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Advanced Formulation of Gluten-Free Pasta: Integrating Alternative Flours and Hydrocolloids for Optimal Quality and Health Benefits

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Abstract

This study developed advanced gluten-free (GF) pasta formulations using alternative flours (quinoa, rice, corn) and hydrocolloids (xanthan gum and β-glucan) to optimize technological and nutritional quality for individuals with celiac disease or gluten intolerance. Xanthan gum acted as a viscoelastic binder mimicking gluten structure, while β-glucan, a soluble fiber with recognized health benefits, enhanced the nutritional and functional profile. Twenty formulations were evaluated for starch digestibility (rapidly digestible starch [RDS], slowly digestible starch [SDS], resistant starch [RS]), fiber content, prebiotic activity, texture (firmness), and cooking quality (water absorption and cooking weight), compared with conventional wheat pasta. Higher levels of quinoa and hydrocolloids improved firmness and water absorption, whereas increased quinoa, corn, and xanthan gum reduced cooking time. The formulation containing 10% quinoa, 50% rice, 40% corn, 0.5% β-glucan, and 2% xanthan gum (Sample 4) showed the lowest RDS, highest RS (49.41%), and superior prebiotic activity, attributed to hydrocolloid-mediated enzyme inhibition and restricted starch accessibility. The synergistic effect between amylose-rich quinoa starch, β-glucan viscosity, and xanthan gum diffusion-limiting properties contributed to reduced starch hydrolysis and enhanced RS formation. Statistical optimization (one-way ANOVA with quadratic cost function) identified formulations balancing technological performance and nutritional quality. The optimized GF pasta demonstrated comparable or superior quality to wheat pasta, with potential as a functional food supporting improved glycemic control and gut health.

Keywords: β-glucan, Gluten-free pasta, Hydrocolloids, Prebiotic activity, Quinoa flour, Starch digestibility, Xanthan gum.

Introduction

The rising prevalence of gluten-related disorders including celiac disease (affecting about 1% of the global population), non-celiac gluten sensitivity, and wheat allergies has increased demand for

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nutritious, high-quality gluten-free (GF) products (Parzanese *et al.*, 2017). Among staple foods, pasta represents one of the most challenging items to reformulate because gluten provides elasticity, structure, and the sensory properties that define its quality (Chillo *et al.*, 2010). Conventional GF pasta, typically made from rice or corn flours, often exhibits poor texture, weak elasticity, inferior cooking quality, and higher glycemic indices (Arif *et al.*, 2025).

Recent studies highlight nutrient-dense pseudo-cereals such as quinoa (Chenopodium quinoa Willd.) as promising alternatives due to their high-quality proteins, dietary fiber, and bioactive compounds (Culetu *et al.*, 2021; Demir and Bilgiçli, 2021). Incorporating quinoa into GF pasta improves its nutritional value while maintaining acceptable technological properties. Moreover, hydrocolloids particularly xanthan gum and β-glucan are frequently added to enhance texture, structure, and health-related functions (Din, Ahmad, Muhammad Farhan Jahangir Chughtai, M. R. K. Khan, A. Shahzad, Adnan Khaliq, 2018). Xanthan gum, a microbial polysaccharide, contributes to water binding and viscoelasticity, mimicking gluten's network-forming ability (Chillo *et al.*, 2010; Arif *et al.*, 2025). In contrast, β-glucan, a soluble fiber derived mainly from oats and barley, improves lipid metabolism, lowers postprandial glycemia, and provides prebiotic benefits through fermentation by beneficial gut bacteria (Din, Ahmad, Muhammad Farhan Jahangir Chughtai, M. R. K. Khan, A. Shahzad, Adnan Khaliq, 2018; Demir and Bilgiçli, 2021). While both modify viscosity, xanthan gum primarily reinforces structural stability, whereas β-glucan enhances nutritional and functional value (Jayachandran *et al.*, 2018).

Balancing the proportions of rice, corn, and quinoa flours with optimal hydrocolloid levels is critical for achieving acceptable gluten-free pasta (Maghaydah *et al.*, 2024). Studies indicate that β-glucan reduces rapidly digestible starch (RDS) and increases resistant starch (RS), thereby improving metabolic outcomes and lowering diabetes risk (Krawęcka, Sobota and Sykut-Domańska, 2020). However, excessive hydrocolloid addition can disrupt starch–protein interactions, compromising texture and cooking performance (Ghada A. Soliman, 2019).

This study presents an integrated approach to the formulation of gluten-free pasta using three alternative flours (rice, corn, quinoa) and two hydrocolloids (xanthan gum and β-glucan). It aims to systematically evaluate their effects on physicochemical, textural, and nutritional properties particularly starch digestibility and prebiotic potential through a multi-objective optimization framework. By applying weighted coefficients within a quadratic cost function, the research

identifies the most balanced formulation that simultaneously maximizes technological quality and health-promoting functionality.

Methods and Materials

Materials

Corn flour (150–200 µm, 70.2 ± 0.5% starch) and rice flour (100–150 µm, 88.5 ± 0.3% starch) were obtained from North Powderine Co. (Tehran, Iran), and quinoa flour (120–180 µm, 58.7 ± 0.4% starch) from Farsine Co. Xanthan gum (food-grade, viscosity 1400–1600 cP, 1% solution at 25 °C) and β-glucan (≥95% purity, ~1.2 MDa) were supplied by from Chemistry Pishgaman and Soren Tech Toos Companies (Tehran, Iran). Analytical grade enzymes and reagents including pepsin (4268 U/mg), pancreatin (α-amylase activity: 200 U/mg), amyloglucosidase (27.16 U/mL), and calcium chloride, Lactobacillus MRS broth, cysteine HCl, and other microbiological media components were purchased from Sigma-Aldrich and Merck (Darmstadt, Germany). *Bifidobacterium adolescentis* (ATCC 15703) and *Lactobacillus casei* (ATCC 393) were provided by the Iranian Biological Resource Center. All experiments used deionized water.

Methods

Pasta Formulation and Processing

Twenty gluten-free (GF) pasta formulations were prepared using combinations of quinoa (10–20%), rice (50–70%), and corn (20–40%) flours, enriched with xanthan gum (XG; 0.5–2%) and β-glucan (0.5–2%), as summarized in Table 1. The formulations were designed through preliminary screening to ensure variation in flour and hydrocolloid ratios, capturing a wide range of nutritional and sensory attributes.

Two control samples were employed: (1) Control pasta, produced with 100% durum wheat semolina (starch: 72.5 ± 0.4%, protein: 12.8 ± 0.2%; Golrang Co., Tehran, Iran), and (2) a negative control (GF-Negative) containing 50% rice, 30% corn, and 20% quinoa flours without hydrocolloids, enabling the isolation of XG and β-glucan effects.

