

## Article

# Short-Range Starch Order, Pasting Behavior, and Gelatinization of Native and Sprouted Wheat and Rice Flour Systems

Paloma Lopez-Sarmiento \*  and Julián de la Rosa-Millán \* 

Tecnologico de Monterrey, FEMSA Biotechnology Center, School of Engineering and Sciences, Ave. Eugenio Garza Sada 2501, Monterrey 64700, Mexico

\* Correspondence: a01746059@tec.mx (P.L.-S.); juliandlr@tec.mx (J.d.l.R.-M.)

## Abstract

This study evaluates the effects of germination on the composition, starch architecture, thermal profiles, short-range molecular order, and pasting behavior of wheat and rice flour systems and their corresponding isolated starch fractions. Germination significantly reduced total starch content in wheat flour (74.81–56.74%) and rice flour (85.55–64.00%). Additionally, amylose content decreased from 24.70 to 18.77% in wheat flour and from 20.83 to 19.69% in rice flour. Amylopectin A-chains increased in germinated wheat flour (33.98%) and germinated rice flour (31.49%), suggesting starch depolymerization and molecular restructuring. These structural changes are associated with lower gelatinization enthalpy values, which decreased from 8.82 to 4.78 J/g in wheat flour and from 9.99 to 6.90 J/g in rice flour, reflecting reduced thermal stability after sprouting. ATR-FTIR analysis showed that germinated flours had the highest short-range molecular order (1047/1023 ratios of 0.75 and 0.71 for wheat and rice, respectively), despite their lower starch content. Germination also affected the development of viscosity; peak viscosity decreased from 2482.5 to 217.0 cP in wheat flour and from 4830.0 to 1039.5 cP in rice flour. Germination influenced protein secondary structure: it increased relative  $\alpha$ -helix content in flour systems (from 35.23% to 36.16% in wheat, and from 36.26% to 37.58% in rice) but decreased it in isolated starches (from 42.05% to 33.17% in wheat, and from 39.60% to 36.29% in rice), suggesting a matrix-specific response. Germination-induced depolymerization and reorganization altered starch structure, thermal properties, and viscosity. These findings provide an integrated structural and functional framework that links starch architecture, molecular order, gelatinization, and the development of viscosity in germinated cereal matrices for food applications.

**Keywords:** germinated flours; starch structure; starch depolymerization; short-range molecular order; cereal matrices; wheat flour; rice flour



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## 1. Introduction

Cereal starch is a major determinant of the functional performance of grain-based systems because its molecular and supramolecular organization governs hydration, swelling, gelatinization, pasting behavior, and retrogradation during processing [1,2]. In cereal flours, however, starch functionality cannot be interpreted in isolation, since proteins, lipids, fiber, and minerals also modulate thermal transitions and the development of viscosity by altering water competition, granule mobility, and starch reorganization during heating and cooling [3–5]. As a result, differences between isolated starches and flour systems are not

only compositional but also structural, reflecting the complexity of cereal matrices and the interactions among their components.

The relative contribution and organization of amylose and amylopectin strongly influence the functionality of starch-based cereal systems. Beyond the amylose-to-amylopectin ratio, structural features such as chain architecture, molecular order, and interactions with non-starch components can substantially modify starch behavior [5,6]. Therefore, understanding cereal functionality requires evaluating starch not only as a purified polymer, but also as part of the native flour matrix where its behavior is constrained or promoted by surrounding constituents.

Germination is a particularly relevant biological process in cereal science because it activates endogenous hydrolytic enzymes that reshape grain macromolecules and, consequently, their technological functionality. During sprouting, amylolytic, proteolytic, and lipolytic activities promote the degradation and reorganization of starch, proteins, and lipids, thereby altering composition, chain arrangement, and matrix architecture [7–9]. These modifications may alter pasting and gelatinization behavior, as well as the balance between structural disruption and molecular reorganization. In this sense, germination should not be viewed only as a degradative process, but also as a transformation that may generate cereal materials with distinct physicochemical and functional attributes.

Previous studies have characterized starch and flour systems from cereals such as wheat and rice. For example, Singh et al. [3] examined thermal and physicochemical differences among rice grain, flour, and starch, whereas Yu et al. [2] reported cultivar-dependent variations in the physicochemical properties of rice starches and flours. Kumar and Khatkar [4] described the thermal and pasting properties of wheat starch granules. In addition, Noda et al. [10] evaluated physicochemical changes in partially digested starch from sprouted wheat grain.

Recent studies have continued to explore the effects of germination and other post-harvest treatments on cereal starches and flours. Perveen et al. [11] compared physicochemical and rheological traits of whole and sprouted wheat flour. Similarly, Zhu et al. [12] reported that germination altered the nutritional composition, physicochemical properties, and functional characteristics of black rice flour. Mendoza Moreno et al. [13] studied the impact of wheat grain germination time on the physicochemical and textural properties, as well as the protein hydrolysis rate, of bread. Beyond germination, Wang et al. [14] evaluated the effects of long-term storage on the multi-scale structure and properties of starch isolated from rice grains, and Huang et al. [15] investigated the impact of retrogradation on the molecular structure and short-range order of wheat starch.

Although these studies provide valuable information, comparative evidence integrating cereal type, matrix type, and germination status within a single framework remains limited. There is still insufficient understanding of how germination-induced changes in cereal matrices are reflected simultaneously in the short-range order of starch, protein secondary structure, gelatinization, and pasting behavior.

A more integrated characterization is relevant because wheat and rice differ markedly in botanical origin, grain composition, and matrix organization, which may lead to different responses to germination and starch isolation. Comparing native starches, native flours, sprouted flours, and the starches isolated from these flour systems provides an opportunity to distinguish matrix effects from starch-specific effects and to identify whether germination primarily promotes structural degradation, molecular reorganization, or both. Such information is useful not only for understanding cereal functionality but also for guiding the selection of raw materials for food applications where viscosity development, thermal behavior, and structural organization are critical.

Therefore, this study aims to compare wheat and rice matrices at different structural levels, including native flours, sprouted flours, and starches isolated from native and sprouted flours, to evaluate how germination modifies their composition, starch molecular order, protein secondary structure, thermal transitions, and pasting behavior, using commercially available flours as representative cereal systems under practical processing conditions. Rapid Visco Analyzer (RVA), Differential Scanning Calorimetry (DSC), and Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy (ATR-FTIR) were used to identify physicochemical changes associated with germination. We hypothesized that germination would not only reduce the thermal energy required for gelatinization through partial hydrolysis but also promote structural rearrangements in cereal matrices that modify short-range starch order and viscosity development in a grain-dependent manner.

## 2. Materials and Methods

### 2.1. Materials

Wheat flour (WF) was obtained from Espiga, Trimex Group (Miguel Hidalgo, Mexico City, Mexico), and rice flour (RF) from Mi Granero, Alday Ingredients (San Pedro Cholula, Puebla, Mexico). Germinated wheat flour (GWF) and germinated rice flour (GRF) were supplied by Functional Bread, FV NextFuture Foods (Estado de Mexico, Mexico). According to the supplier, the products were manufactured from germinated cereal grains and commercialized as germinated flour products. The flours were used as received. Starch fractions were isolated from native and germinated flours. Accordingly, starches isolated from native wheat and rice flours were designated as WSI and RSI, respectively. In contrast, starches isolated from germinated wheat and rice flours were referred to as GWSI and GRSI, respectively.

### 2.2. Rice Starch Extraction from Native and Sprouted Flours

Rice starch was extracted from native and sprouted flours following the alkaline extraction method described by De Souza et al. [16], with modifications. A flour-to-solution ratio of 1:10 (*w/v*) was used. Briefly, 45 g of rice flour was dispersed in 450 mL of 0.045 M NaOH and stirred for 30 min to ensure homogenization. The suspension was then distributed into Falcon tubes and centrifuged at  $3300 \times g$  for 15 min in a Thermo Scientific Sorvall ST16R centrifuge (Thermo Fisher Scientific, Longwood, FL, USA). The temperature was set at 4 °C, and both the acceleration and deceleration rates were adjusted to 9. After centrifugation, the supernatant was discarded, and the pellets were recovered.

The pellets were suspended in 150 mL of 0.045 M NaOH and centrifuged again under the same conditions. Subsequently, the recovered pellets were transferred to a beaker containing 85 mL of distilled water, and the suspension was neutralized to pH 7.25 with 0.1 M HCl. The neutralized suspension was centrifuged again under the same conditions. The resulting pellets were then washed with 150 mL of 70% ethanol and centrifuged for 10 min using the same settings. Finally, the pellets were collected and dried overnight (approximately 18 h) in an oven (Shel Lab 1350 GX, Cornelius, OR, USA) at 50 °C.

