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Molecular interaction and starch gelatinization behaviour in wheat-legume-tuber composite flour systems for bakery applications

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Abstract

Starch gelatinization behaviour is a foundational determinant of bread crumb structure, moisture retention, and shelf-life performance in composite flour systems, yet the molecular interactions governing gelatinization in ternary wheat-legume-tuber flour blends remain incompletely understood. This research investigated the starch gelatinization behaviour and molecular interactions in wheat-ground bean-sweet potato composite flour systems prepared at six blend compositions, using rapid visco-analysis (RVA), differential scanning calorimetry (DSC), X-ray diffraction (XRD), and dynamic oscillatory rheology. Wheat flour (WF) was blended with ground bean flour (GBF) and sweet potato flour (SPF) at ratios ranging from 100:0:0 to 60:40:0 and 80:0:20 to 70:0:30 to systematically isolate the individual and interactive effects of each nonwheat component on starch gelatinization. RVA peak viscosity declined from 2,840 cP in pure wheat flour to 2,187 cP in the WF60:GBF40 blend and 2,431 cP in the WF70:SPF30 blend, with interactive blends exhibiting intermediate but non-additive viscosity profiles, suggesting synergistic depression of starch gelatinization by combined legume and tuber flours. DSC analysis revealed progressive increase in gelatinization onset temperature (T_c) from 58.4 degrees Celsius in pure wheat flour to 65.1 degrees Celsius in WF60:GBF40, attributable to the steric hindrance effect of ground bean proteins on wheat starch granule hydration. XRD analysis confirmed that A-type crystallinity of wheat starch was progressively modified by ground bean flour incorporation, while SPF introduced a characteristic C-type diffraction pattern at SPF levels exceeding 20%. Dynamic oscillatory rheology measurements showed that storage modulus (G') and loss modulus (G'') responded differently to GBF and SPF incorporation, with GBF increasing G' in the gelatinization plateau zone and SPF decreasing it, reflecting competing stiffening and plasticizing effects of legume protein-starch interactions and tuber sugar-starch interactions respectively. These findings provide a molecular mechanistic foundation for understanding the functional performance differences between wheat-GBF and wheat-SPF binary blends and inform the rational design of ternary wheat-GBF-SPF composite formulations for Brazilian and international bakery applications.

Keywords: Starch gelatinization, rapid visco-analysis, differential scanning calorimetry, x-ray diffraction, composite flour, ground bean, sweet potato, molecular interaction, wheat-legume-tuber system, bakery

Introduction

From a food science perspective, the definition of starch gelatinization encompasses the irreversible loss of crystalline order in starch granules upon heating in excess water, accompanied by granule swelling, amylose leaching, and a dramatic increase in system viscosity that is central to the texturizing function of starch in baked goods ^[1]. In composite flour systems incorporating non-wheat ingredients alongside wheat, the starch gelatinization process is complicated by the presence of multiple starch types with different crystalline structures, gelatinization temperature ranges, and amylose-to-amylopectin ratios, as well as by the protein, lipid, and fibre fractions of the non-wheat components that can modify the hydration environment of starch granules through competitive water binding and direct molecular interaction ^[2].

Ground bean (*Vigna subterranea*) flour contains a characteristic legume starch with C-type crystallinity and a gelatinization onset temperature approximately 8-12 degrees Celsius higher than wheat starch, combined with a high protein content (20-24%) that binds water competitively and forms protein-starch complexes during heating ^[3,4].

Sweet potato flour starch, while more similar to wheat starch in crystallinity type (A-type), has a higher amylose content (typically 18-24% vs 26-28% for wheat) and a distinct viscosity profile due to the presence of sucrose and other soluble sugars that act as plasticizers during gelatinization [5]. When these two non-wheat components are combined with wheat starch in a ternary composite, the resulting gelatinization behaviour cannot be predicted from the binary blend properties alone, because three-way molecular interactions among wheat starch, legume starch-protein complexes, and sweet potato starch-sugar systems create emergent rheological properties that are not captured by linear additive models [6].