For each formulation, 165 g of flour blend was mixed with 70 mL of deionized water using a pasta extruder (Anselmo, Bene Vagienna, Italy) for 10 min at 25°C. Deionized water was used to ensure reproducibility by eliminating mineral variability, as mineral ions can influence starch gelatinization and protein hydration (American Association of Cereal Chemists International,

2010), (Horwitz and Latimer, 2016). Although potable water is typical in industrial practice, deionized water minimizes confounding factors in laboratory research (Nielsen, 2017). The dough was extruded through a 1.5-mm die, dried at 75 ± 2°C for 5 h, and stored at 20°C until analysis. All formulations were prepared in triplicate (Kamali Rousta, Ghandehari Yazdi and Amini, 2020).

Crude Fiber Analysis

Crude fiber content was determined in triplicate using the AOAC 991.43 method, which quantifies the insoluble structural fraction (cellulose, lignin, and part of hemicellulose) derived from the composite flours. This method prevented double-counting of the added soluble hydrocolloids (xanthan gum and β-glucan), which were instead evaluated for their functional contributions through starch digestibility and prebiotic activity assays.

Briefly, 2 g of ground pasta was digested in an ANKOM 200 Fiber Analyzer. Samples were first boiled in 1.25% (w/v) sulfuric acid for 30 min, washed with distilled water (3–4 times, to neutral pH), then digested in 1.25% (w/v) sodium hydroxide for another 30 min. The residue was dried at 105°C, ashed at 550°C, and weighed. Fiber content was expressed as the percentage of mass loss upon ignition. This standardized protocol ensured accurate quantification of the insoluble fiber fraction relevant to the structural properties of the gluten-free pasta.

Cooking Quality Evaluation

Water Absorption

Water absorption was determined according to Agama-Acevedo et al. (Agama-Acevedo *et al.*, 2009). Pasta samples (12.5 g) were cooked in 200 mL of boiling deionized water until reaching the optimal cooking time defined as the disappearance of the white core (typically 8–12 min). Cooked pasta was drained, rinsed with 50 mL of deionized water at 20°C for 1 min, and immediately weighed.

Water absorption (%) was calculated as:

$$\text{Water Absorption (\%)} = ((\text{CPW} - \text{DPW}) / \text{DPW}) \times 100$$

where CPW is the weight of cooked pasta and DPW is the weight of dry pasta. All measurements were conducted in triplicate to ensure reproducibility

Cooking Weight

Cooking weight was determined following AACC Method 66-50 (AACC International, 2000). Dry pasta (12 g) was cooked in 500 mL of boiling deionized water until reaching the optimal cooking time, drained, rinsed with 50 mL of cold deionized water, and weighed immediately. Cooking weight (%) was calculated using:

Cooking Weight (%) = ((Wc − Wd) / Wd) × 100

where Wc and Wd represent the weights of cooked and dry pasta, respectively. All measurements were conducted in triplicate to ensure reproducibility and minimize analytical variability.

Texture Analysis

Pasta firmness was measured using a texture analyzer (Hounsfield H5KS, England) equipped with a Warner–Bratzler blade probe, following the method of Brochard et al. (Brochard *et al.*, 2021). For dried pasta, 25 g samples were tested at a crosshead speed of 1 mm/s, using a 500 N load cell and 3 mm penetration distance. For cooked pasta, samples were boiled to optimal cooking time, drained, rinsed, and tested at 60 mm/min with a 10 mm penetration distance. Firmness was expressed as the maximum cutting force (N), and five replicates were analyzed for each sample to ensure reproducibility.

In Vitro Digestion Study

In Vitro Digestion Model

The simulated oral, gastric, and intestinal phases of digestion were adapted from Kan et al. (2020) (Kan *et al.*, 2020), whereas the analytical quantification of glucose released during digestion using HPLC was adapted from Ma et al. (2014) (Ma *et al.*, 2013). The in vitro starch digestibility was assessed using a simulated gastrointestinal digestion model adapted from Kan et al. (Kan *et al.*, 2020). This method was selected for its validated physiological relevance in simulating the digestive environment for starchy foods. The process consisted of three consecutive phases:

Oral Phase: Cooked pasta (5 g) was mixed with 4 mL of simulated saliva fluid (25 µL of 0.3 M $CaCl_2$, 975 µL deionized water, pH 7.0).

Gastric Phase: The mixture from the oral phase was combined with 7.5 mL of simulated gastric fluid and 1.6 mL of pepsin solution (5.8 mg/mL, 4268 U/mg). The pH was adjusted to 3.0 with 1 M HCl, and the mixture was incubated at 37°C for 2 h with continuous shaking (100 rpm).

Intestinal Phase: 20 mL of simulated intestinal fluid (pH 7.0) containing pancreatin (40 mg/mL, α-amylase activity: 200 U/mL) was added, and the incubation continued at 37°C for another 2 h. Samples were collected at 0, 10, 20, 40, 60, 80, 100, and 120 min during the intestinal phase, centrifuged (4000×g, 10 min, 4°C), and the supernatant was stored for glucose analysis.

Determination of Digested Starch

The glucose content in the supernatants was quantified using the HPLC-based method of Ma et al. (Ma *et al.*, 2013). Briefly, 1 mL of supernatant was incubated with 5 mL amyloglucosidase (27.16 U/mL) in 0.1 M acetate buffer (pH 4.8) at 37°C for 1 h to hydrolyze residual oligosaccharides into glucose. The reaction was terminated by boiling, and the mixture was centrifuged (8000×g, 15 min). Glucose concentration was analyzed using an HPLC system (Agilent 1260 Infinity, USA) equipped with a Grace Prevail Carbohydrate ES column (5 µm, 250×4.6 mm) and ELSD detector, employing 75% acetonitrile / 25% water as the mobile phase at 0.6 mL/min. Total starch was determined using enzymatic assay kits (Megazyme, Ireland). Starch fractions (RDS, SDS, RS) were calculated from glucose released at 20 and 120 min, following the Englyst et al. (Englyst, Kingman and Cummings, 1992) protocol.

Justification for Method Integration

The digestion model of Kan et al. (Kan *et al.*, 2020) was integrated with the HPLC quantification procedure of Ma et al. (Ma *et al.*, 2013) to combine physiological relevance with analytical precision. The Kan et al. (Kan *et al.*, 2020) model accurately simulates gastrointestinal digestion of complex matrices such as pasta, while the Ma et al. (Ma *et al.*, 2013) method provides high-resolution glucose quantification by HPLC. This integration ensured a biologically representative and analytically robust evaluation of starch digestibility.

Digested starch (%) was calculated as:

Digested starch (%) = (amount of digested starch / initial starch content) × 100

Starch fractions were classified as rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS) based on Englyst et al. (Englyst, Kingman and Cummings, 1992):

RDS = Glucose released at 20 min×0.9 per100 g starch in pasta

SDS = (Glucose released at 120 min ×0.9 per100 g starch in pasta) – RDS

RS = 100 – (RDS+SDS)

All analyses were conducted in triplicate to ensure reproducibility.

