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Investigation of Cooking Quality, Textural and Sensory Characteristics of Rice and Corn Flour-Based Gluten-Free Pasta Fortified with Xanthan Gum and Lyophilized Trout Flour (*Oncorhynchus mykiss*)

Hülya IŞIK[1], Hülya GÜL[2]*

[1] *Süleyman Demirel University, Graduate School of Natural and Applied Sciences, Department of Food Engineering, Isparta/Türkiye*
[2] *Süleyman Demirel University, Faculty of Engineering and Natural Sciences, Department of Food Engineering, Isparta/Türkiye*
**Corresponding author: H. Gül e-mail: hulyagul@sdu.edu.tr*

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RESEARCH ARTICLE

Abstract

This study investigated the technological, nutritional, and sensory properties of gluten-free pasta (GFP) made from a rice (60%) and corn (40%) flour blend fortified with 1% xanthan gum and 2.5–10% lyophilized trout flour (LTF; *Oncorhynchus mykiss*). Proximate composition, cooking quality, including optimum cooking time (OCT), cooking loss (CL), volume increase (VI), and water absorption capacity (WAC); color; textural properties of dried-uncooked and cooked GFP; and sensory attributes were evaluated. Increasing LTF significantly decreased moisture and water activity while increasing protein, ash, dietary fiber, and titratable acidity ($p < 0.05$). Cooking quality parameters such as OCT, WAC, and VI improved, though CL also rose but remained acceptable. Color analysis showed increased lightness and yellowness, with slight redness. Texture analysis revealed decreased dry hardness at ≥7.5% LTF and softer, stickier cooked pasta with reduced springiness. Sensory acceptability declined due to fishy odor and flavor; however, vinegar pretreatment combined with lyophilization enhanced palatability and color. Formulations with up to 7.5% LTF, especially 2.5–5%, maintained acceptable sensory and cooking qualities. Adding xanthan gum enabled the production of nutritionally enhanced GFP with satisfactory technological and sensory properties.

Key words: Fish; *Oncorhynchus mykiss*; celiac; gluten-free; pasta.

INTRODUCTION

Grains and grain products are fundamental components of human nutrition worldwide, serving as primary sources of carbohydrates, proteins, vitamins, and minerals. Their widespread availability, affordability, and ease of storage, primarily due to their starch content, position them as staple foods that fulfill over half of the daily energy requirements of many populations worldwide. The primary grain types, predominantly in the Gramineae family, include wheat, rice, corn, oats, barley, rye, and millet, with wheat, rice, and corn being the most widely consumed. It is projected that global cereal utilization will increase by 2.2% in the 2025/26 season, with cereal food consumption anticipated to rise by 1.1% annually, reaching 1.28 billion tons by 2034 (OECD-FAO, 2025; FAO, 2026;). Processed grain products, such as bread, cakes, noodles/pasta, biscuits and pastries, are widely

consumed worldwide. The International Pasta Organization projected a global pasta consumption of 19.5 million metric tons in 2023 (Business Wire, 2025). Pasta, a traditional grain-based food, has seen an increase in global consumption owing to its convenience, palatability, and nutritional value (Bustos et al., 2015). It is considered one of the most preferred foods after bread (Özgören & Yapar 2015). In Türkiye, the monthly per capita pasta consumption was reported to be 913.45 g in Isparta's urban areas in 2017 (Gül et al. 2017). The versatility of pasta, characterized by its low cost, extended shelf life, and broad appeal, makes it an ideal candidate for enrichment with functional ingredients, allowing for diverse product development (Bustos et al., 2015; Konuray & Erginkaya, 2020). The ease of its production process and its broad appeal further highlight its suitability for enhancement with a variety of components (Ghandehary Yazdi et al., 2020; Gül et al., 2023a; Gül et al., 2023b; Acun et al., 2025; Çoban et al., 2026; Toktabek et al., 2026a; Toktabek et al., 2026b).