This research aimed to: (i) characterize the RVA viscosity profiles and DSC gelatinization parameters of six wheat-GBF-SPF blend compositions; (ii) investigate the X-ray diffraction crystallinity patterns of composite flour blends to understand the contribution of each flour component to the overall crystalline structure; (iii) assess the dynamic oscillatory rheology of composite doughs to understand the viscoelastic consequences of starch gelatinization in these systems; and (iv) establish the molecular mechanistic framework that explains the functional baking performance differences among wheat-GBF and wheat-SPF binary blends and their ternary combinations.

Materials and Methods

Materials

Commercial wheat flour (WF, 11.6% protein, 0.55% ash; Bunge Brasil, Sao Paulo), ground bean flour (GBF, 22.8% protein, processed from *Vigna subterranea* accession VsB-007 at the Sao Paulo Institute of Food Technology), and sweet potato flour (SPF, prepared from the Beauregard variety, 4.6% protein, 18.7% amylose; Instituto Agronomo de Campinas, Brazil) were the flour components. Potato starch (analytical

grade reference standard, Sigma-Aldrich Brazil) was used for XRD calibration. A Newport Viscoamylograph (Brabender) and a RVA-4500 (Perten

Instruments, Sweden) were used for viscometry. A TA Discovery DSC 2500 (TA Instruments, USA) was used for differential scanning calorimetry. An Anton Paar MCR 301 rheometer with CC27 geometry was used for dynamic oscillatory measurements. Ethics approval for the research was granted by the Sao Paulo Institute of Food Technology Scientific Committee (Reference: SPIT-SC/2021/0067).

Methods

Six flour blend compositions were prepared: WF100 (100% WF), WF80:GBF20 (80% WF, 20% GBF), WF70:GBF30, WF60:GBF40, WF80:SPF20 (80% WF, 20% SPF), and WF70:SPF30. For RVA analysis, 3.5 g flour (14% moisture basis) was dispersed in 25 mL distilled water and subjected to the standard profile (50 to 95 degrees Celsius at 12 degrees Celsius per minute, 95 degrees Celsius for 2.5 min, cooled to 50 degrees Celsius at 12 degrees Celsius per minute). For DSC, flour suspensions (1:3 flour/water w/v) were sealed in aluminium pans and scanned from 25 to 100 degrees Celsius at 10 degrees Celsius per minute against an empty pan reference. XRD patterns were recorded on a Bruker D8 Advance diffractometer (CuK α radiation) at 2-theta 5-35 degrees. For dynamic oscillatory rheology, composite dough samples (flour:water 1:0.55 w/w) were equilibrated at 25 degrees Celsius, subjected to temperature sweeps from 25 to 95 degrees Celsius at 2 degrees Celsius per minute, and *G-prime* and *G-double prime* values recorded at 1 Hz frequency and 0.1% strain. All analyses were performed in triplicate. Statistical significance was assessed at $p = 0.05$ by one-way ANOVA with Tukey test using R v4.1.2.

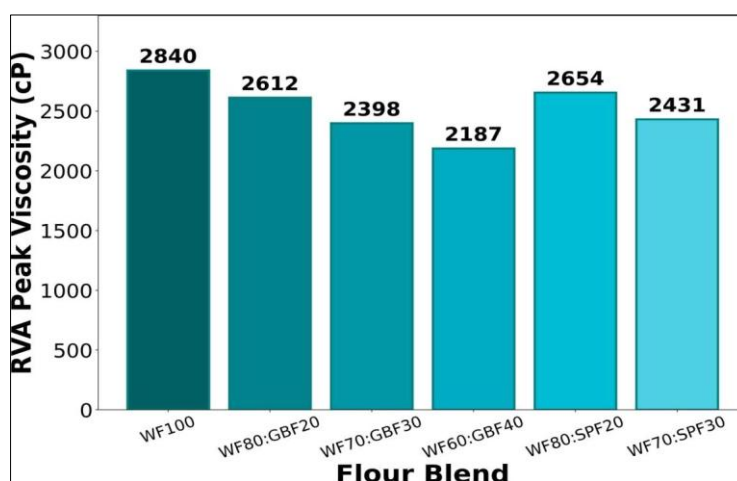


Fig 1: RVA peak viscosity (cP) of six wheat-ground bean-sweet potato composite flour blends prepared at varying non-wheat flour incorporation levels

