

Original Research

Germination-Induced Starch Influence on Structural Characteristics of Water-Soluble Extracts from Purple Pericarp Creole Corn

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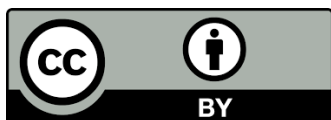
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Abstract

Germination is a viable, cost-effective, and environmentally friendly biotechnological process that enhances the industrial applications of cereals. This study evaluated the impact of germination (86 hours at 30°C) on soluble solids, proximate composition, and total phenolic compounds (TPC) in water-soluble extracts. It also included imaging analyses using scanning electron microscopy (SEM), X-ray diffraction (XRD), apparent amylose content, and starch pasting properties of purple pericarp Creole corn (PPCC) from Couto Magalhães de Minas, MG. Germination significantly increased the total solids content of the germinated PPCC sample to 5 times that of the control ($p < 0.05$). Notably, the germination process significantly reduced protein and lipid content in the water-soluble extracts by 17.72% and 34%, respectively, compared to the non-germinated sample, providing important nutritional insights. This reduction in protein and lipid content is a noteworthy finding that may be of interest to professionals in the field. Additionally, germination reduced sedimentation in the beverages



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by approximately eightfold compared to the control, which may be relevant to beverage technologists. SEM images revealed the action of amylolytic enzymes synthesized during germination, evidenced by pores in the starch granules, resulting in a 9.18% decrease in relative crystallinity compared to the control. However, germination did not alter the samples' crystalline pattern or pasting temperature. However, the degradation of amylose and amylopectin led to a reduction in hot paste viscosity and final viscosity of the starch gels, consequently decreasing the viscosity of both germinated and non-germinated corn starch samples. Therefore, germination is a promising technology for producing water-soluble extracts and modifying PPCC starch, offering a means to preserve and add value to Creole corn varieties.

Keywords

Agrobiodiversity; biotechnological processes; amylolytic enzymes; bioactive compounds

1. Introduction

Corn is one of the most extensively cultivated monocultures globally and plays a vital role in agribusiness, being a staple food due to its macronutrient content, including starch, proteins, fibers, lipids, beta-carotene, and minerals such as magnesium, zinc, phosphorus, and copper, with estimates indicating that up to 25% of the total calories consumed worldwide come from this cereal [1]. Furthermore, corn holds significant social importance, particularly Creole varieties, which are part of the genetic and cultural heritage of traditional communities. These varieties are seeds that have been traditionally cultivated in specific localities and are native, without gene flow from genetically modified varieties [2-5]. These seeds are typically passed down through generations, exchanged among neighbors, and naturally adapted to local climatic conditions, ensuring subsistence and food sovereignty in the communities where they are grown [6]. Therefore, studying and utilizing regional raw materials in food matrices is a way to highlight, promote, and preserve these seeds, with Creole corn standing out as a valuable raw material for producing starch extraction and water-soluble extracts.

Germination is a clean and safe technology for producing modified starches, offering a sustainable alternative to conventional methods. Techniques such as solvent modification, for instance, can leave toxic residues in the final product, while enzymatic processes are often costly. During germination, the action of endogenous amylolytic enzymes promotes the hydrolysis of starch, leading to a reduction in paste temperature and an increase in digestibility compared to native starch. This process enhances the functional properties of the starch, making germination a beneficial and eco-friendly approach [7]. A thorough understanding of the physicochemical properties of starch is essential for predicting its behavior in various food matrices. In water-soluble extracts, starch contributes not only to viscosity but also to colloidal stability. It may be associated with an undesirable gritty mouthfeel, attributed to the size of its granules.

Currently, there is a growing number of individuals with physiological restrictions to milk consumption, such as lactose intolerance or milk protein allergies, as well as those who abstain from dairy due to philosophical or personal reasons, including social values or lifestyle choices. This trend

has increased the demand for plant-based extracts from alternative sources as milk substitutes [7]. Purple pericarp Creole corn (PPCC), in addition to being a promising option for developing milk substitutes, represents an underutilized source of starch and bioactive compounds. These bioactive compounds, such as anthocyanins, offer nutritional and functional benefits, including antioxidant and anti-inflammatory effects, making them an attractive alternative for the development of food products [8]. Moreover, the development of food products or the extraction of ingredients from PPCC offers a promising strategy to enhance the visibility of Creole corn traditionally grown in the region of Couto Magalhães de Minas. This approach not only aids in the preservation of local landraces and the maintenance of regional agrobiodiversity but also provides a potential source of income for smallholder farmers who are responsible for the conservation and cultivation of these traditional varieties.

