstrou um alto conteúdo de amido resistente (16 %), o que a torna um material relevante para a produção de ingrediente funcional em termos da manutenção da eubiose da microbiota intestinal tornando possível sua aplicação em produtos alimentícios e com apelo nutricional.

As perspectivas de futuros trabalhos devem incluir o estudo das propriedades emulsificantes sob diferentes pHs, temperaturas, adição de solutos como açúcares e

sais, propriedades espumantes e propriedades de barreira como embalagens biodegradáveis para alimentos.

8 Appendix

Table 1. Thermal properties, particle properties and relative crystallinity of seventeen samples cassava starches.

Starch type	Thermal properties				Particle properties				Relative crystallinity (%)
	To (°C)	Tp (°C)	Tc (°C)	ΔH (J/g)	ρ_{ap} (kg/m³)	D[4,3] (μm)	D_{10} (μm)	D_{90} (μm)	
1403	57.37±0.11 *j*	64.24±0.52 *h*	83.00±1.29 *efgh*	18.39±2.47 *a*	651.8±4.5 *de*	15.83±0.55 *cd*	7.47±0.38 *a*	24.06± 0.73*cde*	31.84± 2.7 *a*
1586	60.36±0.07 *i*	67.84±0.08 *ef*	82.54±0.07 *fgh*	19.23±1.99 *a*	597.6±1.4 *c*	15.13±0.32 *cd*	5.82±0.55 *bcdef*	23.07± 0.33*de*	33.71± 0.23 *a*
1103	62.42±0.05 *fg*	62.42±0.05 *i*	83.81±0.57 *defgh*	18.82±0.34 *a*	626.7±6.6 *ef*	12.16±0.35 *g*	3.44±0.31 *i*	18.96± 0.37*h*	31.75± 3.4 *a*
0144	63.29±0.04 *def*	69.02±0.17 *de*	81.90±1.42 *gh*	17.70±0.76 *a*	639.8±10.5 *bc*	13.90±0.45 *ef*	4.98±0.75 *efgh*	21.17± 0.35*fg*	35.45± 1.86 *a*
1044	62.66±0.06 *ef*	70.38±0.68 *bc*	88.11±0.36 *ab*	19.94±0.55 *a*	586.2±6.2 *ef*	13.11±0.22 *fg*	4.46±0.06 *fghi*	20.14± 0.38*gh*	33.73± 1.59 *a*
0953	61.42±0.16 *h*	67.63±0.03 *fg*	81.03±0.50 *h*	16.50±1.59 *a*	637.0±0.3 *def*	15.10±0.08 *cde*	5.33±0.29 *cdefgh*	23.20± 0.05*de*	32.11± 2.24 *a*
0985	64.14±0.35 *cd*	69.26±0.52 *cd*	82.88±0.54 *fgh*	17.42±1.25 *a*	589.2±1.7 *abc*	15.83±0.19 *cd*	6.53±0.08 *abcd*	24.17± 0.35*cde*	33.68± 1.58 *a*
0011	64.91±0.18 *bc*	70.74±0.17 *b*	86.64±0.00 *abcd*	19.38±0.66 *a*	573.7±3.5 *d*	15.41±0.03 *cd*	6.71±0.20 *abc*	23.17± 0.03*de*	34.99± 1.64 *a*
0867	63.33±0.45 *def*	70.04±0.26 *bcd*	84.62±0.57 *cdefg*	19.75±1.49 *a*	574.1±3.5 *def*	16.21±0.60 *abc*	5.29±0.46 *defgh*	25.22± 1.06*abc*	32.17± 0.15 *a*
0975	63.44±0.02 *de*	72.45±0.37 *a*	88.45±0.28 *ab*	18.91±1.48 *a*	632.1±0.1 *ef*	15.94±0.10 *bc*	6.20±0.51 *abcde*	24.42± 0.03*bcd*	34.74± 1.38 *a*
0914	64.51±0.22 *bc*	69.26±0.06 *cd*	83.61±0.86 *defgh*	20.16±1.16 *a*	619.4±0.8 *bc*	15.21±0.20 *cd*	6.22±0.08 *abcde*	23.09± 0.40*de*	32.7± 2.24 *a*
0061	65.35±0.13 *ab*	71.06±0.36 *b*	86.69±0.35 *abcd*	18.65±0.88 *a*	644.8±4.5 *abc*	15.85±0.15 *bcd*	6.18±0.06 *abcde*	24.14± 0.28*cde*	33.86± 0.57 *a*
0069	65.30±0.11 *ab*	70.12±0.03 *bcd*	89.01±0.21 *a*	19.24±0.18 *a*	627.9±0.1 *ab*	14.63±0.40 *de*	4.31±0.26 *ghi*	22.46± 0.67*ef*	29.73± 1.46 *a*
1406	61.58±0.17 *gh*	66.47±0.28 *g*	86.14±2.14 *abcde*	18.36±0.50 *a*	574.2±0.8 *a*	15.49±0.08 *cd*	6.08±0.24 *abcde*	23.35± 0.07*de*	36.03± 0.15 *a*
7788	62.72±0.47 *ef*	70.05±0.25 *bcd*	87.04±0.14 *abc*	19.15±0.37 *a*	577.6±9.3 *def*	17.08±0.13 *ab*	5.65±0.33 *bcdefg*	26.11± 0.19*ab*	36.53± 0.61 *a*
7737	65.94±0.45 *a*	70.13±0.33 *bcd*	82.70±0.43 *fgh*	16.41±0.30 *a*	577.4±9.3 *f*	14.64±0.01 *de*	4.13±0.13 *hi*	22.42± 0.01*ef*	36.04± 0.31 *a*
0729	62.76±0.09 *ef*	70.75±0.11 *b*	85.73±0.00 *bcdef*	18.07±1.22 *a*	567.2±4.2 *abc*	17.35±0.38 *a*	6.75±0.30 *ab*	26.65± 0.81*a*	33.31± 2.18 *a*