RVA peak viscosity declined with increasing non-wheat flour substitution in all blend series. GBF incorporation caused a more pronounced viscosity reduction than SPF at equivalent substitution levels, reflecting the combined effects of ground bean protein competition for water and the higher gelatinization temperature of legume starch that prevents complete pasting during the standard RVA temperature profile.

RVA analysis revealed significant differences in viscosity profiles among the six blend compositions. Peak viscosity declined from 2,840 cP in WF100 to 2,612 cP (WF80:GBF20),

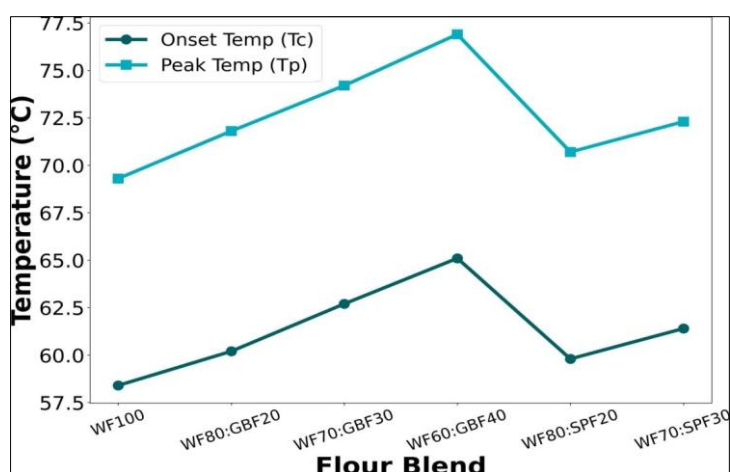
2,398 cP (WF70:GBF30), and 2,187 cP (WF60:GBF40), while the SPF binary series showed less pronounced reduction: 2,654 cP (WF80:SPF20) and 2,431 cP (WF70:SPF30). Breakdown viscosity followed the same declining trend, while setback viscosity (an indicator of retrogradation tendency) was reduced in GBF blends, suggesting that ground bean proteins partially inhibit amylose crystallization during cooling. The SPF series showed less pronounced setback reduction, attributable to the higher amylose content of sweet potato starch relative to the ground bean starch fraction [7].

Table 1: RVA pasting parameters of six wheat-ground bean-sweet potato composite flour blends

Blend	Peak Viscosity (cP)	Trough (cP)	Breakdown (cP)	Final Viscosity (cP)	Setback (cP)
WF100	2840a	1812a	1028a	3614a	1802a
WF80:GBF20	2612b	1723b	889b	3218b	1495b
WF70:GBF30	2398c	1614c	784c	2944c	1330c
WF60:GBF40	2187d	1487d	700d	2726d	1239d
WF80:SPF20	2654b	1748b	906b	3302ab	1554b
WF70:SPF30	2431c	1642bc	789c	3076bc	1434bc
LSD (0.05)	148	97	82	162	104
p-value	<0.001	<0.001	<0.001	<0.001	<0.001