Grain germination is a cost-effective method for enhancing the nutritional value of cereals. This bioprocessing technique involves conditioning grains in high-humidity and moderate-temperature environments to activate hydrolytic enzymes. These enzymes convert the endosperm into a source of energy and nitrogen. This process leads to the partial hydrolysis of macromolecules, releasing the nutrients and energy required for embryo development [8]. Germination of PPCC can improve its starch properties and the quality of water-soluble extracts in an environmentally friendly and cost-effective manner, without the need for solvents or harsh chemical processes. Compared to other processing methods, such as those using chemical solvents, germination is a sustainable alternative, as it does not produce toxic by-products and consumes less energy, thereby contributing to environmental preservation [9]. In this context, the present study aimed to evaluate the impact of germination on the starch paste properties and structural characteristics, based on amylose content and SEM evaluation, as well as the physicochemical and nutritional characteristics of water-soluble extracts from purple pericarp Creole corn cultivated in the region of Couto Magalhães de Minas.

2. Materials and Methods

2.1 Raw Material

Purple pericarp Creole corn (*Zea mays* L.) seeds were sourced from the Crioulo Corn Project at the Institute of Agricultural Sciences, Federal University of Vales do Jequitinhonha and Mucuri, from the 2019 harvest. The seeds originated from Viçosa, MG, and were reproduced at the Rio Manso Experimental Farm in Couto de Magalhães de Minas, MG, located at 18°4'44.55" S and 43°27'23" W, with an altitude of 721 m. The project is registered under number A5C29C1 in the National System for the Management of Genetic Heritage and Associated Traditional Knowledge (SisGen) of Brazil's Ministry of the Environment. The seeds were stored in polyethylene terephthalate containers at a moisture content of 13% for nine months before the experimental procedures.

2.2 Methods

2.2.1 Germination Process

The germination process of PPCC seeds was performed following the method described by Andressa et al. [10]. Initially, 200 g of seeds were sanitized in a 200 ppm chlorine solution for 15 minutes, followed by rinsing with flowing deionized water to remove any residual chlorine.

Following sanitation, the samples were macerated in deionized water for 24 hours at a ratio of 1:5 (water:seed, w/v) at 20°C. After maceration, the liquid was drained, and the samples were placed on aluminum trays (0.120 m²) and covered with a layer of cotton (approximately 80 g) on both the top and bottom. The samples were then incubated at 30°C for 96 hours in a TF-33A incubator (Telga, Belo Horizonte, Brazil) with a relative humidity of 75-80%. After germination, the samples were dried at 45°C in a TE-394/1 dryer (Tecnal, Piracicaba, Brazil) with air renewal and forced circulation for 12 hours. The drying temperature was based on previous studies with germinated seeds, aiming to minimize the possible negative impacts of drying on the grains, particularly the loss of bioactive compounds. The germination process was conducted in triplicate. Non-germinated corn seeds were used as the control.

2.2.2 Flour Development

The germinated and non-germinated seeds were ground using a Multi Grains disc grinder (Malta, Caxias do Sul, Brazil) for two consecutive cycles at medium speed to reduce particle size. Grinding was performed at room temperature (25°C). Immediately after grinding, the resulting flours were stored in bi-oriented polypropylene packaging under refrigeration (4°C) and protected from light, for 7 days until the analyses were performed.

2.3 Analysis of Control and Germinated Corn Flours

2.3.1 Preparation of Water-Soluble Corn Extracts

The flours from germinated and non-germinated PPCC were mixed in an SL-150 water bath (Solab, Piracicaba, Brazil) at 65°C for 60 minutes in a dry ratio of 1:6 (40 g of corn flour: 240 g of potable water). During mixing, the soluble solids content of the samples was measured every 5 minutes using method 80 51.01 [11].

After mixing, the samples were blended with a PMX-700 mixer (Philco, Curitiba, Brazil) at 2.000 rpm for 2 minutes and filtered through a 0.88 mm mesh. The final volume was adjusted using a 250 ml volumetric flask with potable water to compensate for any possible evaporation that may have occurred during mashing. The samples were stored in polypropylene packaging and frozen in a DFN41 freezer (Electrolux, Curitiba, Brazil) at -18°C, protected from light, until further analysis.

2.3.2 Proximate Composition

The water-soluble extracts were analyzed for total solids content (method 44-20.01), total reducing sugars (method 80-68.01), pH (method 02-52.01), total titratable acidity (method 02-31.01), ash content (method 08-01), and protein content (method 46-11.05; N = 6.25) according to AACCI [11] standards. Analyses were performed in triplicate, and the results were expressed in °Brix for soluble solids, the percentage for total solids and proteins, and the percentage of ferulic acid for total titratable acidity.

2.3.3 Free Glucose

The free glucose content in the extracts obtained from control and germinated corn was quantified using the GOPOD enzymatic kit (glucose oxidase + peroxidase), according to method 80-10.01 [11]. The analysis was performed in triplicate, expressing the results in $\text{g} \cdot 100 \text{ g}^{-1}$.