### 2.3. Wheat Starch Extraction from Native and Sprouted Flours

Starch from native and sprouted wheat flours was extracted according to the method of Guo et al. [17], with adaptations. Briefly, 45 g of wheat flour was washed manually with distilled water to remove the insoluble gluten protein fraction, and the resulting suspension was passed through a 100-mesh strainer. The retained gluten fraction was discarded, and the filtrate was centrifuged in a Thermo Scientific ST16R centrifuge (Longwood, FL, USA) at  $4000 \times g$  for 15 min at 4 °C, with acceleration and deceleration rates set to 9. The supernatant was discarded, and the pellets were collected.

The starch pellets were washed twice by centrifugation under the same conditions. The first wash was performed with 99.5% ethanol, followed by a second wash with 70% ethanol. After washing, the pellets were collected and dried overnight (approximately 18 h) in an oven (Shel Lab 1350 GX, Cornelius, OR, USA) at 50 °C.

#### 2.4. Starch Extraction Yields

Starch extraction performance was evaluated by calculating both starch yield and recovery yield. Starch yield was calculated as the percentage of starch recovered after extraction with respect to the initial flour weight. Recovery yield was calculated as the proportion of starch recovered relative to the total starch initially present in the flour sample.

The starch yields are calculated using the following formula:

$$\text{Starch yield (\%)} = \frac{\text{Weight of extracted starch (g)}}{\text{Weight of flour (g)}} \times 100\%$$

The recovery yields are calculated using the formula described as follows:

$$\text{Recovery yield (\%)} = \frac{\text{Weight of extracted starch (g)}}{\text{Weight of total starch in flour (g)}} \times 100\%$$

#### 2.5. Proximal Composition Analysis

All starch-based matrices, including native flours, sprouted flours, and starches isolated from native and sprouted wheat and rice flours, were analyzed for protein, lipids, and ash contents according to AACC-approved Methods 960.52, 32-10.01, and 08-17.01, respectively [18]. Carbohydrate content was calculated by difference. Total, insoluble, and soluble dietary fiber contents were determined using a Megazyme K-TDFR assay kit (Neogen, Megazyme, Wicklow, Ireland), following AOAC Method 991.43 [19] and AACC Method 32-07.01. Total starch (K-TSTA) and amylose (K-AMYL) contents were also determined using Megazyme assay kits (Megazyme, Wicklow, Ireland), following the method reported by de la Rosa-Millán [20]. For total starch determination, 500 mg of sample was subjected to amylolysis using thermostable  $\alpha$ -amylase, producing soluble branched and unbranched maltodextrins. Subsequently,  $\alpha$ -glucoamylase hydrolyzed these dextrins into glucose, which was quantified using the glucose oxidase/peroxidase (GOD-POD) method. For Amylose quantification, starch samples were dissolved and treated with Concanavalin-A (Con-A), which selectively binds to the reducing end of amylopectin, forming a complex that facilitates its precipitation. The resulting supernatant, enriched in amylose, was hydrolyzed using  $\alpha$ -amylase and  $\alpha$ -glucoamylase, and the released glucose was quantified using the GODPOD method. All analyses were performed in triplicate.

#### 2.6. Molecular Characteristics and Amylopectin Chain Length Distribution

The procedure described by de la Rosa-Millán [20] was followed. Samples (500 mg) were dissolved in 90% DMSO. The suspensions were stirred for 1 h in a boiling water bath and then maintained at 25 °C for 24 h. For starch precipitation, a 2 mL aliquot of the dispersion was mixed with 8 mL of ethanol (4 volumes). The insoluble starch fraction was recovered by centrifugation at 7000 rpm for 20 min and dissolved in 5 mL of hot water. The resulting solution, at a concentration of 4 mg/mL, was stirred for 30 min in a boiling water bath. The samples were then filtered through a 5.0  $\mu$ m nylon membrane prior to chromatographic analysis.

The molecular weight and radius of gyration ( $R_z$ ) of amylopectin were determined by high-performance size-exclusion chromatography coupled with multi-angle laser-light scattering and refractive index detectors (HPSEC-MALLS-RI) (Waters Corp., Milford, MA, USA). The system consisted of a Waters 1525 binary pump (Waters Corp., Milford, MA,

USA), a multi-angle laser-light scattering detector (Dawn, Wyatt Tech. Co., Santa Barbara, CA, USA), and a Waters 2414 refractive index detector (Milford, MA, USA). Amylopectin was separated from amylose using a Shodex OH Pak KB-guard column and an SB-806 HQ column (Showa Denko KK, JM Science, Grand Island, NY, USA). The sample concentration was 4 mg/mL, and the flow rate was 0.5 mL/min.

For starch debranching, 15 U of isoamylase (E-ISAMY, Megazyme, Wicklow, Ireland) was added, and the samples were incubated at 45 °C for 6 h under stirring. The enzyme was inactivated by boiling the samples in a water bath for 20 min. Immediately after debranching, the solutions were filtered on a 0.45 µm nylon membrane and injected into a CLARET-IR system equipped with a Waters 1525 binary pump (Waters Corp., Milford, MA, USA), a Waters 2414 refractive index detector, and two HR 10/30 columns connected in series, the first packed with Superdex 200 (Marlborough, MA, USA). The analysis was performed using Superdex 30 gel (Amersham Biosciences, Piscataway, NJ, USA) at a flow rate of 0.4 mL/min, with deionized water (18 MΩ) as the mobile phase. Pullulan standards of a known molecular weight (189, 738, 5900, 11,800, 22,800, and 112,000 g/mol) (Agilent, Santa Clara, CA, USA) were used for calibration. Each quantification was performed in triplicate using Empower 3 software (Waters Corp., Milford, MA, USA).

## 2.7. Viscosity Profile of Starches and Flours Monitored by Rapid Visco Analyzer (RVA)

The pasting properties of the different starch-based materials were determined using a Rapid Visco Analyzer (RVA Model 4500, Perten Instruments, Macquarie Park, New South Wales, Australia) according to the AACC International method 76-21.02. The RVA analyses were performed in duplicate. Sample moisture was adjusted to a 10% basis before testing. For each run, 3.26 g of sample and 25.24 g of water were used.

The viscosity profile parameters obtained included peak viscosity, trough viscosity, breakdown, final viscosity, setback, and peak time.

## 2.8. Differential Scanning Calorimetry (DSC)

The thermal properties of the samples were determined using a differential scanning calorimeter (Diamond DSC, PerkinElmer, Norfolk, VA, USA) according to the procedure described by Chávez-Murillo et al. [21]. Briefly, 2 mg of sample, calculated on a total starch basis, was weighed into semi-hermetic anodized aluminum pans (Perkin Elmer, B02190062, Waltham, MA, USA). Samples were hydrated with distilled water at a ratio of three times the sample weight and sealed. The hydrated pans were equilibrated at room temperature (approximately 25 °C) for 30 min before analysis.

Samples were heated from 30 to 120 °C at 10 °C/min. The instrument was calibrated with an indium before each run, and an empty pan was used as the reference. The onset temperature ( $T_o$ ), peak temperature ( $T_p$ ), conclusion temperature ( $T_f$ ), gelatinization range ( $T_f - T_o$ ), and gelatinization enthalpy ( $\Delta H$ ) were calculated using Pyris software version 4.02 (PerkinElmer, Norwalk, CT, USA).

## 2.9. Attenuated Total Reflectance Fourier Transformed Infrared Spectroscopy (ATR-FTIR)

Infrared spectra of the starch-based samples were collected using an ATR-FTIR spectrometer (Spectrum 1; Perkin Elmer, Norwalk, CT, USA) equipped with a diamond crystal (KRS-5: thallium bromide/iodide) and Spectrum software version 5.3.0 (Perkin Elmer). Spectra were recorded in absorbance mode over the mid-infrared region from 4000 to 400  $\text{cm}^{-1}$ , with a resolution of 4  $\text{cm}^{-1}$ , a half-bandwidth of 15  $\text{cm}^{-1}$ , and 64 scans per sample, using air as background correction [22,23].

For starch structural analysis, the fingerprint region was evaluated, particularly the bands associated with amorphous and ordered short-range structures at approximately

1022–1023  $\text{cm}^{-1}$  and 1047  $\text{cm}^{-1}$ , respectively. The absorbance ratio 1047/1023  $\text{cm}^{-1}$  was calculated as an indicator of relative short-range molecular order [24,25].

Protein structural features were analyzed in the amide I region (approximately 1700–1570  $\text{cm}^{-1}$ ) [22,26]. Spectral deconvolution was performed in apodization mode using an enhancement factor of 1.5 to resolve overlapping bands associated with  $\alpha$ -helix structures (approximately 1650  $\text{cm}^{-1}$ , observed at 1647  $\text{cm}^{-1}$ ),  $\beta$ -sheet structures (approximately 1635, observed at 1637  $\text{cm}^{-1}$ ) [27], and aggregated structures (1618  $\text{cm}^{-1}$ ) [22]. The relative contribution of each secondary structure was estimated from the relative intensity of the fitted bands with respect to the total amide I signal [28]. Peak intensity was used as an approximation of relative contribution, which may differ from area-based quantification.