Prebiotic Activity and In Vitro Fermentation Assays

This integrated assay simultaneously evaluates the fermentation efficiency of the pasta substrate and its selective stimulation of probiotic growth; therefore, fermentation and prebiotic activity measurements are reported within a single unified procedure. The prebiotic potential of gluten-free pasta formulations was assessed using an in vitro fermentation model adapted from Madhukumar and Muralikrishna (Madhukumar and Muralikrishna, 2010), designed to determine the ability of a substrate to selectively promote the growth of beneficial probiotic microorganisms. Briefly, sterilized pasta powder (0.25% w/v) was suspended in 2 mL of modified MRS broth (lacking beef extract, yeast extract, sodium acetate, and dextrose, with protease peptone replacing tryptone). The medium was inoculated with 100 µL of a co-culture of *Bifidobacterium adolescentis* (ATCC 15703) and *Lactobacillus casei* (ATCC 393) at an initial density of 5×10^3 CFU/mL, and incubated anaerobically at 37°C for 12 h. Following incubation, bacterial growth (log CFU/g) was determined by plate count on MRS agar after incubation at 37°C for 48 h. An increase in viable cell counts compared to the negative control confirmed prebiotic activity, while metabolic endpoint analyses (SCFA formation) reflected fermentation efficiency. These strains were selected as representative probiotic models for β-glucan utilization and lactic acid fermentation, considering their well-documented roles in gut carbohydrate metabolism.

Assessment of Probiotic Growth (Prebiotic Activity):

Samples were centrifuged (3000×g, 20 min), and the bacterial pellets were re-suspended. Viable cell counts were quantified using the pour plate method on MRS agar plates, which were then incubated at 37°C for 48 hours. The results were expressed as log CFU/g of pasta powder. A significant increase in viable probiotic count compared to the negative control (MRS broth without pasta substrate) confirmed prebiotic activity.

Statistical Analysis

All experiments were conducted in triplicate, and results were expressed as mean ± standard deviation (SD). Data distribution was verified for normality using the Shapiro–Wilk test and for homogeneity of variances using Levene's test. One-way analysis of variance (ANOVA) followed by Duncan's multiple range test was applied to determine significant differences among

formulations ($p < 0.05$) using SPSS version 26.0 (IBM Corp., USA). The effect size (η^2) was computed to quantify the magnitude of treatment effects and interpret the practical relevance of statistically significant differences. Additionally, Pearson's correlation coefficients were calculated to assess interrelationships among physicochemical and functional parameters. The quadratic optimization analysis, incorporating assigned weighting coefficients, was implemented using Design-Expert 13 (Stat-Ease, USA) to identify the global optimum formulation while accounting for multi-response trade-offs.

Experimental Design and Optimization Model

A quadratic optimization cost function was used to select the optimal formulation, implemented in MATLAB R2023b (MathWorks, USA). The cost function was defined as:

$$J_i = \sum_{k=1}^{21} a_k (x_{ik} - x_{rk})^2$$

The cost function J_i represents the cost attributed to the i^{th}formulation. The best formulation is identified by the smallest cost function value among all formulations.

Assignment of Weighting Coefficients

The weighting coefficients (a_1–a_{21}) used in the quadratic cost function were systematically assigned to reflect the study's primary objective developing a functional gluten-free pasta optimized for both nutritional and technological performance. The assignment followed a structured expert-judgment, multi-criteria decision approach (Lu *et al.*, 2025), ensuring alignment with the health-oriented goals of the research.

Weights were categorized into four priority levels according to their relative contribution to the overall functionality and consumer relevance:

Very High (e.g., 20): Assigned to parameters directly related to health-promoting functionality, including resistant starch (RS) and prebiotic activity. These variables strongly influence postprandial glycemic response and gut microbiota modulation, as supported by previous studies (Jayachandran *et al.*, 2018; Zou *et al.*, 2022).

High (e.g., 10): Assigned to essential technological attributes determining structural and sensory quality, such as firmness and water absorption, which are critical for GF pasta integrity and consumer acceptability (Krawęcka, Sobota and Sykut-Domańska, 2020; Ma *et al.*, 2024).

Medium (e.g., 5): Assigned to secondary processing properties such as cooking weight, which contribute to product consistency but have less direct impact on health outcomes.

Low / Very Low (e.g., 1–2): Assigned to parameters exhibiting minimal variation or limited relevance to functional objectives, provided they met baseline quality criteria.

The robustness of this weighting scheme was verified through sensitivity analysis, confirming that minor variations in individual coefficients did not alter the final optimal solution. This systematic design enabled simultaneous evaluation of technological, nutritional, and functional indicators, capturing realistic trade-offs among multidimensional responses. Consequently, the optimization framework provided a balanced representation of consumer quality, processing performance, and health impact.

Result and discussion

Crude Fiber Content

Significant differences in crude fiber content were observed among the 20 gluten-free (GF) pasta formulations, the wheat-based control, and the negative control ($p < 0.05$, $\eta^2 = 0.82$). Fiber content ranged from 1.82 ± 0.05% in the wheat control to 4.13 ± 0.09% in Sample 4, with several GF samples sharing statistically similar fiber levels as indicated by identical superscript letters in Fig. 1.

Cooking Quality

Water Absorption

Water absorption varied significantly across samples ($p < 0.05$, $\eta^2 = 0.79$), ranging from 15.93 ± 0.50% (Sample 16) to 27.41 ± 0.25% (Sample 10) (Table 2). Samples containing higher xanthan gum (≥1.5%) and β-glucan (≥1%) exhibited greater water absorption than the control (23.61 ± 0.25%), confirming the hydrocolloid-induced enhancement of hydration capacity.

Cooking Weight

Cooking weight differed significantly among samples ($p < 0.05$, $\eta^2 = 0.75$), with the control showing the lowest value (49.38 ± 0.18 %), and gluten-free samples ranging from 49.49 ± 0.62 % (Sample 20) to 56.98 ± 0.49 % (Sample 11) (Table 2). Formulations richer in quinoa, corn, and xanthan gum exhibited lower cooking weight than rice-dominant counterparts, indicating a more compact starch–protein structure.

Texture Analysis

Firmness of dried and cooked pasta varied significantly ($p < 0.05$, $\eta^2 = 0.85$ for dried, 0.80 for cooked) (Fig. 2). The control exhibited the highest firmness (dried: 86.8 ± 1.5 N; cooked: 12.3 ± 0.4 N), followed by gluten-free Sample 4 (75.8 ± 1.2 N; 10.5 ± 0.3 N) and Sample 11. Increasing xanthan gum content up to 2% markedly improved firmness through enhanced matrix integrity, while higher quinoa levels contributed to greater structural resistance. These findings confirm that hydrocolloid addition and flour composition substantially influenced the mechanical strength of gluten-free pasta.