2.3.4 Total Phenolic Compounds

The total phenolic content was determined according to the methodology proposed by Cáceres et al. [12], with modifications. Briefly, 1.5 g of each flour was weighed into 50 mL Falcon tubes (in duplicate), and 9 mL of a methanol:water (60:40 v/v) solution was added. The tubes were covered with aluminum foil to protect them from light and agitated at 240 RPM for 16 hours at room temperature (20°C). After this period, the samples were centrifuged at 2500 xg for 10 minutes, and the supernatant was transferred to 10 mL volumetric flasks and brought to volume with extraction solution. For total phenolic analysis, 100 μL of the extract was mixed with 250 μL of 2 N Folin-Ciocalteu phenol reagent, 3 mL of distilled water, and 1 mL of 15% sodium carbonate solution. The tubes were manually shaken and incubated at room temperature, protected from light, for 30 minutes. Absorbance was measured using a UV-M5 1 spectrophotometer (750 nm), using a standard gallic acid curve (0 to 150 mg/L). The results were expressed as mg of gallic acid per 100 g of flour (dry weight basis).

2.4 Structural Characterization of Creole Corn Starch, Control, and Germinated

2.4.1 Starch Isolation

Starch was isolated from native and germinated corn samples as described by Kringel et al. [13]. The samples were macerated in distilled water at a ratio of 1:2 (corn:water – w/w) with the addition of sulfur dioxide (0.3% active SO_2 – w/w) in a water bath (SL-150, Solab, Piracicaba, Brazil) at 52°C for 24 hours. After this period, the maceration liquid was drained, and the samples were washed with distilled water. Distilled water was then added at a ratio of 1:2 (corn:water – w/w), and the samples were ground using a PMX-700 mixer (Philco, Curitiba, Brazil) at maximum speed for 2 minutes. The ground material was filtered through a 0.88 mm mesh cloth and transferred to 50 mL centrifuge tubes, which were then subjected to fraction separation in a Sorvall ST 8 centrifuge (Thermo Fisher Scientific, Jiangsu, China) for 10 minutes at 2500 xg . After centrifugation, the supernatant was discarded, and the protein layer was carefully scraped off with a spatula. For purification, the precipitate was resuspended in distilled water and centrifuged again until the protein layer was removed. The corn starch was then resuspended in analytical-grade ethanol and filtered through filter paper under vacuum using a vacuum pump (model 121, Primatec, Porto Alegre, Brazil). Finally, the starch was dried in a forced-air oven (1 m/s circulation and air renewal, model TE 394/1, Tecnal, Piracicaba, Brazil) at 40°C for 12 hours [10].

2.4.2 Apparent Amylose Content

The apparent amylose content was determined using the colorimetric method with an iodine/potassium iodide solution, according to Method 66470 of the International Organization for Standardization [14]. The analysis was conducted in triplicate, and the results were expressed as a

percentage. The absorbance of the samples was measured using a UV-M5 1 absorption spectrophotometer (Bel Photonics, Monza, Italy) at a wavelength of 620 nm, with three readings taken for each replicate.

2.4.3 Pasting Properties

The viscoamylographic behavior was determined using a Perten viscometer (RVA-4500, Warriewood, Australia) according to method No. 162 [15]. Standard 1 profile was used for the analysis, it begins at 50°C with a 1-minute equilibration, followed by heating to 95°C at 12°C/min, holding at 95°C for 2.5 minutes, cooling back to 50°C at 12°C/min, and a final 2-minute hold at 50°C, totaling 13 minutes. The sample mass was 3.5 g (based on 14% moisture content). The analysis was conducted in triplicate, and the results were expressed in °C for paste temperature and in cP (centipoise) for peak viscosity, viscosity breakdown, final viscosity, and setback viscosity.

2.4.4 X-Ray Diffraction (XRD)

X-ray diffraction analysis was performed based on the methodology of Hayakawa et al. [16] using an XRD-6000 instrument (Shimadzu, Tokyo, Japan). The parameters set for analysis were Cu rotating anode at 40 kV and 80 mA, with diffraction angles ranging from 5 to 70° (2 θ), a step size of 0.02°, and a scan speed of 2°/minute. Relative crystallinity was calculated as the ratio of the total area to the area under the peaks, with ten smoothing points applied to reduce peak noise. The analysis was conducted in triplicate, and the results were expressed as a percentage of crystallinity.

2.4.5 Scanning Electron Microscopy (SEM)

SEM analysis was conducted to examine the morphology of starch granules [10]. In brief, the samples were mounted on a support over a carbon film and examined using a TM3000 scanning electron microscope (Hitachi, Tokyo, Japan). The acceleration voltage applied was 15 kV, with a beam current of 33.9 μ A, to obtain micrographs that were analyzed at a magnification of 5000 $\times$.