### 2.10. Statistical Analysis

Data were analyzed using analysis of variance (ANOVA) at a 95% confidence level, and mean comparisons were performed using Tukey's test when significant differences were detected. Pearson correlation analysis was also conducted at significance levels of  $p \leq 0.05$  and  $p \leq 0.01$  to identify relationships among the evaluated parameters. In addition, Principal Components Analysis (PCA) was performed on standardized variables (correlation matrix) to examine relationships and grouping patterns among variables and samples. All statistical analyses were performed using Minitab Statistical Software 22 (Minitab LLC, State College, PA, USA), except for the clustered heatmap visualization, which was generated in Python (version 3.7.7, Python Software Foundation, Wilmington, DE, USA), using the libraries pandas, seaborn, and matplotlib. Data used for the clustered heatmap were standardized using Z-score normalization prior to analysis, and hierarchical clustering was applied to both samples and variables using Euclidean distance and Ward's linkage. All experiments and analytical determinations were performed in triplicate, unless otherwise indicated.

## 3. Results and Discussion

### 3.1. Extraction Yield, Recovery, and Chemical Composition

The extraction data showed a clear cereal-dependent effect (Table 1). RF gave higher starch yield and recovery (73.06% and 85.40%) than WF (46.71% and 62.43%), and this trend was maintained after germination, with GRSI achieving the highest recovery (95.88%). This is consistent with the greater difficulty of separating wheat starch from the surrounding protein matrix, particularly gluten-associated material, whereas rice starch is generally recovered more efficiently because of its simpler flour matrix and lower protein interference [3,17]. Germination improved recovery in both cereals, especially in wheat, suggesting that sprouting partially disrupted the native grain matrix and facilitated starch separation, even though wheat's absolute starch yield remained lower than that of rice [8]. The compositional results also confirmed the expected distinction between flour matrices and isolated starch fractions. Native and sprouted flours contained significantly higher protein, lipids, and ash than the corresponding isolated starches, while all extracted starch fractions were enriched in carbohydrates (~93.2–95.0%). Previous reports have shown similar trends in starch isolation, resulting in starch-enriched fractions with reduced levels of non-starch components, including proteins, lipids, and minerals [29]. Germination reduced protein, lipid, and ash contents in both flour systems (Table 1). This is consistent with reserve mobilization during sprouting and the restructuring of grain components [8,11]. Similar reductions in protein content after rice starch isolation have been reported by Yue et al. [29], who observed that separating starch from the protein matrix yielded highly purified starch fractions with lower residual protein content than the original flour. Notably, the sprouted starch isolates showed somewhat higher residual protein and lipid contents

than the native starch isolates, particularly in wheat, suggesting that germination may alter matrix disassembly in a way that changes the purity of the recovered starch fraction. Li et al [30] reported lipid (~0.31%), protein (~3%), and ash (~0.4%) contents for native wheat isolated starch. The corresponding values obtained in the present study were higher, possibly due to differences in isolation procedures (Table 1).

**Table 1.** Chemical composition of flours and isolated starches (%).

| Sample        | Yield | Recovery | Protein                  | Lipids                     | Ash                      | Carbohydrates             |
|---------------|-------|----------|--------------------------|----------------------------|--------------------------|---------------------------|
| Wheat systems |       |          |                          |                            |                          |                           |
| WF            | ND    | ND       | 9.73 ± 0.12 <sup>a</sup> | 4.35 ± 0.12 <sup>a</sup>   | 4.63 ± 0.12 <sup>a</sup> | 81.30 ± 0.35 <sup>c</sup> |
| GWF           | ND    | ND       | 6.99 ± 0.13 <sup>b</sup> | 3.62 ± 0.13 <sup>b</sup>   | 4.12 ± 0.13 <sup>a</sup> | 85.26 ± 0.40 <sup>b</sup> |
| WSI           | 46.71 | 62.43    | 1.31 ± 0.11 <sup>d</sup> | 1.27 ± 0.16 <sup>d</sup>   | 2.47 ± 0.14 <sup>b</sup> | 94.95 ± 0.19 <sup>a</sup> |
| GWSI          | 45.13 | 79.54    | 2.46 ± 0.52 <sup>c</sup> | 2.31 ± 0.25 <sup>c</sup>   | 2.05 ± 0.74 <sup>b</sup> | 93.17 ± 1.45 <sup>a</sup> |
| Rice systems  |       |          |                          |                            |                          |                           |
| RF            | ND    | ND       | 7.72 ± 0.29 <sup>a</sup> | 3.67 ± 0.29 <sup>a</sup>   | 4.73 ± 0.29 <sup>a</sup> | 83.87 ± 0.86 <sup>c</sup> |
| GRF           | ND    | ND       | 6.13 ± 0.18 <sup>b</sup> | 2.51 ± 0.18 <sup>b</sup>   | 3.74 ± 0.18 <sup>b</sup> | 87.62 ± 0.54 <sup>b</sup> |
| RSI           | 73.06 | 85.4     | 2.06 ± 0.07 <sup>c</sup> | 1.85 ± 0.12 <sup>c</sup>   | 2.20 ± 0.06 <sup>c</sup> | 93.89 ± 0.19 <sup>a</sup> |
| GRSI          | 61.35 | 95.88    | 1.94 ± 0.29 <sup>c</sup> | 2.00 ± 0.28 <sup>b,c</sup> | 2.07 ± 0.23 <sup>c</sup> | 93.99 ± 0.69 <sup>a</sup> |

WF: wheat flour, GWF: germinated wheat flour, WSI: isolated wheat starch, GWSI: germinated isolated wheat starch. RF: rice flour, GRF: germinated rice flour, RSI: isolated rice starch, GRSI: germinated isolated rice starch. Values are mean ± SD (*n* = 3), except for yield and recovery, which are reported as process outputs. ANOVA was performed separately for wheat and rice systems. Different letters within each cereal system and column indicate significant differences (*p* < 0.05) according to Tukey's test.

### 3.2. Carbohydrate Composition

The carbohydrate composition further differentiated flour matrices from isolated starch fractions and showed a clear effect of germination (Table 2). As expected, isolated starches contained higher total starch than the corresponding flours, reflecting the removal of proteins, lipids, and other non-starch constituents during extraction, as previously reported for rice and wheat systems [3,4]. Germination markedly reduced total starch in both cereals, particularly in GWF, which is consistent with the mobilization of starch reserves by endogenous amylolytic activity during sprouting [8,9]. This reduction was accompanied by a lower amylose content in sprouted materials and a relative increase in the proportion of amylopectin, suggesting that germination altered the starch fraction not only quantitatively but also compositionally. The reduction in amylose content in both systems after germination can be attributed to endogenous amylolytic enzymes that hydrolyze starch reserves [31]. Since amylose is associated with the amorphous regions of the granule, it is more accessible to enzymatic attack [32]. Previous studies have shown that crystallinity alone does not determine starch degradability but also starch molecular organization and enzyme accessibility [31,32]. Structural modifications that involve amylose-content and amylopectin-chain distribution have also been associated with starch molecular remodeling during long-term storage [14].

At the flour level, total dietary fiber was similar across samples. Still, the sprouted flours showed the highest soluble dietary fiber values, indicating that germination may have promoted partial solubilization or redistribution of structural carbohydrates, also observed in sprouted cereal systems [11].

In contrast, all isolated starches showed much lower dietary fiber contents, confirming that extraction yielded starch-enriched fractions with limited interference from fiber-rich components [1,6].

