
Development and characterization of gluten-free bread from optimized cassava, chickpea, and corn composite flours

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Abstract An optimized composite flour blend of cassava, chickpea, and corn flours produced a high-quality gluten-free bread with specific volume, crumb hardness, and springiness comparable to conventional wheat-based bread. Flour composition significantly influenced the physical and sensory attributes of the final product. The incorporation of chickpea flour enhanced the nutritional profile, specifically increasing protein content, while corn flour modified the whiteness index and crumb texture. Rapid Visco Analyzer (RVA) profiles revealed that the viscosity and amylose content of the flour formulations were critical determinants of the bread's structural integrity. The addition of hydrocolloids and emulsifiers further stabilized the crumb structure and extended shelf life. Sensory evaluation scores indicated high consumer acceptance, particularly for the optimized blend, which balanced flavor and mouthfeel effectively. Cassava flour functioned as a viable, sustainable, and cost-effective primary base in the gluten-free system. These results demonstrated that the interaction between cassava, legumes, and maize flours created a synergistic effect that mimicked the viscoelastic properties of gluten. The findings established a successful formulation for a gluten-free bakery product using locally sourced ingredients, providing a functional alternative for the expanding gluten-free market.

Keywords: Gluten-free bread, Cassava flour, Composite flour, Sensory acceptance, Shelf life

Introduction

The increasing global incidence of celiac disease and other gluten-related disorders has led to a significant demand for gluten-free food products, particularly bread (Chen *et al.*, 2026). However, the absence of gluten, a protein network crucial for dough elasticity and gas retention, presented a major challenge in producing gluten-free bread with desirable sensory and nutritional qualities (Jiang *et al.*, 2026). Traditional gluten-free formulations often resulted in products with poor textural properties, low specific volume, and a hard, dense

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crumb structure (Chisenga *et al.*, 2020). This had prompted extensive research into alternative ingredients and processing techniques to replicate the functional properties of gluten. One promising approach was the utilization of composite flours from non-traditional sources (Achalun *et al.*, 2025; Jiang *et al.*, 2026). Root crops, cereals, and legumes, such as cassava (*Manihot esculenta*), corn, and chickpea (*Cicer arietinum*), had emerged as viable alternatives to wheat flour, offering a wide array of starches and proteins that contributed to improved bread quality. The incorporation of cassava flour, for instance, had been shown to improve the quality of gluten-free bread, with its starch influencing dough rheology and loaf volume (Sigüenza-Andrés *et al.*, 2021; Róžańska *et al.*, 2025). Similarly, chickpea flour was a valuable functional ingredient, particularly when used in sourdough fermentation, to enhance nutritional value and improved baking quality attributes such as specific volume and crumb hardness (Jiang *et al.*, 2026). Despite the potential of these ingredients, replicating the viscoelastic properties of gluten remained a significant hurdle. To overcome this, researchers had explored various additives and innovative processing methods. The application of hydrocolloids, such as gum extracts and xanthan gum, had proven successful in improving dough rheology and loaf quality by increasing specific volume (Ndjang *et al.*, 2024; Zhang *et al.*, 2025a). Furthermore, incorporating sourdough fermented with lactic acid bacteria and yeast had been shown to enhance the technological and nutritional quality of gluten-free bread, leading to an increase in specific volume and a reduction in crumb firmness (López *et al.*, 2025). Physical treatments, like radio frequency, had also been investigated to modify flour properties, resulting in a greater specific volume and more uniform crumb structure in the final product (Zhang *et al.*, 2022; Chen *et al.*, 2026). While previous studies had examined the individual effects of these components, it is needed to systematically optimize and characterize a composite flour blend of cassava, chickpea, and corn to produce a high-quality gluten-free bread. This research aimed to address this gap by developing an optimized formulation and characterizing its rheological, physical, and sensory attributes.