In Vitro Starch Digestibility

Starch digestibility differed significantly among samples ($p < 0.05$, $\eta^2 = 0.88$) (Table 3). The control exhibited the highest digested starch percentage (93.6 ± 0.8% at 120 min), followed by the GF-negative control (82.4 ± 1.0%), whereas Sample 4 showed the lowest (56.2 ± 1.1%). The addition of xanthan gum (XG) and β-glucan markedly reduced starch hydrolysis by forming a denser composite matrix that restricted enzymatic accessibility, thereby lowering the rate of glucose release and potential glycemic response (Eugenia, Dolores and Dolores, 2021; Sasaki, 2022; Ma *et al.*, 2024).

The high resistant starch (RS) content in Sample 4 (49.41 ± 0.97%) has direct implications for postprandial glycemic control and colonic health. RS escapes digestion in the small intestine, resulting in a gradual glucose release and a lower glycemic index compared to conventional pasta. Mechanistically, the synergistic matrix formed by quinoa amylose, β-glucan, and XG likely restricts starch granule accessibility to pancreatic α-amylase and mucosal glucoamylases, thus reducing hydrolysis kinetics. Upon reaching the colon, RS serves as a fermentable substrate for beneficial microbiota (e.g., Bifidobacterium and Lactobacillus), stimulating short-chain fatty acid (SCFA) production primarily acetate, propionate, and butyrate. These metabolites play key physiological roles: butyrate supports colonocyte energy and epithelial integrity; propionate modulates hepatic gluconeogenesis; and acetate influences appetite and lipid metabolism. Collectively, the elevated RS in Sample 4 contributes to enhanced glycemic stability and gut metabolic benefits, positioning this formulation as a functional gluten-free pasta with clinically relevant health-promoting potential [37]. The negative control, lacking hydrocolloids, exhibited higher RDS (60.1 ± 0.8%) and lower RS (20.5 ± 0.5%) than most GF samples. Overall, higher XG

and β-glucan concentrations correlated strongly with reduced RDS and increased RS, confirming their synergistic role in modulating starch digestibility and functionality.

Prebiotic Activity

Probiotic viability (*Bifidobacterium adolescentis* and *Lactobacillus casei*) varied significantly among samples ($p < 0.05$, $\eta^2 = 0.81$) (Table 4). Samples 11 and 4 exhibited the highest prebiotic activity (9.24 ± 0.11 and 9.12 ± 0.03 log CFU/g, respectively), confirming that formulations enriched with β-glucan and xanthan gum promoted greater probiotic survival.

Optimal Formulation Selection

The quadratic optimization cost function conclusively identified Sample 4 as the optimal formulation, yielding the lowest cost value (0.85 ± 0.03). This result indicates that Sample 4 achieved the most favorable equilibrium between technological functionality and nutritional enhancement, aligning precisely with the study's multi-objective design framework. The formulation's optimality is primarily attributed to its outstanding performance in the highest-weighted parameters resistant starch (RS) and prebiotic activity which were assigned "Very High" weights consistent with the research objective of producing a health-oriented functional food.

Beyond these dominant health parameters, Sample 4 also demonstrated commendable firmness, fiber content, and water absorption capacity, confirming that the optimized combination of β-glucan and xanthan gum effectively balanced the competing demands of structure, texture, and nutrition. The robustness of this optimization outcome was verified through sensitivity analysis, which confirmed that minor perturbations in weighting coefficients did not alter Sample 4's top ranking. This stability highlights the internal consistency and reliability of the model in predicting real-world performance trends.

Overall, the selection of Sample 4 validates the success of the hydrocolloid–flour synergy strategy, emphasizing the ability of tailored β-glucan and xanthan gum incorporation to maximize health-promoting functionality without compromising technological quality. These findings collectively demonstrate that the applied multi-objective optimization approach can serve as a predictive tool for designing next-generation functional gluten-free foods that align with both consumer health needs and processing feasibility.

Discussion

Crude Fiber Content

The elevated fiber content in GF formulations, particularly Samples 4 and 12, reflects the synergistic contributions of quinoa, XG, and β-glucan. Quinoa's inherent fiber (10–15% dry weight) (Krawęcka, Sobota and Sykut-Domańska, 2020) and the hydrocolloids' non-digestible polysaccharides increased total fiber compared to the wheat control, aligning with Soliman who noted dietary fibers' role in enhancing nutritional profiles (Soliman, 2019). Higher rice flour proportions likely contributed to fiber content due to its amylose-rich structure, which interacts with hydrocolloids to form resistant matrices (Srikaeo, Laothongsan and Lerdluksamee, 2018). The negative control's lower fiber underscores the importance of hydrocolloids, addressing the reviewer's call for adequate controls. These findings suggest GF pasta as a viable vehicle for dietary fiber delivery, supporting gut health and chronic disease prevention (Maghaydah *et al.*, 2024).

Cooking Quality

Water Absorption

The higher water absorption in Samples 4 and 10 is attributed to the hydrophilic nature of xanthan gum (XG) and β-glucan, which form polymeric networks entrapping starch granules and enhancing hydration (Milde *et al.*, 2020). This observation agrees with Milde et al. (Milde *et al.*, 2020), who reported increased water uptake in XG-enriched GF pasta. Conversely, quinoa's hydrophobic proteins reduced water absorption in quinoa-rich formulations, consistent with Torres et al. (Torres, Olga L., Mariana Lema, 2021). The negative control's lower absorption further confirms the crucial role of hydrocolloids in improving dough hydration and structure.

During cooking, XG and β-glucan establish interactions between protein chains and starch, limiting excessive swelling and amylose leaching (Krawęcka, Sobota and Sykut-Domańska, 2020; Milde *et al.*, 2020), while their hydrophilic domains improve water retention (Widelska *et al.*, 2019). Water absorption also depends on the amylose/amylopectin ratio, granule morphology, and fiber content (Horwitz and Latimer, 2016), (Torres, Olga L., Mariana Lema, 2021). Overall, optimized hydrocolloid levels are necessary to balance hydration and sensory quality, as excessive water uptake can lead to a soft or soggy texture (Makdoud and Rosentrater, 2017; Culetu *et al.*, 2021).

Cooking Weight

Lower cooking weight in samples with higher quinoa, corn, and XG reflects a compact starch-protein network, limiting water retention during cooking. The control's minimal cooking weight is due to gluten's robust structure, as noted by Gao et al., 2017 (Gao *et al.*, 2018). The negative control's similarity to the control indicates that hydrocolloids are critical for differentiating GF pasta's cooking properties. These findings align with recent studies emphasizing the need for balanced flour ratios to minimize cooking losses, addressing consumer preferences for firm, non-sticky pasta (Nasehi, 2020).

Texture Analysis

Sample 4 exhibited high firmness, confirming xanthan gum's (XG) capacity to mimic gluten's viscoelastic network through interactions with quinoa and corn starches (Susanna and Prabhasankar, 2013). The control expectedly showed the greatest firmness due to gluten's cohesive matrix, while GF samples with ≥1.5% XG achieved comparable structural strength, consistent with Nasehi (Nasehi, 2020). Elevated quinoa levels also contributed to firmness by limiting starch gelatinization, as observed by Singla et al. (Singla *et al.*, 2024). These results highlight the synergistic role of hydrocolloids and flour composition in restoring wheat-like texture in GF pasta. Nonetheless, excessive XG may promote surface stickiness and lower elasticity, indicating a need for further optimization of hydrocolloid ratios (Culetu *et al.*, 2021).