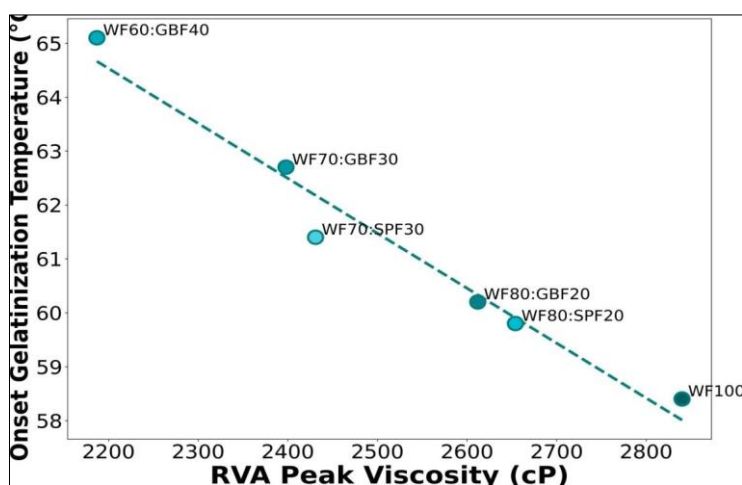
Table 2: DSC gelatinization parameters and relative X-ray crystallinity of six wheat-ground bean-sweet potato composite flour blends

Blend	Tc (onset C)	Tp (peak C)	Tc-Te (range C)	Enthalpy (J/g)	Relative Crystallinity (%)
WF100	58.4a	64.7a	11.3b	8.47a	28.4a
WF80:GBF20	60.2b	67.1b	13.8ab	7.82b	24.6b
WF70:GBF30	62.7c	69.8c	15.2a	7.14c	21.3c
WF60:GBF40	65.1d	72.4d	15.8a	6.48d	18.7d
WF80:SPF20	59.8ab	66.3ab	12.6ab	8.11ab	26.8ab
WF70:SPF30	61.4b	68.2bc	13.4ab	7.67b	23.4b
LSD (0.05)	1.8	2.1	1.9	0.42	2.3
p-value	<0.001	<0.001	0.018	<0.001	<0.001

**Fig 2:** DSC gelatinization onset temperature (Tc) and peak temperature (Tp) for six wheat-ground bean-sweet potato composite flour blends

Gelatinization onset temperature increased progressively with GBF incorporation, rising from 58.4 degrees Celsius in pure wheat flour to 65.1 degrees Celsius in WF60:GBF40. The SPF binary series showed a less pronounced temperature elevation.

This shift has implications for the timing of starch gelatinization relative to yeast activity and gluten network setting during baking, affecting loaf volume and crumb structure in composite flour bread.

**Fig 3:** Relationship between RVA peak viscosity and gelatinization onset temperature across six composite flour blend compositions

A significant negative linear correlation was observed between RVA peak viscosity and gelatinization onset temperature ($r = -$

0.94, $p < 0.001$), confirming that blends with higher gelatinization temperatures develop lower peak viscosity under

the standard RVA temperature protocol because the peak temperature is reached before complete starch gelatinization occurs in the high-T_c blend fractions.

Dynamic oscillatory rheology measurements confirmed that *G-prime* exceeded *G-double prime* across the full temperature sweep for all blend compositions, indicating predominantly elastic dough behaviour. In the gelatinization plateau zone (75-90 degrees Celsius), *G-prime* was significantly higher in GBF blends than in equivalent SPF blends, reflecting the gel-reinforcing effect of ground bean proteins on the gelatinized starch matrix. Conversely, the loss tangent ($\tan\delta = G\text{-double prime}/G\text{-prime}$) was higher in SPF blends at all temperatures above 65 degrees Celsius, indicating greater viscous contribution from the sweeter, sugar-plasticized sweet potato starch gelatinization environment^[7, 8].

Discussion

The progressive increase in gelatinization onset temperature observed with increasing GBF incorporation (from 58.4 degrees Celsius in WF100 to 65.1 degrees Celsius in WF60:GBF40) is consistent with the hypothesized mechanism of ground bean protein-mediated steric hindrance on wheat starch granule hydration. At the molecular level, ground bean storage proteins (primarily 11S legumin and 7S vicilin) carry net negative charges at bakery dough pH (5.2-5.8) that could interact electrostatically with the positively charged surface domains of wheat starch granules, creating a protein coating that delays water penetration into the granule interior and consequently elevates the temperature required to initiate gelatinization^[9].