**Table 2.** Carbohydrate composition of flours and isolated starches (%).

| Sample        | Total Starch              | Amylose                     | Amylopectin                 | Total Dietary Fiber      | Soluble Dietary Fiber    | Insoluble Fiber          |
|---------------|---------------------------|-----------------------------|-----------------------------|--------------------------|--------------------------|--------------------------|
| Wheat systems |                           |                             |                             |                          |                          |                          |
| WF            | 74.81 ± 0.33 <sup>c</sup> | 24.70 ± 0.11 <sup>a</sup>   | 75.30 ± 0.11 <sup>d</sup>   | 5.07 ± 0.28 <sup>a</sup> | 2.35 ± 0.04 <sup>b</sup> | 2.71 ± 0.30 <sup>a</sup> |
| GWF           | 56.74 ± 0.42 <sup>d</sup> | 18.77 ± 0.21 <sup>d</sup>   | 81.23 ± 0.21 <sup>a</sup>   | 5.42 ± 0.29 <sup>a</sup> | 3.58 ± 0.25 <sup>a</sup> | 1.84 ± 0.22 <sup>b</sup> |
| WSI           | 91.37 ± 0.09 <sup>b</sup> | 23.51 ± 0.22 <sup>b</sup>   | 76.49 ± 0.22 <sup>c</sup>   | 2.15 ± 0.04 <sup>b</sup> | 0.55 ± 0.08 <sup>c</sup> | 1.60 ± 0.08 <sup>b</sup> |
| GWSI          | 93.21 ± 0.55 <sup>a</sup> | 21.95 ± 0.48 <sup>c</sup>   | 78.05 ± 0.48 <sup>b</sup>   | 1.93 ± 0.49 <sup>b</sup> | 0.55 ± 0.08 <sup>c</sup> | 1.38 ± 0.45 <sup>b</sup> |
| Rice systems  |                           |                             |                             |                          |                          |                          |
| RF            | 85.55 ± 0.24 <sup>c</sup> | 20.83 ± 0.35 <sup>a,b</sup> | 79.17 ± 0.35 <sup>a,b</sup> | 5.37 ± 0.31 <sup>a</sup> | 2.47 ± 0.27 <sup>b</sup> | 2.90 ± 0.21 <sup>a</sup> |
| GRF           | 64.00 ± 0.43 <sup>d</sup> | 19.69 ± 0.25 <sup>b</sup>   | 80.31 ± 0.25 <sup>a</sup>   | 5.82 ± 0.21 <sup>a</sup> | 3.60 ± 0.13 <sup>a</sup> | 2.22 ± 0.34 <sup>a</sup> |
| RSI           | 90.51 ± 0.57 <sup>b</sup> | 21.69 ± 0.38 <sup>a</sup>   | 78.31 ± 0.38 <sup>b</sup>   | 1.39 ± 0.11 <sup>b</sup> | 0.69 ± 0.34 <sup>c</sup> | 0.70 ± 0.40 <sup>b</sup> |
| GRSI          | 92.40 ± 0.46 <sup>a</sup> | 19.45 ± 0.91 <sup>b</sup>   | 80.55 ± 0.91 <sup>a</sup>   | 1.67 ± 0.61 <sup>b</sup> | 0.69 ± 0.34 <sup>c</sup> | 0.99 ± 0.27 <sup>b</sup> |

WF: wheat flour, GWF: germinated wheat flour, WSI: isolated wheat starch, GWSI: germinated isolated wheat starch. RF: rice flour, GRF: germinated rice flour, RSI: isolated rice starch, GRSI: germinated isolated rice starch. Values are mean ± SD ( $n = 3$ ). ANOVA was performed separately for wheat and rice systems. Different letters within each cereal system and column indicate significant differences ( $p < 0.05$ ) according to Tukey's test.

### 3.3. Thermal Characteristics

The thermal data showed that both matrix type and germination strongly affected starch gelatinization behavior (Table 3). The results for native wheat isolated starch align with literature values [30]. Native flour samples exhibited lower gelatinization enthalpy than the corresponding isolated starches, indicating that the flour matrix did not behave as a purely starch-dominant system and that the measured transition reflected the presence of non-starch components together with a lower starch concentration, as previously reported for cereal flours and starches [3,4]. In contrast, isolated native starches exhibited the highest  $\Delta H$  values, suggesting a greater proportion of ordered structures that must be disrupted during heating (Table 3). This is further supported by the positive correlation between gelatinization enthalpy and total starch ( $r = 0.709$ ,  $p = 0.022$ ), which reinforces that systems with higher starch content exhibit a more ordered structure.

**Table 3.** Thermal properties of flours and isolated starches.

| Sample        | T <sub>0</sub> (°C)       | T <sub>p</sub> (°C)       | T <sub>f</sub> (°C)       | T <sub>f</sub> -T <sub>0</sub> (°C) | ΔH (J/g)                  |
|---------------|---------------------------|---------------------------|---------------------------|-------------------------------------|---------------------------|
| Wheat systems |                           |                           |                           |                                     |                           |
| WF            | 65.22 ± 0.32 <sup>a</sup> | 67.80 ± 0.27 <sup>b</sup> | 69.64 ± 0.27 <sup>c</sup> | 4.42 ± 0.08 <sup>b</sup>            | 8.82 ± 0.29 <sup>b</sup>  |
| GWF           | 56.17 ± 0.45 <sup>b</sup> | 59.75 ± 0.16 <sup>c</sup> | 63.46 ± 0.16 <sup>d</sup> | 7.29 ± 0.40 <sup>a</sup>            | 4.78 ± 0.40 <sup>c</sup>  |
| WSI           | 65.80 ± 0.66 <sup>a</sup> | 69.50 ± 0.51 <sup>a</sup> | 74.02 ± 0.37 <sup>a</sup> | 4.46 ± 0.20 <sup>b</sup>            | 12.70 ± 0.53 <sup>a</sup> |
| GWSI          | 65.51 ± 0.61 <sup>a</sup> | 69.91 ± 0.67 <sup>a</sup> | 72.84 ± 0.16 <sup>b</sup> | 7.33 ± 0.50 <sup>a</sup>            | 7.72 ± 0.44 <sup>b</sup>  |
| Rice systems  |                           |                           |                           |                                     |                           |
| RF            | 66.72 ± 0.18 <sup>a</sup> | 68.79 ± 0.35 <sup>b</sup> | 71.51 ± 0.35 <sup>c</sup> | 4.79 ± 0.48 <sup>c</sup>            | 9.99 ± 0.16 <sup>b</sup>  |
| GRF           | 63.24 ± 0.09 <sup>b</sup> | 65.68 ± 0.21 <sup>c</sup> | 68.87 ± 0.21 <sup>d</sup> | 5.62 ± 0.27 <sup>c</sup>            | 6.90 ± 0.18 <sup>c</sup>  |
| RSI           | 64.19 ± 0.74 <sup>b</sup> | 72.11 ± 0.76 <sup>a</sup> | 75.43 ± 0.29 <sup>a</sup> | 7.48 ± 0.10 <sup>b</sup>            | 13.88 ± 0.20 <sup>a</sup> |
| GRSI          | 63.31 ± 0.56 <sup>b</sup> | 72.35 ± 0.47 <sup>a</sup> | 73.63 ± 0.51 <sup>b</sup> | 10.33 ± 0.44 <sup>a</sup>           | 6.97 ± 0.71 <sup>c</sup>  |

WF: wheat flour, GWF: germinated wheat flour, WSI: isolated wheat starch, GWSI: germinated isolated wheat starch. RF: rice flour, GRF: germinated rice flour, RSI: isolated rice starch, GRSI: germinated isolated rice starch. Values are mean ± SD ( $n = 3$ ). ANOVA was performed separately for wheat and rice systems. Different letters within each cereal system and column indicate significant differences ( $p < 0.05$ ) according to Tukey's test.

RSI also showed the highest  $T_p$  and one of the widest gelatinization ranges, consistent with previous reports indicating that starch source and granule organization strongly influence thermal transitions [1].

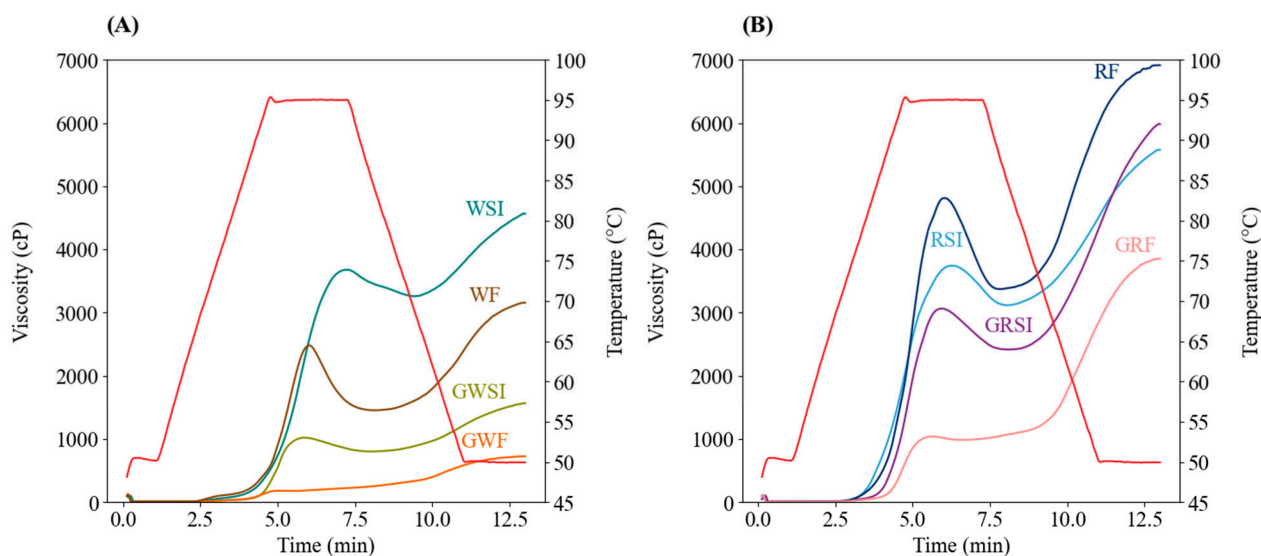
Germination reduced the thermal stability of both flour and starch systems, particularly the enthalpy. GWF showed the lowest  $T_0$ ,  $T_p$ ,  $T_f$ , and  $\Delta H$  values, while both sprouted starches (GWSI and GRSI) exhibited enthalpies considerably lower than those of their

native counterparts (WSI and RSI). This behavior aligns with the partial hydrolysis and structural disruption promoted by endogenous enzymes during sprouting, thereby reducing the energy required for gelatinization [8,9]. At the same time, the broader gelatinization ranges observed in sprouted starches, especially in RSI, suggest a more heterogeneous structural organization after germination. Wang et al. [33] showed that increased molecular organization may be associated with higher gelatinization enthalpy due to enhanced structural stability. However, in the present study, germinated flour exhibited higher short-range molecular order (Section 3.6) and lower  $\Delta H$  values, further supporting the idea that germination may have promoted molecular reorganization while reducing the amount of intact starch available for gelatinization.