Materials and methods

Study of basic formulation and process for cassava composite flour bread production

The composite flours used in this study consisted of cassava flour (CA), corn flour (CR), and chickpea flour (CP). The quality of those flours was analyzed using the following methods moisture content, protein content, fat content and amylose content (AOAC, 2000; Hoover and Ratnayake (2005).

Color (L^* , a^* , and b^*) was measured using the CIE Lab system, and the whiteness index (WI) was calculated using the Eq.1. The viscous properties were analyzed with Rapid Visco Analyzer (RVA). Peak viscosity (RVU), Final viscosity (RVU), and Breakdown (RVU) were reported.

$$WI = 100 - [(100 - L)^2 + a^2 + b^2]^{1/2} \quad (1)$$

Development of cassava composite flour bread formulation from a reference gluten-free recipe

The mixing of CA, CR, and CP was detailed in Table 1. The resulting cassava composite flour was then analyzed for its viscosity behavior and compared with wheat flour. The starting gluten-free bread recipe was selected from three reference recipes (Ryczek, 2016; Bell, 2018). These recipes were then subjected to sensory and physical evaluations. The quality of the initial gluten-free bread recipes was evaluated based on sensory evaluation using a 9-point hedonic scale, batter viscosity and bread texture using a Stable Micro System (Plus-Upgrade) texture analyzer, specific volume (Ho *et al.*, 2013), and crust and crumb analysis was performed.

Table 1. Proportion of cassava flour, corn flour, and chickpea flour used for cassava composite flour production

Sample	CA (%)	CR (%)	CP (%)	Sample	CA (%)	CR (%)	CP (%)
CF-1	100	0	0	CF-14	75	20	5
CF-2	90	10	0	CF-15	75	15	10
CF-3	90	0	10	CF-16	75	10	15
CF-4	85	15	0	CF-17	75	5	20
CF-5	85	10	5	CF-18	75	0	25
CF-6	85	5	10	CF-19	70	30	0
CF-7	85	0	15	CF-20	70	25	5
CF-8	80	20	0	CF-21	70	20	10
CF-9	80	15	5	CF-22	70	15	15
CF-10	80	10	10	CF-23	70	10	20
CF-11	80	5	15	CF-24	70	5	25
CF-12	80	0	20	CF-25	70	0	30
CF-13	75	25	0				

Study of the effect of production process on cassava composite flour bread quality

The cassava composite flour bread was produced using the Straight Dough method. The effect of the mixing process on batter volume expansion was analyzed using Rheofermentographic analysis with a Rheofermentometer

(Chopin, Villeneuve-la-Garenne, France), modified from Czuchajowska and Pomeranz (1993). The effect of fermentation time on the quality of cassava composite flour bread was studied by evaluating the specific volume (cm^3/g) of the cassava composite flour batter using a Digital Imaging Technique (Figure 1), modified from Elmeahdi *et al.* (2003, 2007). The images were analyzed using the APS ASSESSR software. The specific volume of the dough was calculated using Eq. 2, and a standard graph was created using a 3600 mm^2 square calibration card.

$$Y_t = L \times A_t / m \quad (2)$$

where: Y_t = Specific volume of the dough at any given time L = Dough thickness (constant at 2.11 mm) A_t = Dough area at any given time (from image analysis) m = Dough weight

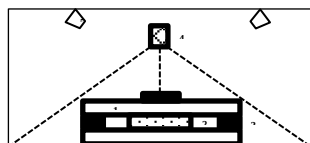


Figure 1. Apparatus for evaluating the specific volume of dough

Study on the quality improvement of cassava composite flour bread

Psyllium husk was added at concentrations of 5.0 to 15.0%. Hydrocolloids and emulsifiers were added at desired concentrations. The quality of the cassava composite flour bread was evaluated by assessing its specific volume.

Statistical analysis

A Completely Randomized Design (CRD) was employed. Data from three replicates were subjected to ANOVA using SPSS software. Treatment means were compared using DMRT at a significance level of $P < 0.05$.