2.5 Statistical Analysis

The experiment was conducted in three replicates, and each replicate was analyzed in triplicate (n = 9). Results were expressed as mean $\pm$ standard deviation. Means were evaluated by analysis of variance (ANOVA) using the t-test for mean comparison at a 5% significance level with Statistica software, version 7.0 (StatSoft Inc., USA).

3. Results and Discussion

3.1 Water-Soluble Extracts

3.1.1 Soluble Solids and Total Solids

It was observed that the germination process resulted in an increased soluble solids content in corn grains during the mashing process, as illustrated in Figure 1. In contrast, the absence of active enzymes in the non-germinated sample resulted in lower total soluble solids content throughout the maceration time. This phenomenon is expected because germination activates dormant

enzymes, such as amylase, glucanase, and proteases, to utilize the seed reserves for plant development. As a result, these enzymes hydrolyze macronutrients, such as starch, proteins, and lipids, providing low-molecular-weight sugars, nitrogen, and amino acids to support root growth [17]. Mashing enhances enzymatic activity and promotes greater diffusion of solids into the continuous phase, thereby increasing the total soluble solids content in the germinated corn-based sample compared to the control [18].

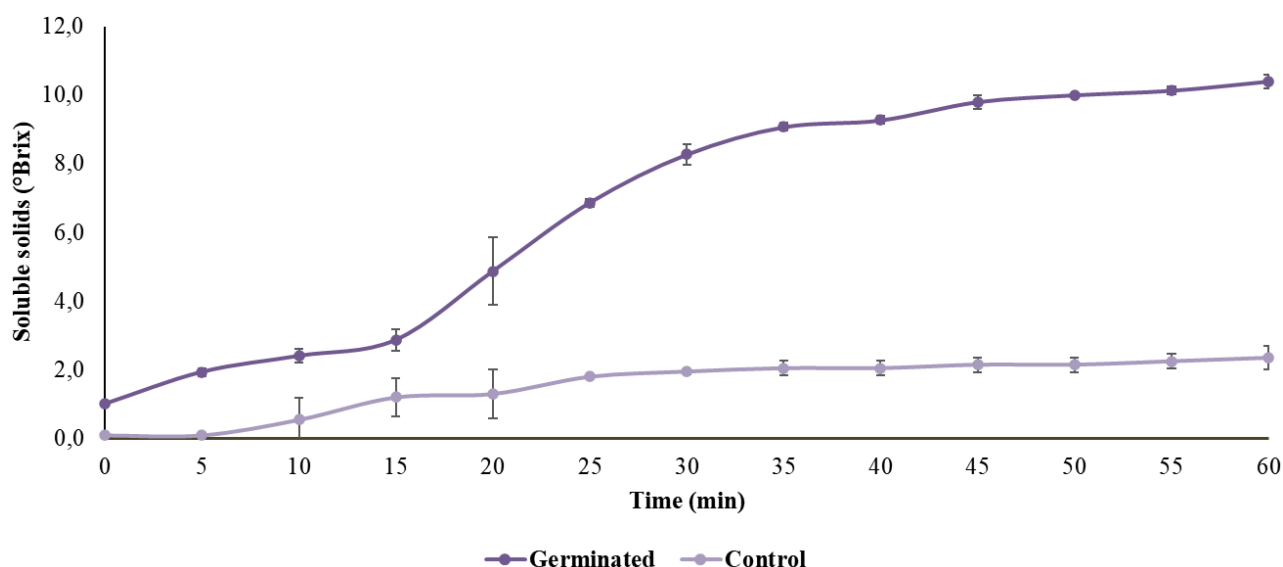


Figure 1 Mashing curve of extracts based on germinated and non-germinated purple creole corn.

Figure 1 demonstrates that mashing rapidly increased soluble solids, particularly in the initial minutes of the process. However, after the first 25 minutes, the control sample exhibited a minimal increase in solids, unlike the germinated corn-based sample, which showed a significant increase.

This behavior has been widely reported in the literature for various raw materials, such as corn and sunflower seeds. The gradual increase in temperature creates a favorable environment for the endogenous enzymes present in the germination process, promoting the solubilization of macronutrients from the plant matrix into the aqueous medium [19]. Among the main endogenous enzymes involved, the following can be highlighted: hemicellulases, with an optimal temperature range between 40 and 45°C; exopeptidases, with an ideal temperature range between 40 and 50°C; endopeptidases, which function optimally between 50 and 60°C; dextrinases, with an optimal temperature range between 55 and 60°C; β -amylases, which work best between 60 and 65°C; and α -amylases, with an optimal temperature range between 70 and 75°C [20].

3.1.2 Centesimal Composition

The centesimal composition of water-soluble extracts based on germinated and non-germinated purple pericarp Creole corn (PPCC) is presented in Table 1.

Table 1 Centesimal composition of water-soluble extracts based on purple pericarp creole corn.