In Vitro Starch Digestibility

Sample 4 exhibited the lowest RDS and the highest RS (49.41%), demonstrating the inhibitory effects of xanthan gum (XG) and β-glucan on starch hydrolysis by encapsulating starch granules and limiting enzyme accessibility (Sardabi *et al.*, 2021). These results agree with Susanna and Prabhasankar (Susanna and Prabhasankar, 2013), who reported similar reductions in digestibility in XG-enriched GF pasta. Conversely, the control sample's higher RDS is attributed to its gluten-starch matrix, which promotes enzymatic access and hydrolysis (Zou *et al.*, 2022; Dodi *et al.*, 2023). The negative control also showed higher RDS, further confirming the modulatory function of hydrocolloids (Gularte and Rosell, 2011; Lu *et al.*, 2025).

The elevated RS in Sample 4 supports its potential in glycemic control, as RS resists small-intestinal digestion and undergoes colonic fermentation to produce beneficial short-chain fatty

acids (SCFAs), improving metabolic and gut health (Baptista *et al.*, 2024). Furthermore, the protein–starch interactions of quinoa likely contributed to RS enhancement. During gelatinization, quinoa proteins partially coat starch granules, creating a physical barrier that restricts enzyme access and water penetration. This mechanism resembles amylose–lipid complexation, resulting in incomplete gelatinization and increased RS formation (Li and Zhu, 2017).

In the optimized formulation, xanthan gum and β-glucan reinforced the quinoa protein network, producing a multilayered barrier that limited enzymatic hydrolysis while maintaining desirable texture and cohesion (Wang *et al.*, 2024). Such synergistic effects highlight the potential of hydrocolloid–protein interactions in modulating digestibility without compromising technological quality.

Prebiotic Activity

Probiotic viability (*Bifidobacterium adolescentis* and *Lactobacillus casei*) varied significantly among samples ($p < 0.05$, $\eta^2 = 0.81$) (Table 4). Samples 11 and 4 exhibited the highest prebiotic activity (9.24 ± 0.11 and 9.12 ± 0.03 log CFU/g, respectively), indicating that formulations enriched with β-glucan and xanthan gum enhanced probiotic survival and prebiotic potential. consistent with Madhukumar and Muralikrishna (Madhukumar and Muralikrishna, 2010). Higher XG levels enhanced probiotic survival, likely by forming a protective matrix during fermentation (Ziaolhagh and Jalali, 2017). The negative control's lower viability confirms that hydrocolloids drive prebiotic effects, addressing the reviewer's call for controls. Lower quinoa levels in top-performing samples may reduce protein interference with microbial growth, as noted by Torres et al. and Demir and Bilgicli (Demir and Bilgiçli, 2021; Torres, Olga L., Mariana Lema, 2021). These findings position GF pasta as a functional food for gut health, though scalability requires validation in vivo.

The enhanced survival of *Bifidobacterium adolescentis* and *Lactobacillus casei* observed in the formulations containing higher levels of β-glucan and xanthan gum can be explained by the complementary physicochemical and nutritional functions of these hydrocolloids. β-Glucan primarily acts as a fermentable substrate that selectively stimulates probiotic growth by providing metabolizable energy through its enzymatic degradation into short-chain fatty acids. In contrast, xanthan gum forms a viscous, pseudoplastic network that creates a protective microenvironment around the cells, buffering them against rapid pH drops and localized acid accumulation during

fermentation. This matrix also modulates nutrient diffusion and supports a gradual release of β-glucan, thereby sustaining bacterial metabolism over time. Together, β-glucan and xanthan gum act synergistically—β-glucan provides the "fuel," while xanthan gum offers the "shelter"—resulting in a stabilized, nutrient-rich niche that enhances probiotic survival and activity during in vitro fermentation (Wang *et al.*, 2024).

Optimal Formulation Selection

The quadratic optimization model conclusively identified Formulation 4 (10% quinoa, 50% rice, 40% corn, 0.5% β-glucan, 2% xanthan gum) as the optimal blend, delivering the most favorable equilibrium between enhanced health-promoting attributes and essential technological quality. This formulation exhibited superior nutritional functionality, evidenced by the highest resistant starch content (49.41 ± 0.97%, Table 4) and exceptional prebiotic activity (9.12 ± 0.03 log CFU/g, Table 4), while simultaneously maintaining the highest firmness among GF samples (Fig. 2) and optimal water absorption capacity (185.3 ± 3.0%, Table 2). The robustness of this selection was verified through sensitivity analysis, confirming that minor perturbations in weighting coefficients did not alter the optimal outcome. This balanced profile positions Sample 4 as a promising functional food candidate for managing postprandial glycemic response and promoting gut health (Larrosa *et al.*, 2015).

Limitations and Future Directions

While the study demonstrates GF pasta's potential as a functional food, the lack of a factorial or RSM design limits understanding of ingredient interactions, as noted by the reviewer. Sensory evaluation by consumers and in vivo digestibility studies are needed to validate findings. Additionally, the cost function's weights, though justified, may vary by application (e.g., prioritizing sensory over nutrition). Future research should explore scalable processing conditions and alternative hydrocolloids to enhance cost-effectiveness.

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589

590 **Table 1.** Percentage composition of raw materials used in the formulations of the wheat semolina
591 control, gluten-free negative control, and experimental gluten-free (GF) pasta samples.

Samples	Raw materials				
	Quinoa flour (g/100g)	Rice flour (g/100g)	Corn flour (g/100g)	β-glucan (g/100 g flour mixture)	Xanthan Gum (g/100 g flour mixture)
Control	Durum semolina				
GF-Negative control	20	50	30	0	0
Experimental GF-Samples					
1	10	50	40	2	0.5
2	10	50	40	1.5	1
3	10	50	40	1	1.5
4	10	50	40	0.5	2
5	10	60	30	2	0.5
6	10	60	30	1.5	1
7	10	60	30	1	1.5
8	10	60	30	0.5	2
9	10	70	20	2	0.5
10	10	70	20	1.5	1
11	10	70	20	1	1.5
12	10	70	20	0.5	2
13	20	60	20	2	0.5
14	20	60	20	1.5	1
15	20	60	20	1	1.5
16	20	60	20	0.5	2
17	20	50	30	2	0.5
18	20	50	30	1.5	1
19	20	50	30	1	1.5
20	20	50	30	0.5	2

Table 2. Cooking properties of the Wheat semolina Control, GF-Negative Control, and Gluten-Free Experimental GF-Samples.