Results

Chemical composition and physical properties of selected flour

The chemical composition analysis of various flour types, as presented in Table 2, revealed differences in their nutritional profiles. Wheat flour, CA, and CR exhibited moisture contents of 11.36%, 12.22%, and 10.45% respectively,

which fell within the expected range for flour products. Notably, CP demonstrated superior protein content at 22.22%, markedly higher than its counterparts. In contrast, CA contained the least amount of protein among the examined samples. With regard to amylose content, CR leads with 24.64%, while chickpea flour contained the lowest percentage at 11.7%.

Table 2. Chemical composition of wheat flour, CA, CR, and CP (n=3)

Flour Sample	Moisture (%)	Protein (%)	Amylose Content (%)
Wheat Flour	11.36±0.06	17.77±0.07	22.39±0.48
CA	12.2±0.01	1.29±0.07	19.5±0.07
CR	10.45±0.01	7.70±0.02	24.64±0.05
CP	7.53±0.05	22.2±0.70	11.7±0.10

The study on the color quality of the flours (wheat, CA, CR, and CP) revealed that the L^* of CA CR and CP were 91.13±1.18, 90.58±0.50 and 88.16±0.32, respectively, all exhibited higher lightness (L^*) values compared to wheat flour. Analysis of the WI showed that the values for wheat, CA, CR, and CP were 76.93, 80.54, 82.23, and 75.49, respectively. Specifically, CA and CR possessed a higher whiteness index than both wheat and CP. This difference was primarily attributed to the varying protein content among the four flour types.

Table 3. Color values and WI of wheat flour, CA, CR, and CP

Flour Sample	Color Value			WI
	L^*	a^*	b^*	
Wheat Flour	77.98±2.65	0.54±0.07	5.96±0.79	76.93
CA	91.13±1.18	-0.15±0.09	17.32±0.89	80.54
CR	90.58±0.50	0.11±0.01	15.06±0.42	82.23
CP	88.16±0.32	0.51±0.07	21.46±0.65	75.49

Pasting behavior of CA composite flour bread production

Based on the analysis of the properties, CR and CP were identified as potential ingredients to be blended with CA in various proportions to create the composite flour for subsequent CA bread production. Analysis of the pasting behavior of the various composite flour formulas showed that the Peak Viscosity of the blends changed according to the proportions of CA, CR, and CP (Figure 2A). Formula CF-2 showed a decrease in peak viscosity. Formula CF-3 also showed a reduction in peak viscosity compared to pure cassava flour. When examining the Final Viscosity (Figure 2B), the value changed according to the amount of corn and chickpea flours in the blend and became closer to that of wheat flour when the composite blend contained various ratios. Regarding the

Breakdown (Figure 2C), the difference between peak and trough viscosity, which indicates the collapse of the starch structure after gelatinization, wheat flour had a breakdown of 57.2 ± 1.8 RVU, while cassava flour had 268.5 ± 2.2 RVU. This confirms that the cassava starch gel collapses more readily. The addition of corn and chickpea flours successfully reduced the breakdown value, suggesting less product collapse after gelatinization. Based on the analysis of pasting behavior, it was concluded that blending corn and chickpea flours changed the viscous behavior of cassava flour. Formulas CF-13 to CF-18 and CF-19 to CF-25 exhibited the lowest breakdown values. To maintain the distinct identity of cassava bread while optimizing structural stability, formulas CF-13 to CF-17 were considered. By jointly assessing the low breakdown value and the peak viscosity (related to porous structure formation), the composite flour formula CF-15 was selected for subsequent study.