Parameter	Water-soluble extract from control corn (g/100 g)	Water-soluble extract from germinated corn (g/100 g)
Total solids	8.90 ± 0.11	9.59 ± 0.74
Proteins	0.79 ± 0.01	0.65 ± 0.01
Starch	64.96 ± 2.65 ^{ns}	67.01 ± 2.05 ^{ns}
Ash	1.58 ± 0.05 ^{ns}	1.14 ± 0.02 ^{ns}
Lipids	0.50 ± 0.00	0.33 ± 0.02
Dietary fibers	0.50 ± 0.00	0.33 ± 0.02
Free glucose	1.23 ± 0.37	7.61 ± 0.60
Total reducing carbohydrates (g/100 g, as glucose)	0.29 ± 0.01	3.73 ± 0.25
Total phenolic compounds (g/100 mL, as gallic acid)	3.49 ± 0.25	7.62 ± 0.51
Solid sedimentation (mL/100 mL)	43.33 ± 4.25	5.56 ± 1.96

Legend: ns - not significant (p > 0.05).

Based on Table 1, germination significantly influenced the centesimal composition of the water-soluble extracts. The increase in total solids (from 8.90 to 9.59 g/100 g) appears to be closely linked to the significant rise in free glucose, which is likely to result from the enzymatic breakdown of macronutrients during germination. The sharp increase in free glucose (from 1.23 to 7.61 g/100 g) is mirrored by the rise in total reducing carbohydrates (from 0.29 to 3.73 g/100 g), supporting the idea that starch was hydrolyzed into simpler sugars throughout the process.

The samples exhibited protein and lipid content differences, which can be attributed to the catabolism of macronutrients to support radicle development. The amino acids and fatty acids produced from hydrolysis serve as sources of nitrogen and energy, respectively, for the seed during germination, leading to a reduction in protein and lipid content in the water-soluble extracts of germinated corn compared to the control [21].

Conversely, the inherent protein hydrolysis process during germination may enhance digestibility and bioaccessibility in the human body, as evidenced in studies on other grains, such as sorghum [22], soybeans [23], sesame [24], and chickpeas [25], among others [26-28]. The reduction in lipid content represents a technological benefit for obtaining water-soluble extracts, as it gives the beverage a more homogeneous appearance, without phase separation and greater stability. The dietary fiber content tends to increase with germination, as reported in some studies [29], because of the development of new cell wall constituents. However, the determination of fiber content was done by mass balance, and since the other compounds, mainly carbohydrates, of the soluble extract increased considerably, the fiber content decreased proportionally.

3.1.3 Total Phenolic Compounds

Total phenolic compounds are synthesized during the secondary metabolism of plants and play an essential role in their defense mechanisms, particularly against ultraviolet radiation. These

compounds are recognized for their diverse bioactive properties, such as antioxidant, antimicrobial, anticarcinogenic, anti-inflammatory, and antimutagenic actions, rendering them significant for human health [8]. However, their typically low solubility in aqueous media limits their effectiveness and bioavailability. Additionally, many phenolic compounds are bound to macromolecules within the plant matrix, such as polysaccharides and proteins, which further hinders their release into the aqueous phase [19]. Germination can be a crucial step in increasing the solubility and bioavailability of these compounds, as the enzymes produced during this process, such as cellulases, phytases, xylanases, proteases, and amylases, help degrade the cell wall and release phenolic compounds into the aqueous phase [18]. This enzymatic breakdown enhances the solubilization and potential absorption of bioactive constituents. In the current study, a 118.33% increase in the water-soluble extract from germinated purple pericarp Creole corn was observed compared to the control (Table 1). This increase also contributes to the solids content, which may have a positive effect on the system's stability.

Similar studies have been conducted with other food sources such as soybeans [18], corn [30], sunflower [19], and pumpkin seeds [31]. Some researchers suggest that germination not only increases the amount of free phenolic compounds but also makes them more available for the body. Phenolic compounds present in the food matrix can be found in either free or bound forms. In their free form, these compounds are more readily available for extraction and absorption. However, when bound to macromolecules such as cell wall polysaccharides, proteins, and lipids, their solubility in aqueous media is reduced, significantly limiting their bioavailability. These interactions may occur through covalent bonds (e.g., esters and ethers) or non-covalent interactions (e.g., hydrogen bonds and hydrophobic forces). To improve the release of these compounds from the plant matrix, processing techniques such as germination, fermentation, or enzymatic hydrolysis have been widely employed. These methods promote the structural breakdown of the matrix and facilitate the release of phenolic compounds, thereby enhancing their bioaccessibility and potential biological effects [32]. However, the impact of the germination process can vary significantly depending on the specific characteristics of each seed type, which makes individualized analysis essential for each plant matrix [33, 34]. The plant's composition, enzyme activity, and even environmental factors during germination are crucial in determining the amount and bioavailability of phenolic compounds. Therefore, although germination can generally improve the nutritional and functional properties of foods, it is essential to evaluate its effects on a case-by-case basis for each type of seed.