These results indicate that starch isolation concentrated the more thermally resistant fraction, whereas germination weakened molecular organization and reduced the energetic demand of gelatinization. Similar reductions in starch structural stability during long-term storage have been associated with modifications in molecular organization that altered gelatinization behavior and thermal transitions [14].

### 3.4. Viscosity Characteristics of Flours and Starches

The pasting profiles of the flours and isolated starch systems for both cereals are presented in Figure 1. Similar pasting profiles were reported by Li et al. [30] for isolated native wheat starch. Previous studies on rice starch have demonstrated that modifications to starch molecular structure, regardless of origin, are reflected in altered pasting behavior, including changes in peak and final viscosities [14]. Similar relationships between structure and functionality were observed in the present study.



**Figure 1.** Viscograms of flours and isolated starches. The red line represents the temperature profile. (A) Wheat systems; (B) rice systems. WF: wheat flour, GWF: germinated wheat flour, WSI: isolated wheat starch, GWSI: germinated isolated wheat starch. RF: rice flour, GRF: germinated rice flour, RSI: isolated rice starch, GRSI: germinated isolated rice starch.

The amylose–amylopectin balance provides insights into viscosity behaviors during the RVA. Amylose, due to its smaller and linear-helical structure, leaches out of the granule during heating, while amylopectin remains largely within the granule and is the main contributor to peak viscosity prior to granule breakdown [34,35]. However, similar amylopectin contents do not necessarily result in similar viscosity profiles. For instance, WF and WSI exhibited similar amylopectin contents (75.30% and 76.49%, respectively), yet their peak viscosities differed significantly (2482.50 vs. 3645.5 cP), highlighting the

influence of additional structural and compositional factors beyond the proportion of starch fractions (Figure 1A). In addition, isolated starches of the wheat system achieved higher peak viscosities than their corresponding flours (Figure 1A). In contrast, in the rice system, this trend was observed only in germinated samples, as RF showed higher peak and final viscosities than RSI. This behavior may be associated with differences in swelling capacity, as previous studies have reported that rice flour can exhibit swelling power comparable to or even higher than that of rice starch, particularly at elevated temperatures [3]. The positive correlation between gelatinization enthalpy and peak viscosity ( $r = 0.837$ ,  $p = 0.009$ ) aligns with these results, indicating that systems requiring higher energy for gelatinization tend to develop greater viscosity during pasting. This behavior may be associated with differences in structural organization and the presence of non-starch components, such as proteins and lipids, in flour systems, indicating restricted granule swelling and reduced development of viscosity compared to isolated starch fractions.

Additionally, amylose content alone does not fully determine final viscosity. Although RSI and GRF showed similar amylose contents (21.69% and 19.69%, respectively) and comparable final viscosities, no proportional relationship was observed across all samples, particularly given the relatively narrow amylose range (~6%). This may indicate that other factors, such as molecular structure and matrix composition, play a dominant role.

Interestingly, GWF exhibited the lowest overall viscosity profile (Figure 1A), despite not having the lowest protein, lipid, or ash contents. Instead, its low viscosity is likely linked to its reduced molecular weight and structural weakening caused by germination [8,9]. In contrast, GRF, although also hydrolyzed, maintained a higher final viscosity, supporting reassociation during cooling [35].

This interpretation is supported by molecular weight data, where sprouted flours (GWF:  $1.08 \times 10^8$  g/mol; GRF:  $1.71 \times 10^8$  g/mol) exhibited reduced chain length, potentially increasing chain mobility and reorganization capacity [9]. This phenomenon could explain why GRF achieved a final viscosity comparable to RS despite its lower structural integrity. Additionally, the observed correlation between molecular weight and peak viscosity ( $r = 0.754$ ,  $p = 0.012$ ) reflects the impact of structural degradation during germination, which reduces both parameters, rather than indicating a direct causal relationship [8,9].

### 3.5. Molecular Characteristics Related to Starch Architecture

The molecular descriptors in Table 4 indicate that germination induced substantial, grain-dependent changes in amylopectin architecture. Similar modifications in the amylopectin branch-chain distribution have been reported in rice starch systems undergoing molecular changes during long-term storage. Wang et al. [14] observed that reductions in long amylopectin chains and redistribution toward shorter chains were associated with alterations in starch molecular organization. In general, native wheat systems showed higher molecular weight and larger radius of gyration than their corresponding sprouted systems; rice showed the same overall trend but with different magnitudes. The reduction in Mw after germination was particularly evident in the isolated starches, decreasing from 2.16 to  $0.95 \times 10^8$  g/mol in wheat and from 1.52 to  $0.76 \times 10^8$  g/mol in rice. This strongly suggests the depolymerization of amylopectin during sprouting, consistent with the action of endogenous amylolytic enzymes on starch reserves during germination [9,10,12]. The concurrent decrease in Rz in sprouted materials further supports the idea of a more compact or fragmented molecular population, particularly in wheat sprouted systems. Similarly, the radius of gyration was positively correlated with peak viscosity ( $r = 0.709$ ,  $p = 0.049$ ), reinforcing the notion that more spatially expanded amylopectin structures favor enhanced swelling behavior. These results indicate that germination did not simply reduce starch content but altered the supramolecular size of the remaining amylopectin fraction.

**Table 4.** Molecular weight, radius of gyration, density, and amylopectin branch dimensions of flours and isolated starches.

| Sample        | Mw<br>( $\times 10^8$ g/mol)   | Rz (nm)                        | $\rho$ (gmol/nm <sup>3</sup> ) | A%                            | B1%                           | B2%                           | B3%                           |
|---------------|--------------------------------|--------------------------------|--------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|
| Wheat systems |                                |                                |                                |                               |                               |                               |                               |
| WF            | 2.15 $\pm$ 0.36 <sup>a</sup>   | 215.63 $\pm$ 0.36 <sup>b</sup> | 2.15 $\pm$ 0.35 <sup>a</sup>   | 12.68 $\pm$ 0.36 <sup>d</sup> | 26.72 $\pm$ 0.36 <sup>b</sup> | 39.77 $\pm$ 0.36 <sup>a</sup> | 20.83 $\pm$ 1.08 <sup>a</sup> |
| GWF           | 1.08 $\pm$ 0.30 <sup>b</sup>   | 167.29 $\pm$ 0.30 <sup>c</sup> | 2.30 $\pm$ 0.62 <sup>a</sup>   | 33.98 $\pm$ 0.48 <sup>a</sup> | 32.89 $\pm$ 0.48 <sup>a</sup> | 12.94 $\pm$ 0.48 <sup>d</sup> | 20.20 $\pm$ 1.43 <sup>a</sup> |
| WSI           | 2.16 $\pm$ 0.02 <sup>a</sup>   | 230.61 $\pm$ 0.72 <sup>a</sup> | 1.76 $\pm$ 0.04 <sup>a</sup>   | 18.13 $\pm$ 0.16 <sup>c</sup> | 32.18 $\pm$ 0.25 <sup>a</sup> | 27.05 $\pm$ 0.08 <sup>b</sup> | 22.65 $\pm$ 0.05 <sup>a</sup> |
| GWSI          | 0.95 $\pm$ 0.12 <sup>b</sup>   | 163.96 $\pm$ 8.36 <sup>c</sup> | 2.15 $\pm$ 0.10 <sup>a</sup>   | 28.47 $\pm$ 0.14 <sup>b</sup> | 31.90 $\pm$ 0.56 <sup>a</sup> | 18.03 $\pm$ 0.09 <sup>c</sup> | 21.60 $\pm$ 0.77 <sup>a</sup> |
| Rice systems  |                                |                                |                                |                               |                               |                               |                               |
| RF            | 2.06 $\pm$ 0.25 <sup>a</sup>   | 194.78 $\pm$ 0.25 <sup>b</sup> | 2.79 $\pm$ 0.33 <sup>b</sup>   | 21.73 $\pm$ 0.36 <sup>d</sup> | 32.94 $\pm$ 0.36 <sup>b</sup> | 24.98 $\pm$ 0.36 <sup>a</sup> | 20.35 $\pm$ 1.09 <sup>a</sup> |
| GRF           | 1.71 $\pm$ 0.24 <sup>a,b</sup> | 155.66 $\pm$ 0.24 <sup>d</sup> | 4.54 $\pm$ 0.61 <sup>a</sup>   | 31.49 $\pm$ 0.31 <sup>a</sup> | 24.68 $\pm$ 0.31 <sup>c</sup> | 23.80 $\pm$ 0.31 <sup>b</sup> | 20.04 $\pm$ 0.93 <sup>a</sup> |
| RSI           | 1.52 $\pm$ 0.2 <sup>b</sup>    | 200.37 $\pm$ 2.19 <sup>a</sup> | 1.89 $\pm$ 0.26 <sup>b,c</sup> | 25.09 $\pm$ 0.21 <sup>b</sup> | 36.95 $\pm$ 0.61 <sup>a</sup> | 17.88 $\pm$ 0.24 <sup>c</sup> | 20.09 $\pm$ 0.70 <sup>a</sup> |
| GRSI          | 0.76 $\pm$ 0.06 <sup>c</sup>   | 183.86 $\pm$ 2.92 <sup>c</sup> | 1.22 $\pm$ 0.10 <sup>c</sup>   | 23.32 $\pm$ 0.27 <sup>c</sup> | 36.70 $\pm$ 0.51 <sup>a</sup> | 25.20 $\pm$ 0.10 <sup>a</sup> | 14.78 $\pm$ 0.53 <sup>b</sup> |