Gelatinization temperatures: To, onset; Tp, peak; Tc, conclusion; ΔH: gelatinization enthalpy change; ρ_{ap}: apparent density; D[4,3]: mean volume diameter; D_{10}: small granule size; D_{90}: large granule size. Different lowercase letters in the same column show a significant statistical difference at $p < 0.05$ by Tukey's test

Table 2. Pasting viscosity parameters from viscosity curves of seventeen cassava starch suspensions.

Starch type	PT °C		PV cP		TV cP		BV cP		MCV cP		SV cP		FV cP	
1403	65.88 ± 0.18	*i*	5479.0 ± 14.1	*a*	1547.0 ± 21.2	*a*	3932.0 ± 7.1	*a*	5090.0 ± 107.5	*abc*	3543.0 ± 86.3	*ab*	4223.5 ± 92.6	*abc*
1586	69.50 ± 0.14	*fgh*	5215.5 ± 4.9	*ab*	1571.0 ± 7.1	*a*	3644.5 ± 2.1	*ab*	4782.5 ± 20.5	*bcde*	3211.5 ± 13.4	*bc*	4302.0 ± 0.0	*abc*
1103	71.03 ± 0.25	*cdef*	4887.0 ± 90.5	*bc*	1402.5 ± 26.2	*bc*	3484.5 ± 64.3	*cb*	5101.0 ± 63.6	*ab*	3698.5 ± 89.8	*a*	4528.0 ± 80.6	*a*
0144	70.68 ± 0.53	*defg*	4787.5 ± 43.1	*cd*	1564.0 ± 12.7	*a*	3223.5 ± 55.9	*cd*	5080.0 ± 1.4	*abc*	3516.0 ± 11.3	*ab*	4303.5 ± 51.6	*abc*
1044	70.90 ± 0.42	*cdef*	4743.5 ± 33.2	*cd*	1555.5 ± 10.6	*a*	3188.0 ± 43.8	*cde*	5333.0 ± 70.7	*a*	3777.5 ± 60.1	*a*	4401.0 ± 12.7	*ab*
0953	70.50 ± 0.42	*defg*	4656.5 ± 156.3	*cde*	1554.0 ± 19.8	*a*	3102.5 ± 136.5	*de*	4764.0 ± 83.4	*cde*	3210.0 ± 63.6	*bc*	4196.5 ± 10.6	*abc*
0985	71.30 ± 0.42	*bcde*	4426.5 ± 10.6	*def*	1544.0 ± 72.1	*a*	2882.5 ± 61.5	*defg*	4566.0 ± 35.4	*e*	3022.0 ± 107.5	*c*	4117.0 ± 18.4	*bc*
0011	73.00 ± 0.78	*a*	4362.5 ± 36.1	*efg*	1400.5 ± 29.0	*bc*	2962.0 ± 65.1	*def*	5017.5 ± 6.4	*abc*	3617.0 ± 22.6	*a*	4317.5 ± 37.5	*abc*