3.2 Physical Stability

Water-soluble extracts are colloidal systems composed of a continuous phase (water) and one or more dispersed phases (such as starch, proteins, or fibers). As a result, these products typically exhibit low colloidal stability, primarily due to the density of the constituents within the dispersed phase [35, 36]. In the present study, an 87.16% reduction in sedimentation was observed in the sample obtained from germinated PPCC compared to the control (Table 1). This significant increase in the colloidal stability of the beverage is evidenced by Figure 2.

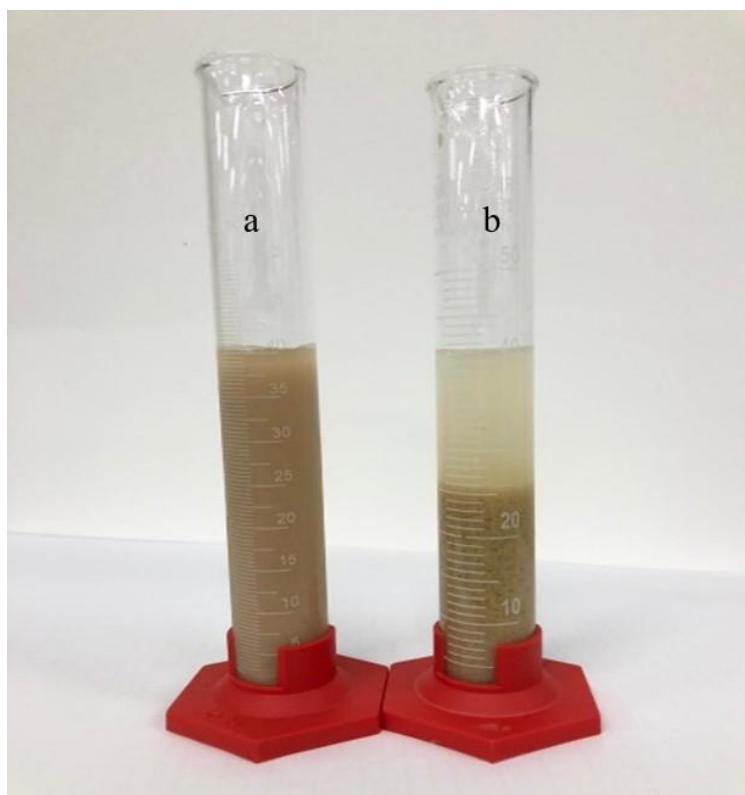


Figure 2 Sedimentation of water-soluble extracts obtained from germinated (a) and ungerminated (b) PPCC.

The hydrolysis of grain macronutrients facilitated by the germination process reduces particle size, allowing particles to remain in suspension rather than settling, thereby positively influencing the colloidal stability of the system (Figure 2). In corn, starch is the primary macronutrient responsible for macroscopic phase separation, accounting for approximately 70% of the grain.

In addition to enhancing the physical stability of the system, the starch hydrolysis inherent in germination promotes the release of reducing sugars into the medium. The control sample exhibited a reducing sugar content of 0.29 ± 0.01 (g/100 g, in glucose), while the germinated sample contained 3.73 ± 0.25 (g/100 g, in glucose). These reducing sugars indicate that the final product is significantly sweeter compared to the control sample. This can reduce the need for added sucrose in the medium and also provide a favorable substrate for fermentation by probiotic microorganisms.

3.2.1 Properties of Starch

The action of endogenous enzymes during the germination process was evidenced in the SEM images (Figure 3), which show the presence of pores in the germinated corn starch granules, in contrast to the control sample. However, despite the enzymatic activity observed in the SEM images, no significant change in starch content was detected between the samples (Table 1). This is likely due to the limitations of the methodology used, which is not sensitive enough to differentiate between starch intact chains and low molecular weight molecules produced from hydrolysis.