WF: wheat flour, GWF: germinated wheat flour, WSI: isolated wheat starch, GWSI: germinated isolated wheat starch. RF: rice flour, GRF: germinated rice flour, RSI: isolated rice starch, GRSI: germinated isolated rice starch. Values are mean  $\pm$  SD ( $n = 3$ ). ANOVA was performed separately for wheat and rice systems. Different letters within each cereal system and column indicate significant differences ( $p < 0.05$ ) according to Tukey's test.

Changes in molecular density also suggest that germination modified polymer packing. GRF exhibited the highest  $\rho$  value, whereas GRSI showed the lowest, indicating that the matrix context influenced the expression of amylopectin reorganization. Because density reflects the relationship between molecular mass and spatial expansion, these differences suggest that flour systems retained interactions with non-starch components, which may have constrained or compacted the remaining amylopectin. In contrast, isolated starches more directly reflected changes in the starch fraction itself. This distinction is important because cereal starch functionality is shaped not only by chain degradation, but also by the spatial arrangement of the polymer and its interaction with the surrounding matrix [6,36].

The branch-chain distribution provides additional evidence that germination shifted amylopectin toward shorter architectures. In both GWF and GWS, the proportion of A chains increased markedly, while B2 chains decreased relative to their native counterparts. A similar trend was observed in GRF, although GRSI retained relatively high levels of B1 and B2 and had the lowest B3 value among all samples. These results suggest that germination preferentially reduced longer and more highly connected branch populations in some systems, particularly in wheat, whereas rice showed a somewhat different re-distribution pattern. Since longer B chains contribute to cluster interconnection and the integrity of amylopectin architecture, their reduction is consistent with partial enzymatic trimming of the branched glucan network during sprouting [10,36]. At the same time, the relative enrichment in the A chain in sprouted systems is compatible with a more fragmented structure composed of shorter external branches. A strong negative correlation was observed between A chains and Rz ( $r = -0.869$ ,  $p = 0.005$ ), suggesting that an increase in the number of shorter external chains is associated with a reduction in molecular size. This supports the idea that germination promotes fragmentation of amylopectin, resulting in a more compact molecular structure.

Differences in amylopectin chain-length distribution are consistent with the activity of endogenous debranching enzymes during germination. In wheat, a pullulanase-type limit-dextrinase has been reported to be activated during sprouting [37], whereas in rice, isoamylase and pullulanase have been identified as the responsible debranching enzymes [38].

In addition, a significant negative correlation between amylose content and A-chain proportion ( $r = -0.824$ ,  $p = 0.012$ ) was observed, indicating that samples with higher proportions of short amylopectin chains tended to exhibit lower apparent amylose content. This trend is consistent with the structural modifications induced by germination. This behavior is particularly evident in germinated flours, for instance, GWF (33.98% A chains;

18.77% amylose) and GRF (31.49% A chains; 19.69% amylose), compared to their native counterparts WF (12.68% A chains; 24.70% amylose) and RF (21.73% A chains and 20.83% amylose) (Tables 2 and 4). These results reflect coordinated starch depolymerization and restructuring, rather than a direct conversion between amylose and amylopectin fractions [8,9,12].

From a functional standpoint, these structural changes help explain why sprouted materials differed from native ones in later thermal and pasting behavior. Lower molecular weight and reduced long-chain connectivity would be expected to weaken the integrity of starch granules and modify their swelling and reorganization behavior during heating and cooling. In this study, the structural changes were especially pronounced in sprouted wheat materials, which align with their lower thermal stability and weaker development of viscosity. By contrast, the rice sprouted systems retained some branch features associated with structural connectivity, which may help explain why the rice matrices preserved a more distinctive functional response after germination. Therefore, Table 4 represents a central mechanistic element of the study: it shows that germination restructured amylopectin beyond a simple loss of starch, and that this restructuring occurred differently in wheat and rice.

### 3.6. Short-Range Order Changes in Starch Architecture Analyzed by ATR-FTIR

Figure 2 shows the FTIR spectra results of the samples. The carbohydrate wavelength range corresponds to the 1200–900  $\text{cm}^{-1}$  region [22]. The band at 1300–900  $\text{cm}^{-1}$  is sensitive to starch granule C-O-H bending [6]. In addition, the amide I band (1700–1570  $\text{cm}^{-1}$ ) is highly relevant to analyze the high-level structure of a protein, as it suggests the stretching vibration of C=O belonging to the amide groups that are weakly coupled with in-plane NH bending and CN stretching [7,39].

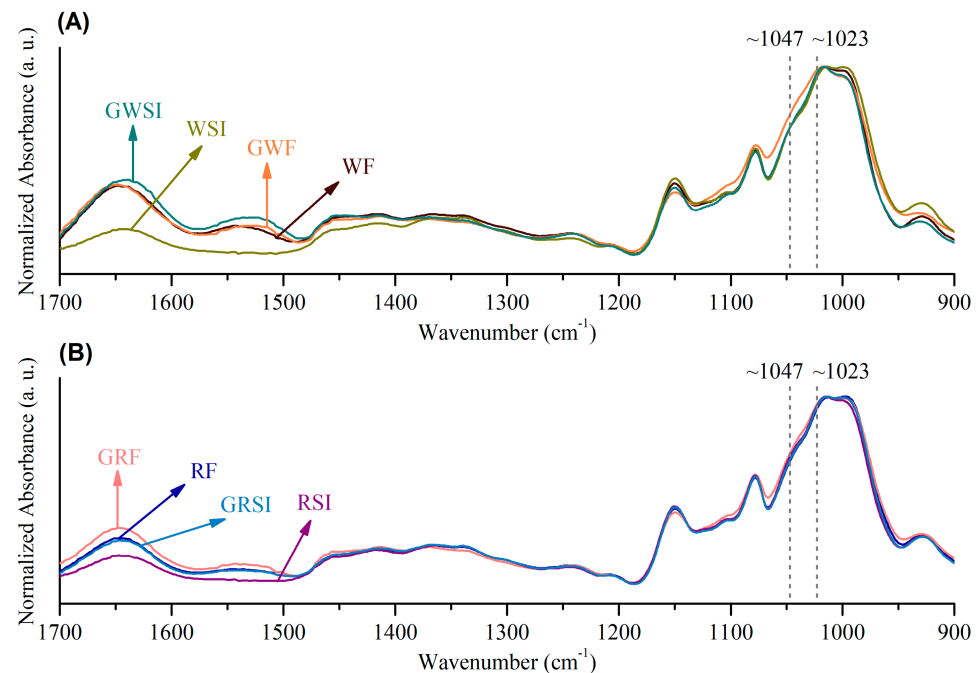
The 1047/1023 ( $\text{cm}^{-1}$ ) ratio was calculated from deconvoluted FTIR spectra results to evaluate changes in the short-range molecular order of starch among the different samples (Figure 3). Short-range order refers to the organization of the double-helical structure within starch granules [24]. It is commonly assessed by the ratio of bands associated with ordered (crystalline starch regions at  $\sim 1047 \text{ cm}^{-1}$ ) to unordered (amorphous starch regions, usually at  $\sim 1022\text{--}1023 \text{ cm}^{-1}$ ) structures [24]. A higher 1047/1023 ( $\text{cm}^{-1}$ ) ratio indicates a greater degree of molecular order (crystallinity), whereas lower values reflect disruption of these structures [25].