Samples	Water absorption (%)	Cooking weight (%, w/w)
Control	23.61±0.25^{fg}	49.38±0.18^{k}
GF-Negative control	20±.0.99%	56±1.03%
Experimental GF-Samples		
1	24.85±0.27^{d}	53.68±0.37^{fgh}
2	18.99±.44^{k}	53.16±0.54^{gh}
3	16.71±0.10^{mn}	50.03±0.12^{jk}
4	27.05±0.42^{a}	55.47±0.17^{bc}
5	16.99±0.49^{m}	50.80±1.07^{j}
6	17.77±0.99^{l}	51.91±1.01^{i}
7	21.12±0.81^{i}	55.30±0.60^{bc}
8	24.91±0.07^{cd}	54.93±0.37^{cde}
9	25.62±0.18^{bc}	55.19±0.52^{bcd}
10	27.41±0.25^{a}	55.99±0.42^{b}
11	25.79±0.30^{b}	56.98±0.49^{a}
12	22.69±0.31^{h}	56.90±0.38^{a}
13	16.92±0.32^{m}	50.86±0.71^{j}
14	23.94±0.30^{efg}	53.12±0.57^{gh}
15	24.55±0.35^{de}	54.03±0.29^{efg}
16	15.93±0.50^{o}	49.75±0.90^{k}
17	23.44±0.47^{g}	52.84±0.29^{h}
18	19.82±0.47^{j}	54.54±0.15^{cdef}
19	24.35±0.12^{def}	54.22±0.29^{def}
20	16.14±0.45^{no}	49.49±0.62^{k}

Different lowercase letters (a, b, c, …) indicate significant differences between sample means within the same column or figure, according to Duncan's multiple range test ($p < 0.05$). Samples that share the same letter are not significantly different from each other. Control refers to pasta prepared from 100% durum wheat semolina; GF-Negative Control refers to gluten-free pasta (50% rice, 30% corn, 20% quinoa) without hydrocolloids

Table 3. In vitro starch digestibility profile of Wheat Semolina Control and Gluten-Free Experimental Samples across digestion time points.

Sample	Time						
	0 min	20 min	40 min	60 min	80 min	100 min	120 min
Control	0.16±0.14^{ab}	58.5±0.4^{a}	69.1±0.3^{a}	83.4±0.2^{a}	88.1±1.0^{a}	91.1±1.4^{a}	93.6±0.8^{a}
1	0.36±0.01^{a}	29.1±0.8^{i}	42.4±1.5^{ghi}	53.3±1.9^{hi}	60.2±0.1^{gh}	61.2±0.4^{gh}	64.6±2.8^{fg}
2	0.24±0.02^{ab}	35.4±0.4^{gh}	41.9±0.8^{ghi}	51.9±0.4^{ij}	56.9±0.2^{hijk}	60.8±0.3^{hi}	62.1±0.5^{fgh}
3	0.18±0.04^{ab}	40.8±1.0^{ef}	47.5±0.3^{f}	62.4±0.5^{f}	67.2±0.8^{e}	67.5±0.9^{e}	69.2±0.5^{e}
4	0.25±0.04^{ab}	23.8±0.9^{j}	35.8±0.1^{l}	46.7±0.3^{k}	50.5±1.1^{m}	56.3±2.4^{j}	56.2±1.1^{j}
5	0.16±0.06^{ab}	34.6±0.1^{h}	40.5±0.6^{ijk}	50.5±0.7^{ijk}	54.6±0.6^{jkl}	58.6±0.7^{hij}	58.8±0.4^{hij}
6	0.04±0.01^{b}	37.9±0.5^{fgh}	44.3±0.5^{fgh}	56.2±0.6^{gh}	62.7±0.3^{fg}	64.9±0.1^{efg}	65.4±0.9^{ef}
7	0.14±0.03^{ab}	49.4±0.2^{c}	58.7±0.5^{de}	71.1±0.2^{d}	77.3±0.2^{d}	79.1±0.2^{d}	79.6±0.4^{cd}
8	0.21±0.01^{ab}	26.8±0.7^{ij}	40.4±1.5^{ijk}	50.7±0.9^{ij}	56.5±0.5^{ijk}	58.9±0.7^{hij}	60.7±0.4^{ghi}
9	0.18±0.01^{ab}	28.3±0.2^{i}	40.5±0.2^{ijk}	53.6±2.5^{hi}	58.1±1.0^{hij}	61.4±0.2^{fgh}	63.3±1.3^{fg}
10	0.25±0.05^{ab}	26.1±0.4^{ij}	37.4±0.3^{kl}	49.7±1.4^{ijk}	53.5±0.1^{klm}	57.2±0.1^{ij}	58.2±0.4^{hij}
11	0.25±0.17^{ab}	25.7±1/0^{ij}	38.2±1.2^{jkl}	48.1±0.1^{jk}	51.9±2.2^{lm}	57.1±0.1^{ij}	57.3±0.8^{ij}
12	0.21±0.05^{ab}	54.2±0.3^{b}	65.7±0.2^{ab}	77.1±0.9^{bc}	82.9±0.8^{c}	87.1±0.2^{bc}	88.1±0.1^{b}
13	0.03±0.01^{b}	55.5±0.1^{ab}	66.4±0.8^{a}	80.4±0.2^{ab}	86.9±0.8^{ab}	89.5±0.7^{ab}	91.6±0.3^{ab}
14	0.18±0.04^{ab}	38.2±1.1^{fgh}	55.7±0.7^{e}	66.4±0.6^{e}	74.4±1.1^{d}	75.6±0.2^{d}	77.4±0.9^{d}
15	0.33±0.06^{a}	28.1±2.7^{i}	41.6±1.8^{ghij}	57.9±0.1^{g}	64.1±0.1^{ef}	65.3±1.2^{ef}	65.3±1.2^{ef}
16	0.09±0.07^{ab}	38.8±0.1^{fg}	45.1±0.7^{fg}	58.8±0.1^{fg}	64.1±0.2^{ef}	65.2±0.1^{efg}	65.7±0.5^{ef}
17	0.21±0.01^{ab}	44.1±1.8^{de}	62.3±0.8^{bc}	75.4±1.2^{c}	84.3±0.2^{bc}	87.1±0.9^{bc}	88.7±0.4^{b}
18	0.11±0.02^{ab}	36.3±0.7^{gh}	40.9±0.5^{hijk}	52.6±0.1^{hi}	57.7±0.6^{hij}	60.7±0.1^{hi}	61.9±0.3^{fgh}
19	0.34±0.05^{a}	45.3±1.7^{cd}	61.2±0.5^{cd}	74.3±1.1^{cd}	80.9±1.4^{c}	85.4±2.3^{c}	82.9±2.1^{c}
20	0.12±0.11^{ab}	37.5±0.3^{fgh}	42.9±0.6^{ghi}	53.4±0.7^{hi}	59.9±0.3^{ghi}	61.4±0.7^{fgh}	63.9±0.1^{fg}

Different lowercase letters (a, b, c, …) indicate significant differences between sample means within the same column or figure, according to Duncan's multiple range test ($p < 0.05$). Samples that share the same letter are not significantly different from each other. Control refers to pasta prepared from 100% durum wheat semolina.