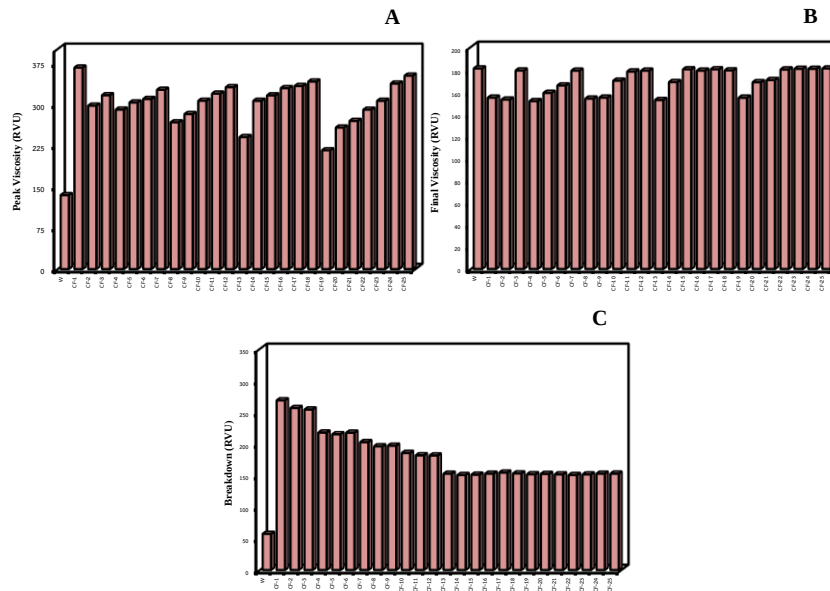


Figure 2. Peak viscosity (A), Final viscosity (B) and Breakdown (C) of wheat flour (W), CA (CF-1), and CA composite flours (CF-2 to CF-25)

Processing conditions on the quality of CA bread using cassava composite flour (CCF)

Rheofermentation analysis of the resulting batter showed that prolonged mixing led to a high initial proofing rate, followed by a sharp decline. Conversely, mixing for a shorter duration resulted in slower gas production but did not exhibit

subsequent structural collapse. The stability of the batter was better maintained with shorter mixing times compared to prolonged mixing (Figure 3(A)), which directly influenced the final specific volume. In comparison, mixing with the leaf-shaped beater at for 2 min. resulted in less gas production but yielded a stronger, non-collapsing structure. Therefore, the optimal mixing time for CCF bread was determined to be mixing for 2 min.

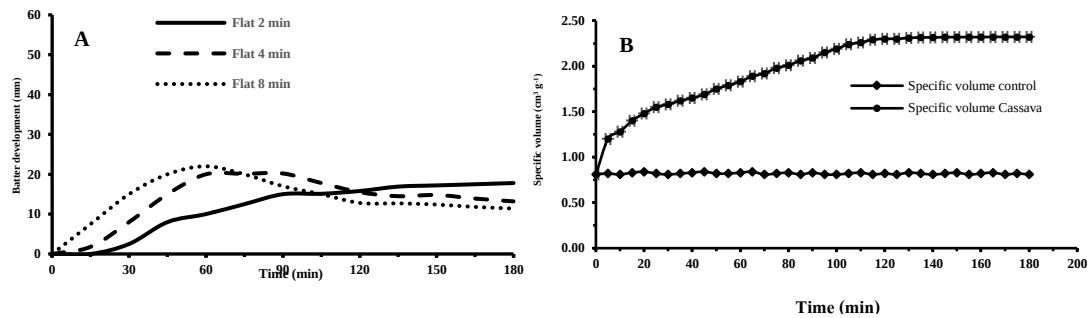


Figure 3. Development of the batter mixed with a Leaf-Shaped Beater at speed 4 (A), Specific Volume (cm^3/g) (B)

A plausible explanation for this result was related to the oxygenation process that occurs during mixing (Figure 3B) which illustrated the change in specific volume (cm^3g^{-1}) of the CA bread batter (utilizing CCF) during the proofing process. The resulted showed that the specific volume of the batter continuously increased throughout the proofing duration, reaching its maximum at 120 minutes, after which it began to stabilize. (The Specific Volume (cm^3g^{-1}) dynamics of the developed batter as a mathematical model when the proofing temperature was kept constant (Figure 4)). The time-dependent behavior of the specific volume was described by a first-order kinetic equation, similar to the growth curve observed for microorganisms. The suitable mathematical equation (Eq. 2) was presented as

$$Y = Y_0 - A_1 \exp^{-t/\mu} \quad (2)$$

Where, Y was the Specific Volume of the batter Y_0 is the Maximum Specific Volume A_1 is the Scaling Parameter for adjustment μ is the Time Constant (related to the rate of change).