While traditional pasta is made from durum wheat semolina, alternative ingredients such as rice, corn, chickpeas, and lentils are increasingly being used, especially to meet specific dietary needs. For example, gluten-free pasta substitutes rice or corn flour for wheat, catering to individuals requiring a gluten-free diet (Kurt, 2018; Turkish Food Codex, 2018). Celiac disease, an autoimmune disorder triggered by gluten consumption, affects approximately 1% of the global population and requires a lifelong gluten-free diet as the only effective treatment (Hayıt & Gül, 2017; Hussein et al., 2023; Mostafa et al., 2022; Stoleru et al., 2022; Machado, 2023;). Beyond celiac disease, the increasing prevalence of non-celiac gluten sensitivity and wheat allergy has further increased the demand for gluten-free products (Khairuddin & Lasekan, 2021). Following a gluten-free diet requires careful attention to ensure adequate intake of essential nutrients, such as B12, folate, iron, calcium, and vitamin D (Küçükazman et al., 2008). Consequently, food manufacturers are increasingly focusing on developing gluten-free alternatives to staple foods such as bread, pasta, and biscuits. Rice (Oryza sativa) and corn flours are preferred gluten-free grains because of their neutral flavor and suitability for processing (Khairuddin & Lasekan, 2021). Rice, which is low in fat, fiber, protein, and sodium and rich in easily digestible carbohydrates, is a common substitute for wheat (Sivaramakrishnan et al., 2004). Corn, with its 7-13% protein content, is also a valuable source of dietary fiber, vitamin B6, and magnesium (Mastromatteo et al., 2011). The demand for gluten-free products is driven by broader healthy eating trends that extend beyond clinical requirements (Bianchi et al., 2021; Irondi et al., 2022).

However, producing gluten-free pasta presents significant technological challenges, primarily because gluten is absent, which typically provides structural integrity, elasticity, and cooking resistance (Mir et al., 2016; Fradinho et al., 2020). This deficiency often results in products with poor structural integrity, brittleness, high stickiness, and suboptimal cooking quality (Mir et al., 2016). To overcome these challenges, gluten-free formulations often incorporate emulsifying agents and hydrocolloids such as xanthan gum, guar gum, and hydroxypropyl methylcellulose (Lai, 2002; Allen & Orfila, 2018; Gül et al., 2018; Dahal et al., 2021). These water-soluble polysaccharides, characterized by their high molecular weights and hydrophilic structures, enhance dough rheology and textural properties by forming gluten-like structures, thereby enhancing water binding capacity, gel formation, and viscosity (Padolino et al., 2016; Allen & Orfila, 2018; Gao et al., 2018; Dahal et al., 2021). Their interactions with starch and proteins help regulate moisture distribution and contribute to product stability (Chen et al., 2023; Zhang et al., 2025). Xanthan gum is extensively used for its robust viscoelastic network-forming ability, which enhances the product volume, reduces firmness, and improves sensory attributes (Widelska et al., 2019; Milde et al., 2020). Its branching structure and pseudoplastic behavior ensure stability across a broad range of pH and temperature conditions, as well as during enzymatic degradation, making it superior to other natural gums (Milas & Rinaudo 1986). Additionally, it is preferred in gluten-free products because of its minimal impact on appearance and taste (Sharma et al., 2006).

Beyond structural concerns, gluten-free pasta often lacks the nutritional density of its wheat-based counterparts, particularly in terms of protein content and essential micronutrients (Mishra et al., 2023). This shortfall has spurred efforts to enrich gluten-free pasta with alternative protein sources such as legumes. Animal protein sources, renowned for their high bioavailability and rich nutritional content, are considered valuable alternatives to plant-based proteins. Fish, for instance, is an excellent source of high-quality protein, essential amino acids, omega-3 fatty acids, vitamins (A, D, B6, B12), and minerals (Berruezo et al., 2005; Mohammed et al., 2020; Golden et al., 2021). The cardiovascular benefits of unsaturated fatty acids (Saini & Keum, 2018; Gladyshev et al., 2022) and the role of amino acids in tissue development (Duan et al., 2020; Wang et al., 2022) further highlight the importance of these compounds in developing functional foods. Rainbow trout (*Oncorhynchus mykiss*) is a notable aquatic species valued for its high nutritional content, including high-quality proteins, essential amino acids, and omega-3 fatty acids (Singh 2020; Singh & Srivastava 2021). While fish-derived ingredients offer substantial nutritional benefits, their direct incorporation into food formulations necessitates careful consideration of sensory characteristics, such as taste and odor, to ensure product acceptability and consumer satisfaction.

Considering these factors, there is a distinct need for comprehensive strategies to enhance the technological and nutritional quality of gluten-free pasta. This study aimed to explore the impact of varying amounts of lyophilized trout flour and xanthan gum (a hydrocolloid) on gluten-free pasta formulations made from rice and corn flour. The chemical composition, cooking properties, textural structure, and sensory characteristics of the

resulting pasta samples were assessed. The findings of this study are anticipated to contribute to the development of gluten-free pasta products that are both functionally and qualitatively enriched, thereby aligning with the evolving consumer expectations.