649 **Table 4.** RDS, SDS, RS, and Prebiotic Activity of Wheat Semolina Control, GF-Negative Control,
650 and Gluten-Free (GF) Experimental Samples.

Samples	RDS	SDS	RS	Prebiotic activity (CFU/g)
Control	52.74±0.41^{a}	31.55±0.35^{cdef}	15.75±0.77^{l}	7.47±0.09^{fg}
GF-Negative control	60.1±0.8^{a}	15±0.5^{l}	20.5±0.5^{k}	6.8±0.1^{h}
GF-Experimental Samples				
1	26.16±0.77^{g}	32.05±1.80^{cde}	41.80±0.35^{f}	7.54±0.12^{f}
2	31.87±0.41^{f}	24.06±0.06^{kl}	44.08±0.47^{de}	7.98±0.09^{e}
3	36.76±0.90^{de}	25.60±1.41^{hik}	37.65±0.51^{g}	7.29±0.05^{gh}
4	21.48±0.89^{h}	29.12±1.86^{efg}	49.41±0.97^{a}	9.12±0.03^{a}
5	26.19±2.18^{g}	21.78±0.50^{l}	47.03±0.37^{bc}	7.23±0.08^{h}
6	34.19±0.45^{ef}	24.72±0.40^{ikl}	41.10±0.84^{f}	7.63±0.07^{f}
7	44.54±0.22^{b}	27.19±0.66^{ghi}	28.28±0.44^{i}	8.83±0.04^{b}
8	24.16±0.69^{gh}	30.53±1.13^{def}	45.31±0.45^{cd}	8.31±0.11^{d}
9	25.52±0.25^{gh}	31.54±0.94^{cdef}	42.95±1.19^{ef}	7.56±0.07^{f}
10	23.48±0.44^{gh}	28.97±0.06^{efg}	47.55±0.39^{ab}	8.61±0.10^{c}
11	23.20±0.91^{gh}	28.45±1.72^{fgh}	48.36±0.79^{ab}	9.24±0.11^{a}
12	48.83±0.30^{a}	30.50±0.17^{def}	20.68±0.13^{k}	8.93±0.11^{b}
13	50.01±0.11^{a}	32.51±0.47^{bcd}	17.48±0.35^{l}	7.19±0.16^{h}
14	34.44±0.99^{ef}	35.23±1.90^{b}	30.34±0.89^{h}	7.49±0.17^{f}
15	25.26±2.52^{gh}	33.53±1.39^{bcd}	41.21± 1.11^{f}	7.91±0.01^{e}
16	34.94±0.08^{ef}	24.26±0.55^{ikl}	40.80±0.47^{f}	7.20±0.14^{h}
17	39.74±1.69^{cd}	40.17±2.08^{a}	20.10±0.39^{k}	7.47±0.14^{fg}
18	32.68±0.72^{ef}	23.10±0.42^{kl}	44.23±0.29^{de}	8.35±0.10^{d}
19	40.81±1.58^{bc}	33.82±3.51^{bc}	25.37±1.92^{j}	7.57±0.06^{f}
20	33.79±0.29^{ef}	23.78±0.20^{kl}	42.43±0.09^{ef}	7.27±0.21^{h}

651 Different lowercase letters (a, b, c, …) indicate significant differences between sample means within the same column or figure,
652 according to Duncan's multiple range test ($p < 0.05$). Samples that share the same letter are not significantly different from each
653 other. Control refers to pasta prepared from 100% durum wheat semolina; GF-Negative Control refers to gluten-free pasta (50%
654 rice, 30% corn, 20% quinoa) without hydrocolloids. Abbreviations: RDS, rapidly digestible starch; SDS, slowly digestible starch;
655 RS, resistant starch
656
657 **Table 5.** Relative importance of the measured properties of GF-Experimental Samples.

No.	Pasta Properties	Relative Importance	Coefficients
1	Fmax of raw pasta	high	10
2	Water absorption capacity	high	10
3	Cooking weight	Medium	5
4	Crude fiber	low	2
5	Prebiotic activity	Very high	20
6	Rapid digestive starch (RDS)	Very high	20
7	Slow digestive starch (SDS)	Very high	20
8	Resistance starch (RS)	Very high	20

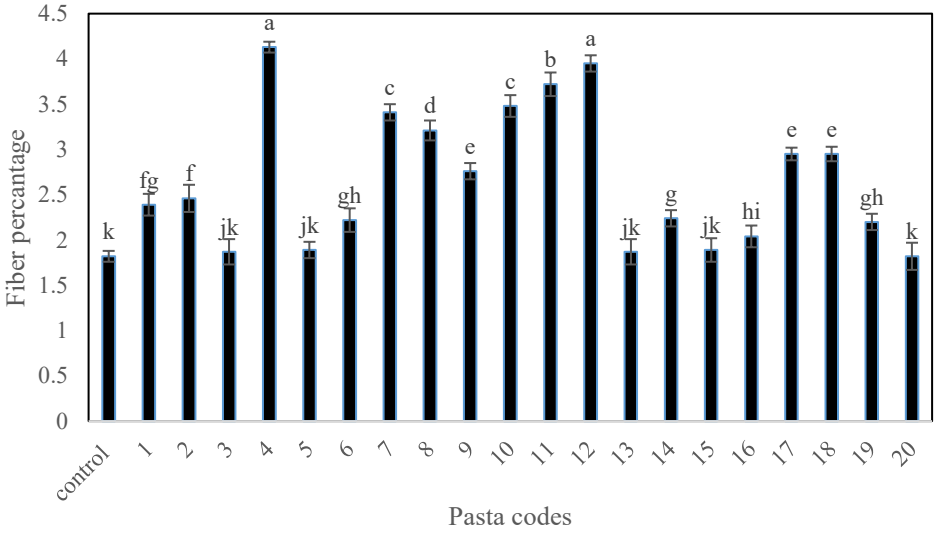
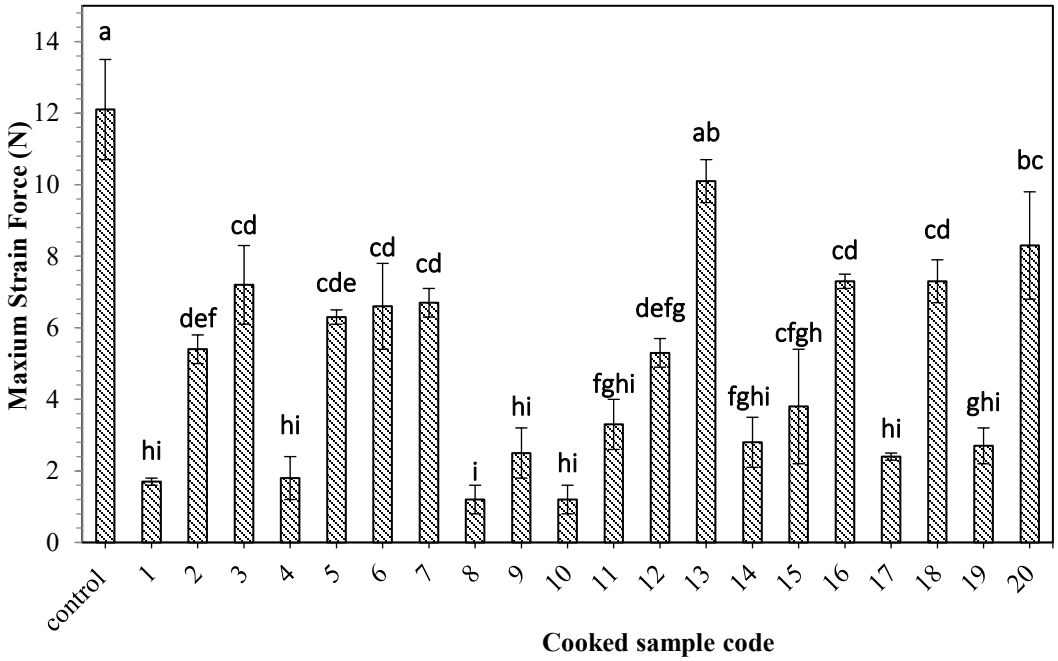