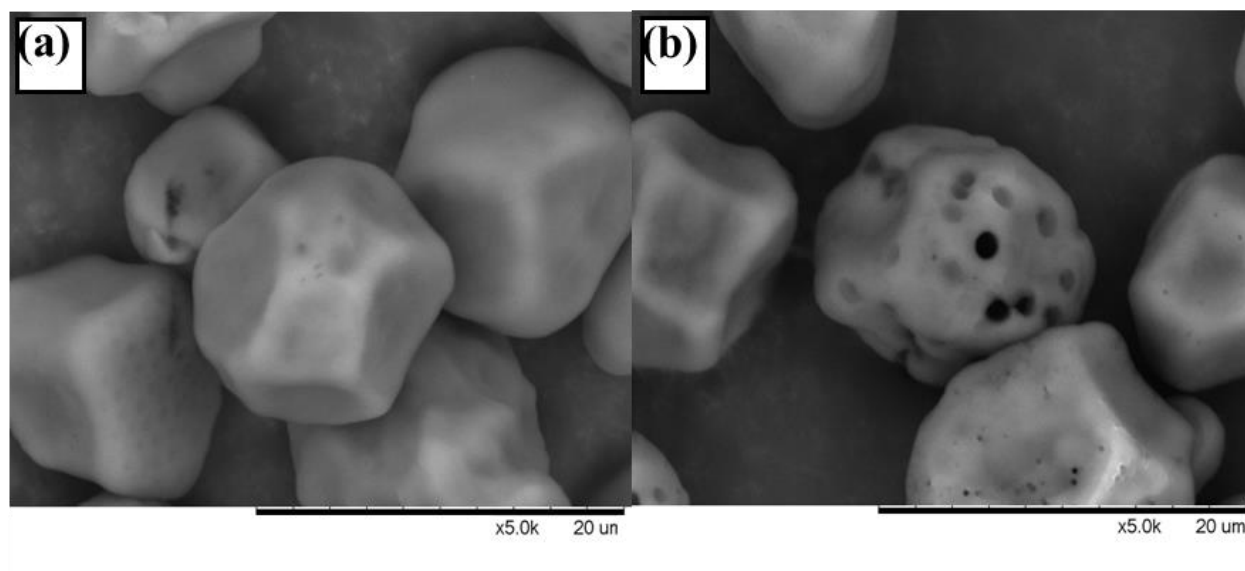


Figure 3 Scanning Electron Microscopy (SEM). a) Creole purple pericarp corn starch; b) Germinated Creole purple pericarp corn starch.

The X-ray diffraction (XRD) parameters of control and germinated corn starch are presented in Figure 4. Both samples exhibited diffraction peaks at 2θ angles of 15° , 17° , 18° , and 23° , indicating a standard A-type polymorphism. Therefore, it can be concluded that germination did not alter the crystalline pattern of the sample. However, it did cause a significant reduction in relative crystallinity ($p < 0.10$) (Table 2) by 9.18%. This reduction can be attributed to the action of inherent amylolytic enzymes in the germination process, which hydrolyze amylose and amylopectin chains, decreasing the starch crystalline region. Amylolytic enzymes, primarily α -amylase, β -amylase, and glucoamylase, act by hydrolyzing the glycosidic bonds in starch molecules, facilitating the breakdown of amylose and amylopectin during germination. These enzymes catalyze the cleavage of α -1,4 and, in some cases, α -1,6 glycosidic linkages, releasing smaller sugar units such as maltose and glucose. α -Amylase acts randomly along the starch chain, while β -amylase removes maltose units from the non-reducing ends, and glucoamylase can cleave both α -1,4 and α -1,6 bonds, producing glucose. The hydrolytic action of these enzymes disrupts the crystalline structure of starch granules, decreasing their relative crystallinity and increasing solubility and digestibility. This enzymatic degradation is especially relevant during germination, where endogenous amylases are activated and contribute to structural modification of starch for energy mobilization [37, 38].