Relationships between short-range molecular order and starch functionality have been reported in rice starch systems, where modifications in molecular organization caused by long-term storage were accompanied by changes in physicochemical properties and starch behavior [14]. For wheat systems, Huang et al. [15] showed that residual short-range molecular order was associated with changes in thermal behavior and starch retrogradation, which highlights the importance of molecular organization beyond crystalline structure.

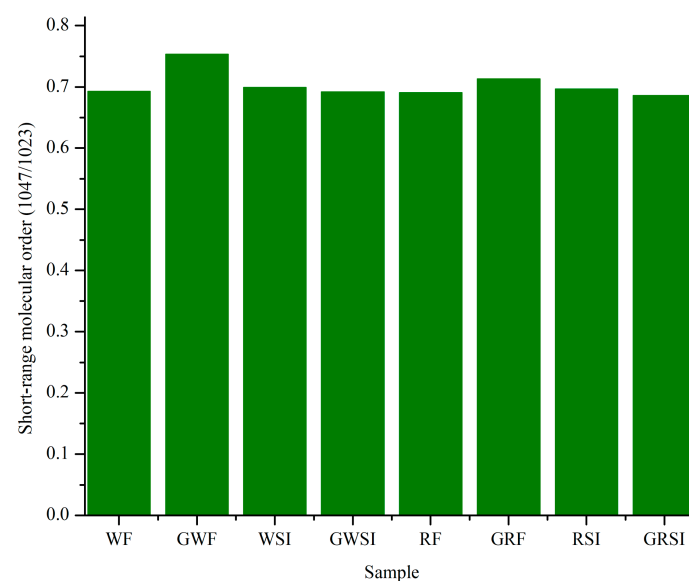
In Figure 3, it is observed that GWF and GRF exhibited the highest 1047/1023 ( $\text{cm}^{-1}$ ) absorbance ratios ( $\sim 0.75$  and  $\sim 0.71$ , respectively) among the samples (Figure 3). Additionally, GRF showed a lower starch content than GWF; this is consistent with the negative correlation between short-range order and total starch ( $r = -0.833$ ,  $p = 0.010$ ).

These results could be attributed to a higher relative organization of double-helical structures, since GRF presented a higher content of A chains (Table 4), rather than a direct measure of crystallinity [24]. This can be supported by the action of amylolytic enzymes, especially in amorphous starch regions, as reflected by the decreased amylose content of sprouted systems (Table 2); in this sense, the hydrolysis of these regions may favor the relative enrichment of shorter amylopectin chains, which could reorganize into more ordered double-helical arrangements [40]. A similar mechanism has been reported for short

amylose chains, which formed double-helical structures and complexes with lipids [41]. Furthermore, non-starch components present in the matrices may also stabilize these ordered structures, although this was not directly evaluated in the study. Therefore, even if total starch decreases (Table 2), the germinated samples exhibited higher short-range order.

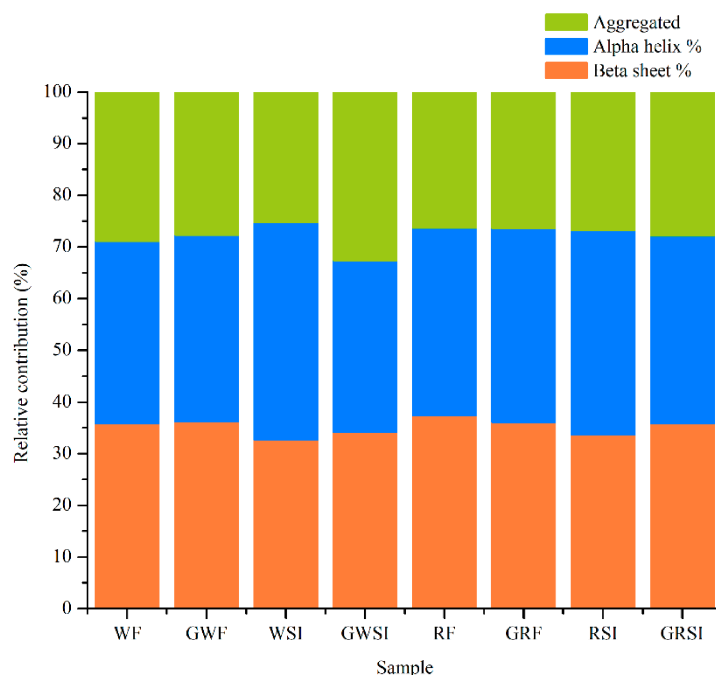


**Figure 2.** ATR-FTIR normalized spectra of starch and protein zone of samples. (A) and (B) amorphous starch region ( $\sim 1023$ ), crystalline starch region ( $\sim 1047$   $\text{cm}^{-1}$ ), and Amide I ( $\sim 1700$ – $1570$   $\text{cm}^{-1}$ ) region of wheat and rice systems, respectively. Y-axis units are arbitrary (a.u.) only to compare regions of interest. WF: wheat flour, GWF: germinated wheat flour, WSI: isolated wheat starch, GWSI: germinated isolated wheat starch. RF: rice flour, GRF: germinated rice flour, RSI: isolated rice starch, GRSI: germinated isolated rice starch.



**Figure 3.** FTIR short-range molecular order ( $1047/1023$   $\text{cm}^{-1}$ ) of flours and isolated starches. WF: wheat flour, GWF: germinated wheat flour, WSI: isolated wheat starch, GWSI: germinated isolated wheat starch. RF: rice flour, GRF: germinated rice flour, RSI: isolated rice starch, GRSI: germinated isolated rice starch.

Since the Amide I band of proteins is a complex composite signal consisting of overlapping contributions from different secondary structures, including  $\alpha$ -helices,  $\beta$ -sheets, and aggregated structures [39], the relative contribution (%) of protein secondary structure was determined through deconvolution of the Amide I region; the results are shown in Figure 4. The relative contribution of  $\beta$ -sheets ranged between 32.64 and 37.41%, while the  $\alpha$ -helix presented a higher variation and ranged between 33.17 and 42.05%; the aggregates of the Amide I protein structure ranged between 26.33 and 32.65% (Figure 4).



**Figure 4.** Relative contribution of protein secondary structures in flours and isolated starches. WF: wheat flour, GWF: germinated wheat flour, WSI: isolated wheat starch, GWSI: germinated isolated wheat starch. RF: rice flour, GRF: germinated rice flour, RSI: isolated rice starch, GRSI: germinated isolated rice starch.

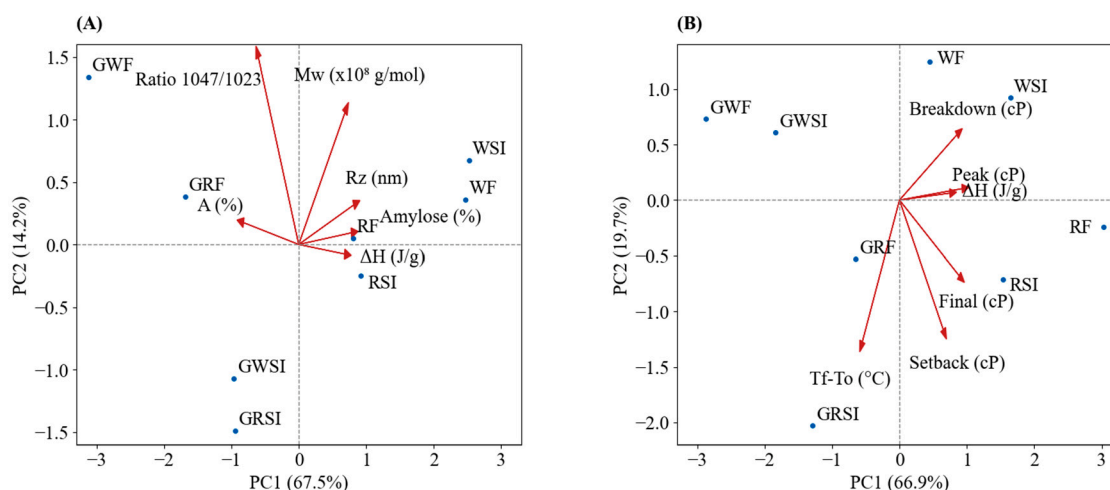
Germination appeared to alter protein secondary structure, as sprouted flours from both cereals exhibited a higher relative abundance of  $\beta$ -sheet structures compared to their native counterparts (Figure 4). Although the gluten matrix in wheat has been reported to promote  $\beta$ -sheet formation [42], the present results indicate a generally higher relative abundance of  $\beta$ -sheet structures in rice systems. This behavior can be attributed to intrinsic differences in protein composition, as rice proteins (mainly glutelins) tend to form more aggregated structures [43]. Notably, this distinction was reduced after germination, with GRF showing a  $\beta$ -sheet relative abundance (36.04%) comparable to GWF (36.16%) (Figure 4). This suggests that germination-induced proteolysis and structural rearrangements could have promoted convergence of protein organization between the two cereal systems studied.