0867	71.28 ± 0.18	*bcde*	4308.5 ± 19.1	*efg*	1207.0 ± 15.6	*fg*	3101.5 ± 3.5	*de*	3082.5 ± 6.4	*h*	1875.5 ± 9.2	*f*	3028.0 ± 0.0	*e*
0975	72.05 ± 0.07	*bacd*	4063.0 ± 8.5	*fgh*	1230.5 ± 6.4	*efg*	2832.5 ± 2.1	*efgh*	3459.5 ± 7.8	*g*	2229.0 ± 14.1	*e*	3189.0 ± 38.2	*e*
0914	70.20 ± 0.07	*efg*	4012.5 ± 19.1	*gh*	1287.5 ± 10.6	*def*	2725.0 ± 8.5	*fghi*	3960.5 ± 21.9	*f*	2673.0 ± 32.5	*d*	3655.0 ± 22.6	*d*
0061	72.40 ± 0.28	*abc*	3937.0 ± 67.9	*h*	1377.0 ± 36.8	*bcd*	2560.0 ± 31.1	*ghij*	4607.0 ± 17.0	*de*	3230.0 ± 53.7	*bc*	4188.5 ± 91.2	*bc*
0069	72.60 ± 0.57	*ab*	3837.5 ± 53.0	*hi*	1336.0 ± 29.7	*cde*	2501.5 ± 82.7	*hij*	4914.0 ± 8.5	*bcd*	3578.0 ± 38.2	*a*	4077.5 ± 156.3	*bc*
1406	68.20 ± 0.14	*h*	3773.0 ± 302.6	*hi*	1336.0 ± 17.0	*cde*	2437.0 ± 285.7	*ijk*	4840.5 ± 290.6	*bcde*	3504.5 ± 273.7	*ab*	4005.0 ± 254.6	*c*
7788	69.30 ± 0.07	*gh*	3507.5 ± 29.0	*ij*	1484.5 ± 46.0	*ab*	2023.0 ± 75.0	*l*	2322.0 ± 19.8	*i*	837.5 ± 65.8	*h*	2216.0 ± 8.5	*f*
7737	70.20 ± 0.78	*efg*	3241.5 ± 47.4	*jk*	1122.5 ± 12.0	*g*	2119.0 ± 59.4	*kl*	1879.5 ± 9.2	*j*	757.0 ± 21.2	*h*	1787.0 ± 4.2	*g*
0729	70.50 ± 0.07	*defg*	3123.0 ± 33.9	*k*	794.5 ± 12.0	*h*	2328.5 ± 21.9	*jkl*	2168.0 ± 22.6	*ij*	1373.5 ± 10.6	*g*	2167.5 ± 23.3	*f*

PT: pasting temperature; PV: peak viscosity; TV: trough viscosity; BV: breakdown viscosity; MCV Maximum cooling viscosity; SV: setback viscosity; FV: final viscosity. Different lowercase letters in the same column show a significant statistical difference at $p < 0.05$ by Tukey's test.

Table 3. Texture parameters from force and area curves of seventeen samples of cassava starch gels at different storage times.