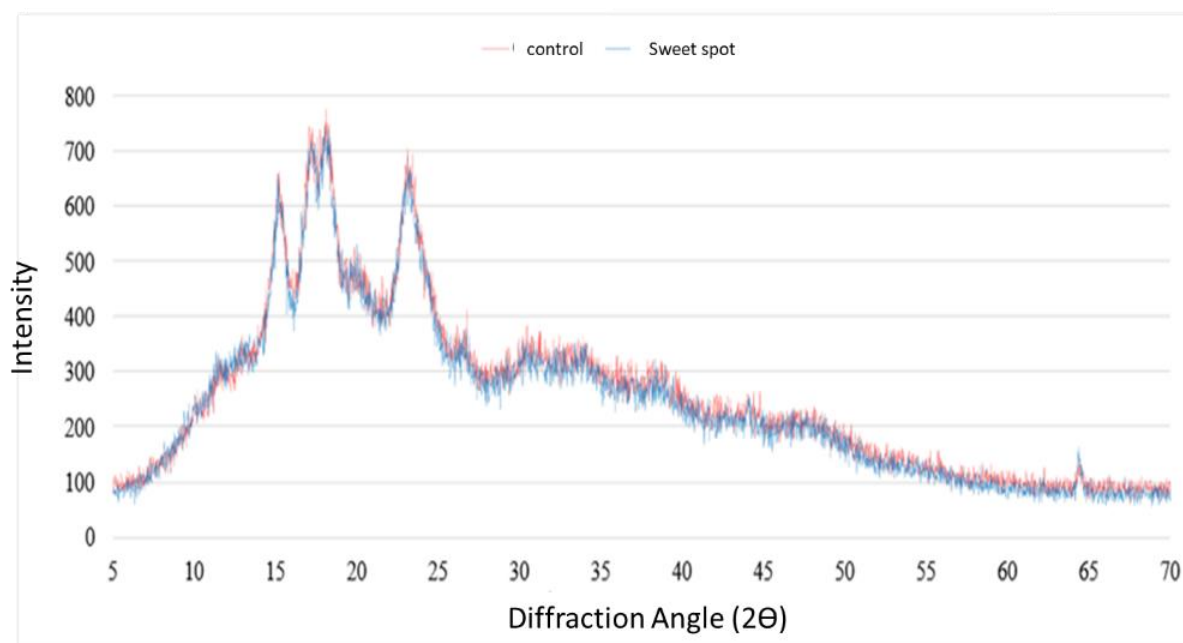


Figure 4 X-ray diffraction patterns of control and germinated Creole purple pericarp corn starch.

Table 2 Apparent amylose content and paste properties of control and germinated corn starches.

Parameter	Control Corn Starch	Germinated Corn Starch
Apparent amylose (%)	19.95 ± 0.02	20.50 ± 0.04
Paste Temperature (°C)	76.72 ± 0.06 ^{ns}	76.17 ± 0.38 ^{ns}
Hot Viscosity (cP)	3892 ± 13.56	3114 ± 6.30
Viscosity Breakdown (cP)	1365 ± 50.86	1074 ± 48.76
Final Viscosity (cP)	4227 ± 38.25	3181 ± 51.38
Retrogradation Tendency (cP)	1291 ± 57.89	1143 ± 43.15
Relative Crystallinity (%)	44.45 ± 1.99	40,37 ± 0.76

Legend: ns – not significant ($p > 0.05$).

Consequently, higher apparent amylose values were observed due to hydrolysis by amylolytic enzymes synthesized during grain germination, indicating the release of high molecular weight dextrans capable of forming complexes with iodine. The starch hydrolysis also resulted in a significant increase in free glucose (Table 1) in the water-soluble extracts of germinated corn compared to the control, along with other reducing carbohydrates throughout germination (Table 1). Despite the increase in low-molecular-weight sugars, no change in the pasting temperature of germinated corn starch was observed compared to the control. This suggests that the level of enzymatic hydrolysis was insufficient to raise sugar concentrations to a level that would affect the pasting temperature. Nevertheless, the presence of sugars from germination could compete with starch for available water, potentially influencing the pasting properties.

Conversely, the degradation of amylose and amylopectin resulted in a reduction in both hot paste viscosity and final viscosity, leading to a decrease in viscosity for both germinated corn starch

and the control sample (Table 2). These changes in the pasting properties of germinated corn starch indicate that germination reduced the thickening capacity of the sample. This finding is corroborated by SEM and XRD analyses, which show that enzymatic activity led to pore formation, causing the starch granules to lose their resistance to swelling during gelatinization and preventing the formation of necessary bonds with water [39]. This outcome is advantageous because excessive viscosity during the thermal treatment of water-soluble extracts can negatively affect processing and stability. The reduction in viscosity through germination helps to avoid such issues, making the processing of the extract more efficient. Furthermore, germination reduced the tendency for starch retrogradation, which may help maintain colloidal stability and extend the final product's shelf life [40]. The starch analyses further confirm the increased physical stability of the final product, as the reduction in starch and protein particle sizes decreases the likelihood of sedimentation, thereby enhancing the beverage's colloidal stability.

4. Conclusions

The germination of Creole purple pericarp corn grains positively influenced the levels of lower molecular weight carbohydrates, as demonstrated by the soluble solids measurements during mashing and in the final product. Similar trends were observed for total solids content and total reducing carbohydrate groups, which can be attributed to increased synthesis and enzymatic activity during germination. The enzymatic hydrolysis of flour components, facilitated by enzymes such as amylases, proteases, lipases, and phytases, led to water-soluble extracts with enhanced physical stability and reductions of 17.72% and 34% in protein and lipid levels, respectively, due to embryo catabolism. Starch extracted from germinated corn exhibited increased granule porosity due to the action of amylolytic enzymes. It showed lower values in pasting properties, though the pasting temperature remained unchanged compared to the control.

This biotechnological germination process is therefore significant for adding value and promoting the use of Creole purple pericarp corn cultivated in the municipality of Couto Magalhães de Minas, while also contributing to the preservation of local agrobiodiversity. Future studies could focus on optimizing the germination of Creole purple pericarp corn using unconventional technologies, such as ultrasound and ultraviolet light, to improve its nutritional value and increase the levels of bioactive compounds like anthocyanins and phenolic compounds, while also reducing antinutritional factors such as phytic acid. These approaches could make corn cultivated in the Couto Magalhães de Minas region more attractive for producing water-soluble extracts. Furthermore, such studies could explore the potential applications of germinated corn starch in the food, chemical, and pharmaceutical industries.

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Author Contributions

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Competing Interests

The authors have declared that no competing interests exist.

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