In addition, the  $\alpha$ -helix relative contribution slightly increased in sprouted flours compared to native flours, in both systems (Figure 4). This behavior could be attributed to proteolysis that occurs during germination, which enhances protein mobility and facilitates conformational rearrangements into helical structures [8,9]. On the contrary, isolated starches of both systems exhibited a decrease in  $\alpha$ -helix relative content after germination (WSI: 42.05% to GWSI: 33.16%; RSI: 39.60% to GRSI: 32.29%), likely due to the removal or reduced contribution of residual proteins during starch isolation. These results suggest a matrix-dependent response, where germination induces protein restructuring in flour systems but leads to a reduction in ordered protein structures in isolated starches.

### 3.7. Statistical Analyses

#### 3.7.1. Principal Component Analyses

The Principal Component Analysis (PCA) revealed clear patterns of variation among the samples (Figure 5). The PCA of structural variables (Figure 5A) showed a clear separation by germination status, as well as a matrix-dependent effect. On the positive side of PC1, native samples were grouped and associated with higher molecular weight, radius of gyration, and amylose content, as well as greater thermal energy requirements for gelatinization. In contrast, germinated samples were located on the negative side of PC1, where the A-chains and short-range molecular-order variables were higher, particularly in sprouted flour samples. This shows structural changes consistent with starch reorganization.



**Figure 5.** Principal Component Analysis (PCA) of flours and isolated starches. **(A)** PCA elucidating depolymerization. **(B)** PCA elucidating processing-related changes induced by germination. WF: wheat flour, GWF: germinated wheat flour, WSI: isolated wheat starch, GWSI: germinated isolated wheat starch. RF: rice flour, GRF: germinated rice flour, RSI: isolated rice starch, GRSI: germinated isolated rice starch.

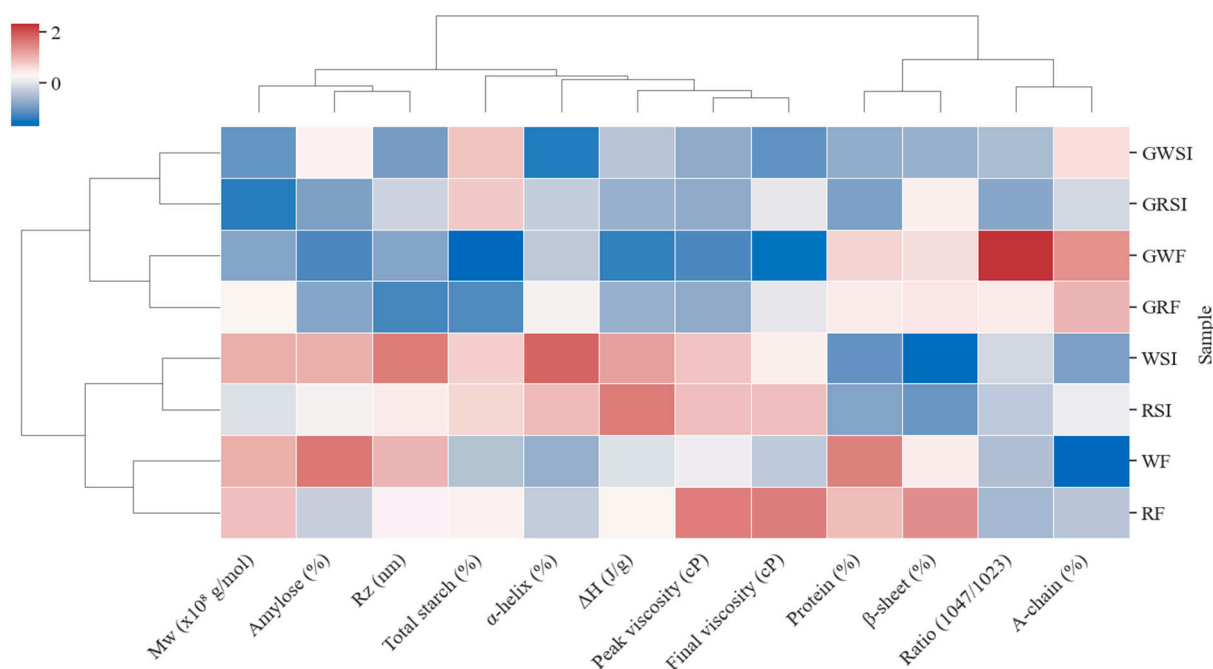
Additionally, separation along PC2 highlighted differences between flour and isolated-starch systems, particularly in germinated samples. Isolated starches (GWSI and GRSI) were located on the negative side of PC2, whereas germinated flours (GWF and GRF) were positioned on the positive side, which suggests that the structural effects of germination are also modulated by matrix composition. This indicates that germination does not affect all systems uniformly; rather, its impact depends on the presence of non-starch components such as proteins and lipids.

Conversely, the PCA based on thermal and pasting properties (Figure 5B) revealed patterns that describe the functional impact of germination. For instance, on the positive side of PC1, native systems were located and associated with higher swelling capacity, viscosity, and thermal energy requirements for gelatinization. However, on the negative side of PC1, sprouted samples were identified and associated with a broader gelatinization range (Tf-To), reflecting increased structural heterogeneity [1].

PC2 was mainly associated with setback, reflecting retrogradation and reassociation behavior during cooling [44]. RSI showed a clear association with this variable; however, GRSI was not aligned with the setback direction, suggesting that germination modifies this behavior rather than directly increasing it. This shows that germination does not simply increase or decrease retrogradation, but it modifies how starch reorganizes.

### 3.7.2. Heat Map Analysis of Structure-Related Variables

The clustered heatmap revealed a clear separation of samples by germination status, with germinated samples grouped in the upper cluster and native samples in the lower cluster (Figure 6), indicating that germination is a dominant factor in sample organization. Within these main clusters, further subgrouping by matrix type was observed, with flour systems (WF, RF) and isolated starches (WSI, RSI) forming distinct subclusters. A similar pattern was observed for germinated samples, which were also separated into flour (GWF, GRF) and isolated starch (GWSI, GRSI) systems. This indicates that, in addition to germination, matrix composition plays a key role in redefining structural and functional properties.



**Figure 6.** Clustered heatmap of flours and isolated starches. WF: wheat flour, GWF: germinated wheat flour, WSI: isolated wheat starch, GWSI: germinated isolated wheat starch. RF: rice flour, GRF: germinated rice flour, RSI: isolated rice starch, GRSI: germinated isolated rice starch.

Regarding variable associations, flour-based systems (WF, RF, GWF, GRF) were primarily characterized by higher protein content, β-sheet relative abundance, and short-range molecular order. In contrast, native starches were associated with higher molecular weight, radius of gyration, and amylose content. A consistent trend across germinated samples (GWF, GRF, GWSI, GRSI) was reduced amylose content, increased A-chain proportion, lower molecular weight and radius of gyration, and decreased viscosity, reflecting enzymatic depolymerization and structural reorganization [12].

Overall, these results demonstrate that germination induces systematic modification in starch-based systems, while the matrix composition modulates how these changes are expressed, highlighting the combined influence of both factors on physicochemical and structural behaviors.

## 4. Conclusions

The results demonstrate that germination disrupts conventional starch behavior, affecting viscosity profiles, the energy required to disrupt granules, compositional attributes, and the distribution of amylopectin chains. Sprouted samples required less energy for gelatinization, suggesting that germination may offer a processing advantage by reducing

thermal demand in starch-based systems. This effect was accompanied by an increase in amylopectin A-chain content and a reduction in amylose, reflecting enzymatic depolymerization of glucan fractions. Despite degradation, sprouted flours exhibited greater short-range molecular order in starch, suggesting that germination promotes subsequent structural reorganization and the formation of ordered structures. At the protein level, germination also influenced secondary structure, as rice systems, typically less associated with  $\beta$ -sheet-rich conformations than wheat systems, reached values comparable to those of wheat after sprouting. Furthermore, a matrix-dependent effect was observed for  $\alpha$ -helix content: germination increased its relative contribution in flour systems, while a decrease was observed in isolated starches, likely due to the reduced presence of residual proteins after extraction. Also, clustered heatmap groups suggested that both germination and matrix type independently and jointly influence the physicochemical and structural properties of starch-based systems.

The results demonstrate that germination promotes both starch depolymerization and molecular reorganization, which leads to measurable changes in starch composition, amylopectin chain-length distribution, short-range molecular order, thermal behavior, and viscosity development. These findings improve the understanding of the relationship between structure and functionality in wheat and rice flour systems and their corresponding isolated starch fractions. This provides insights into how germination modifies cereal matrix functionality, with potential applications in food formulation and nutrition.

**Author Contributions:** P.L.-S.: Writing—review and editing, Writing—original draft, Software, Investigation, Conceptualization, Formal analysis, Data Curation. J.d.I.R.-M.: Writing—review and editing, Writing—original draft, Visualization, Validation, Supervision, Project administration, Methodology, Formal analysis, Data curation, Conceptualization, Resources. All authors have read and agreed to the published version of the manuscript.

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