MATERIALS AND METHODS

Material

Rainbow trout (*Oncorhynchus* mykiss) were obtained from a specialized fish processing plant in Isparta (Türkiye). The rice flour and corn flour used in gluten-free pasta production were purchased from İpek Değirmen (Aksaray, Türkiye), and the xanthan gum was purchased from IFC Aroma Kimya Gıda Sanayi Tic. A.Ş. (İstanbul, Türkiye). All chemicals were of analytical grade.

Methods

Preparation of rainbow trout (*Oncorhynchus mykiss*) flour

Rainbow trout (*Oncorhynchus mykiss*) weighing approximately 300–350 g were obtained from a private fish processing facility in Isparta province and transported to the laboratory under sterile conditions. The trout samples were promptly washed with potable water and separated from the tail, internal organs, skin, fins, and bony structures. The fish was subsequently minced using a meat grinder (Philips Viva Collection HR2726/90, Netherlands) equipped with a 5 mm diameter plate. The minced trout was immersed in a 5% vinegar solution at a ratio of twice its weight for 1.5 h, then drained and rinsed at 10°C to remove odor components. The minced trout was spread to a thickness of 2 cm on sterilized trays and frozen at -20°C for 24 h. The samples were then freeze-dried in a lyophilizer (Teknosem TRST-D-4-4, Turkey) at -55°C under a vacuum pressure of 20 Pa for 48 h. The dried samples were ground using a grinder (Waring Blender, Torrington, CT, USA) to a particle size of less than 1000 µm and processed into flour for use in the pasta formulations. The preparation stages of lyophilized rainbow trout (*Oncorhynchus mykiss*) flour (LTF) are shown in Figure 1. The resultant LTF was preserved in airtight containers at -18°C to prevent moisture and microbial activity.

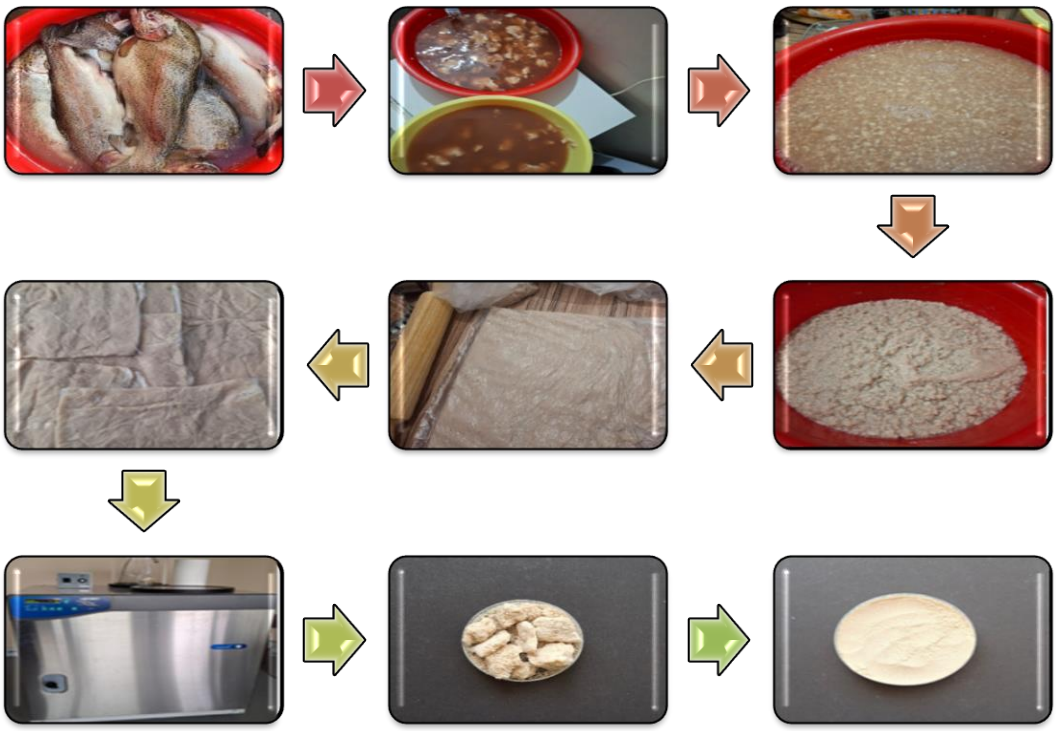


Figure 1. Preparation stages for LTF (Lyophilized rainbow trout; *Oncorhynchus mykiss* flour).