Starch type	Maximum force (N) of cassava starch gel after				Area (N·mm) under the force curve of cassava starch gel after			
	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h
1403	0.087±0.014 *aAB*	0.100±0.014 *cdeA*	0.082±0.000 *fghAB*	0.057±0.001 *hB*	0.994±0.115 *abA*	0.925±0.048 *cdeA*	0.783±0.019 *hijAB*	0.560± 0.008 *hiB*
1586	0.068±0.006 *aB*	0.074±0.010 *deB*	0.164±0.002 *defA*	0.158±0.021 *defghA*	0.766±0.022 *abC*	0.883±0.063 *deBC*	1.298±0.006 *fghAB*	1.537± 0.215 *defghA*
1103	0.089±0.009 *aB*	0.127±0.009 *bcdAB*	0.206±0.028 *cdeAB*	0.222±0.054 *cdefA*	1.001±0.045 *abB*	0.979±0.034 *cdeB*	1.598±0.208 *defA*	1.663± 0.173 *cdefgA*
0144	0.093±0.004 *aB*	0.118±0.003 *cdeAB*	0.144±0.014 *defghA*	0.115±0.009 *fghAB*	1.023±0.031 *abA*	1.041±0.010 *cdeA*	1.087±0.052 *fghijA*	0.988± 0.190 *efghiA*
1044	0.085±0.011 *aC*	0.117±0.012 *cdeBC*	0.203±0.010 *cdeB*	0.324±0.044 *bcA*	0.997±0.146 *abC*	1.008±0.152 *cdeC*	1.608±0.096 *defB*	2.740± 0.179 *abcA*
0953	0.092±0.025 *aA*	0.118±0.023 *cdeA*	0.157±0.009 *defgA*	0.140±0.031 *efghA*	1.026±0.227 *aA*	1.018±0.062 *cdeA*	1.223±0.067 *fghiA*	1.198± 0.246 *efghiA*
0985	0.073±0.016 *aB*	0.079±0.025 *cdeB*	0.103±0.007 *fghAB*	0.154±0.009 *defghA*	0.869±0.154 *abA*	0.796±0.091 *deA*	0.853±0.063 *ghijA*	1.197± 0.169 *efghiA*
0011	0.087±0.012 *aB*	0.146±0.008 *bcB*	0.238±0.015 *cdA*	0.252±0.040 *bcdeA*	0.901±0.070 *abC*	1.096±0.029 *cdB*	1.966±0.009 *cdA*	0.252± 0.040 *iD*
0867	0.055±0.008 *aB*	0.111±0.017 *cdeB*	0.294±0.080 *bcA*	0.344±0.022 *abA*	0.608±0.093 *bB*	0.956±0.027 *cdeB*	2.385±0.416 *bcA*	2.506± 0.467 *bcdA*
0975	0.066±0.010 *aC*	0.102±0.018 *cdeBC*	0.133±0.018 *efghAB*	0.180±0.014 *defgA*	0.693±0.085 *abB*	0.897±0.066 *deB*	1.359±0.115 *efghA*	1.460± 0.107 *defghA*
0914	0.083±0.019 *aC*	0.192±0.015 *abB*	0.266±0.022 *bcB*	0.444±0.016 *aA*	0.797±0.085 *abC*	1.286±0.063 *bcBC*	1.938±0.168 *cdeB*	3.397± 0.307 *abA*
0061	0.095±0.011 *aB*	0.230±0.029 *aB*	0.416±0.024 *aA*	0.451±0.060 *aA*	0.889±0.012 *abB*	1.499±0.073 *abB*	3.260±0.122 *aA*	3.645± 0.701 *aA*
0069	0.056±0.012 *aB*	0.093±0.008 *cdeB*	0.208±0.026 *cdeA*	0.267±0.019 *bcdA*	0.670±0.122 *abB*	0.791±0.014 *deB*	1.576±0.019 *defA*	1.912± 0.249 *cdeA*
1406	0.081±0.012 *aC*	0.099±0.011 *cdeBC*	0.157±0.016 *defgAB*	0.215±0.020 *cdefA*	0.890±0.058 *abB*	0.882±0.001 *deB*	1.373±0.155 *efgAB*	1.747± 0.283 *cdefA*
7788	0.081±0.009 *aA*	0.058±0.011 *eA*	0.056±0.010 *hA*	0.067±0.021 *ghA*	0.788±0.099 *abA*	0.693±0.121 *eA*	0.633±0.095 *jA*	0.755± 0.205 *fghiA*
7737	0.073±0.009 *aA*	0.091±0.029 *cdeA*	0.061±0.008 *ghA*	0.063±0.014 *ghA*	0.740±0.091 *abA*	0.954±0.275 *cdeA*	0.702±0.101 *ijA*	0.623± 0.094 *ghiA*
0729	0.087±0.011 *aC*	0.218±0.013 *aB*	0.359±0.019 *abA*	0.317±0.018 *bcA*	0.892±0.064 *abD*	1.717±0.034 *aC*	2.643±0.167 *bB*	3.138± 0.121 *abA*

F24, F48, F72, F96: maximum force of the starch gels after 24, 48, 72, and 96 h, respectively. A24, A48, A72, A96: area under the force curve of the starch gels after 24, 48, 72, and 96 h, respectively. Different lowercase letters in the same column show a significant statistical difference at $p < 0.05$ by Tukey's test