The fitting results indicated that this mathematical equation was successfully used to predict the potential specific volume generated during the proofing process.

The hydrocolloids included High Methoxyl Pectin (HM), Carboxymethyl cellulose (CMC), Guar gum (GG), Hydroxypropyl Methyl Cellulose (HPMC), and Polygum Carob (PC) were selected. The emulsifiers used included Sodium stearoyl lactylate (SSL), Diacetyl tartaric acid ester of monoglycerides (DATEM), and Soy lecithin (LC). Fig. 5B showed the specific volume of the CCF bread when incorporated with HM, CMC, GG, HPMC, and PC. The reference had a specific volume of $3.10 \text{ cm}^3 \text{ g}^{-1}$. At the 0.1% addition level, the specific volumes were 2.59, 2.68, 2.16, 2.05, and $2.41 \text{ cm}^3 \text{ g}^{-1}$ for HM, CMC, GG, HPMC, and PC, respectively, all of which showed a significant reduction compared to the reference formula ($p < 0.05$). It showed that when CMC was used as the hydrocolloid, the loaf specific volume increased by 23.0% to 27.0%, depending on the emulsifier type and concentration (Figure 5C). The formula with 0.3% DATEM yielded the highest specific volume increase. When HM-Pectin was used, the specific volume increased by 14.0% to 19.0%, with 0.5% SSL providing the maximum increase.

Discussion

Chemical composition and physical properties of flour

The study of raw material quality serves as the fundamental basis for defining the quality of the final product. A critical observation was that CA contains over 10 times less protein than wheat flour. This indicated that using CA as the sole raw material for bread production would be incapable of forming the necessary gas-retaining structure during the fermentation process. Therefore, the composite flour technique was essential for improving the quality of cassava bread. An effective composite flour should have protein and amylose contents similar to wheat flour, as both were crucial for creating the gas-retaining structure in bread and the final porous structure after baking. In conventional bread production, the proteins glutenin and gliadin were key structural components. Glutenin imparts elasticity, while gliadin provided extensibility. The synergy between the physical properties of glutenin and gliadin allowed the protein network to expand and trap the carbon dioxide produced during fermentation. Upon baking, the gas escaped, leaving the characteristic open, porous structure within the bread. Furthermore, the protein content influenced water absorption, resulting in a more cohesive and stronger gluten network. Stronger gluten led to increased bread volume by enhancing the retention of fermentation gases. Given that CA had a low protein content compared to wheat flour, these protein-derived benefits were diminished. Consequently, the composite flour used to develop cassava bread must incorporate flours with a protein content that could

compensate for the protein deficiency in CA. CP demonstrated high protein content which could compensate for the lack of gluten in the traditional bread formula. Numerous studies confirmed the effective use of CP in the production of high-quality gluten-free baked goods (Miñarro *et al.*, 2012; Rostamian *et al.*, 2014; Santos *et al.*, 2020a; Santos *et al.*, 2021; Garske *et al.*, 2023; Kahraman *et al.*, 2022; Gkountenoudi-Eskitzi *et al.*, 2023). As previously noted, amylose content was another chemical factor influencing bread quality. During fermentation, bread volume increased as the resulting carbon dioxide was contained within the elastic protein structure, enclosed by thin protein films and suspended as air bubbles in the continuous phase of the dough. When the dough was heated, this foam structure became unstable and collapsed if the dough viscosity was too low. Collapse was particularly evident when the amylose content fell below 7.00%, resulting in the undesirable "Horn-Like structure." Since CA had a lower amylose content than wheat flour, amylose content, in addition to protein content, was a critical factor when selecting composite flours and other functional ingredients for producing successful gluten-free breads, such as cassava bread. The color values and WI of a flour were indicators of the quality derived from the milling and production processes. Beyond indicating milling and production quality, the whiteness index could be used as a genetic indicator in breeding or for selecting cereal varieties (Ji *et al.*, 2011; Hrušková *et al.*, 2011).