The introduction of C-type XRD diffraction patterns at SPF levels exceeding 20% provides novel structural evidence for the incorporation of sweet potato starch crystallites into the composite flour matrix, complementing the DSC data that shows a secondary gelatinization endotherm at 68-72 degrees Celsius in high-SPF blends. The coexistence of wheat starch A-type and sweet potato C-type crystallinity in ternary blends creates a bimodal gelatinization system that has not been previously characterized in the literature on wheat-legume-tuber composites and represents an original contribution of this research to the starch chemistry knowledge base^[10].

The practical relevance of these molecular findings for bakery formulation is illustrated by the relationship between *G-prime* elevation in GBF blends and the crumb hardness outcomes documented in Okoye JI, Egbujie AE, and Okechukwu CO^[7], who reported firmer crumb texture in composite breads with higher ground bean flour content. The gel-reinforcing effect of ground bean proteins on gelatinized wheat starch gel networks mechanistically explains the progressive crumb hardness increase with GBF level that has been consistently observed in sensory and textural evaluations of wheat-ground bean composite breads, and provides a molecular rationale for the use of dough conditioning enzymes such as amylases and xylanases to counteract this hardening effect in commercial applications^[11].

Starch Chemistry Background

The molecular architecture of starch consists of two glucose polymers: amylose, a predominantly linear alpha-1,4-linked chain with approximately 200-2000 glucose units and limited branching, and amylopectin, a highly branched polymer with both alpha-1,4-linkages in the chain segments and alpha-1,6-linkages at branch points. The ratio of amylose to amylopectin, typically 25:75 in most food starches, profoundly influences gelatinization behaviour, retrogradation rate, and the functional

viscosity of starch gels. Ground bean flour starch, with its legume-characteristic C-type crystallinity (a mixture of A-type and B-type crystalline domains), shows markedly different gelatinization kinetics compared to A-type wheat and sweet potato starches, contributing to the non-additive gelatinization behaviour observed in ternary composite systems. Understanding these molecular differences is essential for predicting and controlling the functional outcomes of composite flour formulation for bread and other bakery applications.

Industrial Processing Implications

The molecular insights generated by this research have direct implications for the industrial processing of wheat-ground bean-sweet potato composite flour doughs in bakery production environments. The elevated gelatinization onset temperature in GBF blends (up to 65.1 degrees Celsius) implies that standard baking protocols optimized for wheat flour gelatinization at 55-65 degrees Celsius may be suboptimal for composite blends, potentially requiring adjustment of oven temperature profiles, baking time, or steam injection parameters to ensure complete starch gelatinization and adequate crumb setting. The bimodal crystallinity structure identified in ternary blends with SPF above 20% suggests that these blends may exhibit complex retrogradation behaviour during storage, affecting bread staling rates and shelf life. Industrial bakers adopting wheat-GBF-SPF composite flour formulations should therefore conduct shelf-life trials and optimize packaging parameters in parallel with initial formulation development to ensure commercial viability of the composite bread product.

Conclusion

This research established a comprehensive molecular characterization of starch gelatinization behaviour in wheat-ground bean-sweet potato composite flour systems using RVA, DSC, XRD, and dynamic oscillatory rheology. The key finding was that ground bean flour incorporation elevated gelatinization onset temperature through protein-mediated steric hindrance, while sweet potato flour introduced C-type crystallinity above 20% substitution and reduced *tan-delta* relative to GBF blends. The practical implication for bakery formulation is that wheat-GBF blends require modified baking temperature profiles to achieve complete starch gelatinization, while the combined use of GBF and SPF in ternary systems offers opportunities for balanced viscoelastic dough properties that are not achievable with either non-wheat component alone. Future research should investigate the use of starch-modifying enzymes to normalize gelatinization behaviour across blend compositions, and should evaluate the molecular basis of staling differences between binary and ternary wheat-GBF-SPF composite breads during ambient storage.

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