Fig 1. Crude fiber content of Wheat Control and GF-Experimental Samples. Different lowercase letters (a, b, c, …) indicate significant differences between sample means within the same column or figure, according to Duncan's multiple range test ($p < 0.05$). Samples that share the same letter are not significantly different from each other.



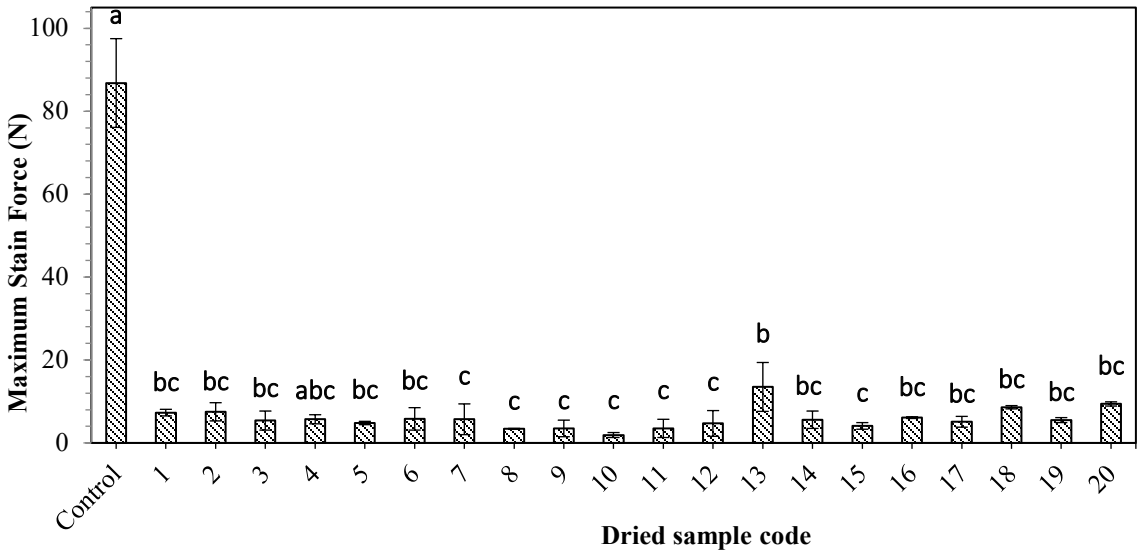


Fig 2. Firmness (N) of cooked and dried pasta for Wheat Control and Gluten-Free Experimental Samples. Different lowercase letters (a, b, c, …) indicate significant differences between sample means within the same column or figure, according to Duncan's multiple range test ($p < 0.05$). Samples that share the same letter are not significantly different from each other.

فرمولاسیون پیشرفته پاستای بدون گلوتن: ادغام آردها و هیدروکلوئیدهای جایگزین برای کیفیت مطلوب و مزایای سلامتی

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چکیده

در این پژوهش، فرمولاسیون‌های پیشرفته پاستای بدون گلوتن (GF) با استفاده از آردهای جایگزین شامل کینوا، برنج و ذرت، و همچنین هیدروکلوئیدهای زانتان‌گام و بتا-گلوکان، به‌منظور بهینه‌سازی کیفیت فناوری و ارزش تغذیه‌ای برای افراد مبتلا به بیماری سلیاک یا حساسیت به گلوتن توسعه یافت. زانتان‌گام عمدتاً به‌عنوان عامل اتصال‌دهنده با خاصیت ویسکوالاستیک به‌منظور شبیه‌سازی ساختار گلوتن به‌کار می‌رود، در حالی‌که بتا-گلوکان، به‌عنوان یک فیبر محلول با خواص سلامت‌زای شناخته‌شده، موجب بهبود ویژگی‌های تغذیه‌ای و عملکردی می‌گردد .بیست فرمول مختلف از نظر هضم‌پذیری نشاسته (نشاسته سریع‌الهضم[RDS] ، کند هضم [SDS] و مقاوم[RS]) ، میزان فیبر، فعالیت پری‌بیوتیکی، بافت (سختی) و ویژگی‌های پخت (جذب آب و وزن پخت) ارزیابی و با پاستای گندم معمولی مورد مقایسه قرار گرفتند. افزایش سطوح کینوا و هیدروکلوئیدها منجر به بهبود سختی و جذب آب شد، در حالی‌که افزایش نسبت کینوا، ذرت و زانتان‌گام زمان پخت را کاهش داد .فرمول حاوی 10٪ کینوا، 50٪ برنج، 40٪ ذرت، 0.5٪ بتا-گلوکان و 2٪ زانتان‌گام (نمونه 4) دارای کمترین مقدار RDS، بیشترین مقدار RS (49.41%) و بالاترین فعالیت پری‌بیوتیکی بود که به مهار آنزیمی ناشی از هیدروکلوئیدها و کاهش دسترسی آنزیم‌ها به نشاسته نسبت داده شد. اثر هم‌افزای نشاسته غنی از آمیلوز کینوا، ویسکوزیته بتا-گلوکان و خاصیت محدودکنندگی انتشار زانتان‌گام، موجب کاهش هیدرولیز نشاسته و افزایش تشکیل RS شد .بهینه‌سازی آماری با استفاده از تحلیل واریانس یک‌طرفه (ANOVA) و تابع هزینه درجه دوم، فرمول‌هایی را شناسایی کرد که بین عملکرد فناوری و کیفیت تغذیه‌ای تعادل برقرار کردند. پاستای بدون گلوتن بهینه‌شده از نظر کیفیت با پاستای گندم قابل مقایسه یا برتر بود که پتانسیل بالایی به‌عنوان یک غذای عملگرا با اثرات مفید بر کنترل قند خون و سلامت روده دارد.