Pasting behavior of raw materials for cassava composite flour (CCF) bread production

The pasting behavior of the component flours suggested the potential for producing bread with minimal post-baking staling. However, certain pasting parameters of CA, such as peak viscosity, breakdown, and setback, significantly differ from wheat flour. Therefore, composite flour technology was necessary to modify the pasting profile to closely match that of wheat flour, thereby improving the final CA bread quality. This modification primarily involves adjusting the content of protein, amylose, and amylopectin, all of which influence the structure, gelatinization, and retrogradation in bread (Oerega-Ojeda and Eliasson, 2001). Reducing the CA content while increasing the CR content resulted in a further decrease in peak viscosity due to the increased amylose content. Conversely, increasing the CP content led to a gradual increase in viscosity at the same level of CA. This raised in viscosity was attributed to the increased protein content contributed by the CP. Research by Zhang *et al.* (2025a) supported this, suggesting that protein was involved in forming a strong structural network within the flour. The addition of corn flour increased the

overall amylose content of the blend. Although this tended to decrease the peak viscosity upon heating, it suggested that the carbon dioxide generated could disperse better due to the reduced system viscosity. Furthermore, this action helped stabilize the foam-like structure, reducing the tendency for structural loss in the bread. Studies had shown that high-amylose batter exhibits higher volume expansion during mixing and fermentation (Hiroko *et al.*, 2019), and amylose content was a critical factor for selecting flours for gluten-free bread production (Yano *et al.*, 2017). The quality of gluten-free bread depended on polysaccharides, which enhanced foam stability by increasing viscosity and preventing the flocculation and coalescence of the aqueous phase, while stabilizing the film structure around the air bubbles (Buresova *et al.*, 2014). The addition of amylose and protein increased the final viscosity due to their influence on the rearrangement of crystalline structure after heating, which affected internal crystalline consistency (Takahashi *et al.*, 2005). Flour viscosity indicated the change in sample viscosity due to heating and shear force. The analysis showed that the amounts of amylose and protein influence the pasting behavior. When amylose content increased, peak viscosity decreased. As heating continued, the starch granules swelled, ruptured, and decreased in size, causing the viscosity to fall until the trough viscosity was reached. A higher trough viscosity indicated better heat resistance. The trough viscosity of the CA-CR-CP composite decreased as the amylose content increased, suggesting less heat resistance compared to pure CA. Upon cooling, the viscosity increased to the final viscosity, which varied based on the composition blend. Starch molecules rearranged into a retrogradation, and setback measurements showed that retrogradation decreased as amylose content increased, though higher protein content could increase retrogradation (Blazek and Copelan, 2008). Final viscosity tends to increase with increasing amylose and protein content (Zeng *et al.*, 1997; Yamamori and Quynh, 2000; Yanagisawa *et al.*, 2004, 2006).

Effect of processing conditions on the quality of CA bread using CCF

The experiment indicated that the appropriate proofing time was dependent upon the volume expansion of the batter during the process. While extended proofing time leads to increased gas generation by yeast, this expansion was controlled by optimal mixing. Excessive mixing weakened the gas-retaining structure; the batter initially expanded well but rapidly lost volume over time. In the production of both gluten-containing and gluten-free bread, the proofing process was a critical factor for achieving optimal product quality. Proofing was the process during which yeast (or leavening agents) produces carbon dioxide (CO₂). The generated gas was trapped within the structure, and when the product

was baked, the hot air caused the entrapped gas to expand and escape, leaving behind air pockets that form the characteristic porous texture. Furthermore, the proofing process significantly influenced the final product volume (Bloskma, 1981). Proofing induced structural changes in the bread dough or, in the case of gluten-free production, the batter, consequently altering the textural properties due to the expansion of the retained gas. The rate of gas generation during proofing followed the stoichiometry and kinetics of the chemical reactions associated with yeast growth (Woo *et al.*, 2023). Therefore, controlling the type of yeast and the proofing time allowed for effective control over CO₂ production during the process (Zhang *et al.*, 2025b). Effective control of carbon dioxide production was, ultimately, effective control of bread quality. However, if the proofing time was extended beyond 180 minutes, the specific volume of the batter tended to decrease. This decline was attributed to the fact that the structural network formed by the CCF was insufficiently strong to retain the gas produced by the yeast, a critical difference when compared to the superior strength provided by gluten protein.

Quality improvement of cassava composite flour bread

The experimental findings demonstrated that Psyllium positively influenced the softness of the CCF bread and was capable of mimicking the function of gluten. Psyllium acted as a natural hydrocolloid with properties that improved the physical characteristics of gluten-free bread. Its functional benefits included water binding, gel formation, and the creation of a strengthening structure within the flour matrix. These properties enhanced dough viscosity and contributed to the structural integrity necessary to support volume expansion during the proofing process (Fratelli *et al.*, 2018; Mancebo *et al.*, 2015; Ziemichód *et al.*, 2019; Santos *et al.*, 2020b). Increasing the hydrocolloid concentration generally led to an increase in specific volume. These findings aligned with previous studies on the addition of Xanthan Gum, HPMC, and κ -carrageenan in wheat bread (Hager and Arendt, 2013), and Pectin and CMC in wheat-free bread (Lazaridou *et al.*, 2007). The loaf volume increased was attributed to the formation of a gel network structure during baking, which strengthened the expanding air cells. This structural reinforcement better retains the generated gas, thereby increasing the specific volume (Bell, 1990). However, some research suggests that using pregelatinized cassava flour also led to an increase in loaf specific volume compared to native cassava flour (Satin, 1988; Olatunji *et al.*, 1992; Hugo *et al.*, 1997). The change in specific volume depends on the type and concentration of the hydrocolloid used and the specific gluten-free bread formula. For instance, some studies found no correlation between

hydrocolloid concentration and bread volume (Horstmann *et al.*, 2016), while others reported that volume was dependent on the level of Locust bean gum, Guar gum, or Sodium alginate. Conversely, high concentrations of Xanthan Gum presented a negative impact on specific volume (Lazaridou *et al.*, 2007). This negative effect was explained by the excessive formation of rigid gels due to hydrogen bonding between the anionic carboxyl groups of Xanthan Gum, water, and starch, which physically restricts the expansion of gas cells during proofing (Chakraborty *et al.*, 2020; Peressini *et al.*, 2011). The emulsifiers enhanced loaf volume due to their ability to strengthen the thin liquid film at the protein-starch interface (Stampfli and Nersten, 1995). The amphiphilic nature of the emulsifiers allowed them to interact with the hydrophobic domains of the proteins in the composite flour, forming hydrogen bonds with glutamine, which strengthens the overall structure (Stampfli and Nersten, 1995; Stampfli *et al.*, 1996; Azizi and Rao, 2004). This stronger structure improves CO₂ retention during fermentation, leading to increased specific volume (Gómez *et al.*, 2004; Moore and Hosney, 1986). This difference was attributed to the varying interactions between CMC, HM-Pectin, and the emulsifiers with the protein components of the composite flour. The study confirmed that hydrocolloids, emulsifiers, or their combination significantly impact the quality of cassava bread.

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Conflicts of interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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