GFP Production

The formulations of the gluten-free pasta (GFP) are presented in Table 1.

Table 1. Formulations of GFP

GFP	LTF (%)	Rice flour (%)	Corn flour (%)	Xanthan Gum (%)	Water (%)
Control	-	60.0	40.0	1	65
GFP-2.5	2.5	58.5	39.0	1	65
GFP-5	5.0	57.0	38.0	1	65
GFP-7.5	7.5	55.5	37.0	1	65
GFP-10	10.0	54.0	36.0	1	65

GFP: Gluten-free pasta, LTF: Lyophilized Trout Flour, GFP-2.5: Gluten-free pasta containing 2.5% LTF, GFP-5: Gluten-free pasta containing 5% LTF, GFP-7.5: Gluten-free pasta containing 7.5% LTF, GFP-10: Gluten-free pasta containing 10%.

Initially, a base gluten-free flour blend was prepared by mixing rice flour (60%) and corn flour (40%). In preliminary trials, rice flour-corn flour ratios of 50/50, 30/70, and 20/80 were also examined. However, the best results were obtained with a 60/40 mixing ratio. This blend was the sole component (100%) in the control samples. To investigate the effects of LTF, the base flour blend was substituted with LTF at ratios of 2.5%, 5%, 7.5%, and 10%. In preliminary trials, higher levels were tested but deemed unacceptable for sensory quality. At these elevated levels, the fishy smell and taste were perceived as overwhelmingly intense, which diminished the sensory acceptability of the pasta product. Consequently, 10% was set as the maximum acceptable limit. In all formulations, xanthan gum was added at a constant level of 1%, and the water content was maintained at 65% as determined in preliminary tests. In preliminary trials, xanthan gum was tested at higher concentrations (2% and 3 %). However, the best results in terms of technological quality of gluten-free pasta were obtained with xanthan gum at 1%. The water ratio was specifically chosen to maintain the dough moisture content within the optimal range of 30-33%, as determined by gravimetric analysis.

The ingredients, proportioned according to the formulations in Table 1, were mixed using a laboratory mixer (Hobart N50CE, USA) for 10 min, followed by 1 min of manual kneading to ensure dough homogeneity. The resulting dough was divided into 100 g portions and allowed to rest in sealed plastic bags at room temperature for 20 min. After resting, the dough was pre-rolled with a rolling pin and then progressively sheeted and cut into the desired shape using a manual pasta machine (Imperia, Italy). For the production of dry pasta, the samples were dehydrated in a drying oven at 55°C until a final moisture content of 12.5–13% was achieved (Figure 2). Finally, the dried pasta samples were stored in moisture-proof packaging (PET+COEX PA) at +4°C until further analysis.

Determination of the proximate composition of GFP

The moisture (AACC Method 44-15.02; Moisture—Air-Oven Methods), ash (AACC Method 08-01.01), total dietary fiber (AACC Method 32-07.01), and titratable acidity (AACC Method 02-31.01) of the GFP samples were determined using AACC standard methods (AACC International, 2000). The protein content was determined using a Dumatherm analyzer (Gerhardt GmbH & Co. KG, Königswinter, Germany) according to the Dumas Method and calculated using a factor of 6.25 (Shea & Watts 1939). The water activity (aw) values of the samples were determined using a water activity analyzer (Novasina Lab Touch-aw, Lachen, Switzerland). The samples were placed in the titrator's chamber, and the value was expected to stabilize. The values were recorded after fixation.

Cooking quality assessment of GFP

The cooking quality of the GFP samples was evaluated based on optimal cooking time (OCT), cooking loss (CL), water absorption capacity (WAC), and volume increase (VI). The OCT, CL, and WAC were determined according to AACC Approved Method 66-50.01 (AACC International, 2000). A 25 g sample of pasta was cooked in 250 mL of boiling distilled water for the predetermined OCT. During cooking, a pasta strand was removed every 30 s and compressed between two transparent glass plates. OCT was defined as the time at which the central, uncooked, opaque core was no longer visible. After cooking, the GFP was drained for 2 min. The drained GFP was weighed, and the WAC was calculated as the percentage increase in weight compared with that of the uncooked sample. CL was performed as follows: the cooking water was collected in a volumetric flask and

made up to a known volume. An aliquot was evaporated to dryness in an oven at 105°C until a constant weight was achieved. CL was expressed as the percentage of dry matter lost from the original pasta samples. WI was determined using the water displacement method described by Özkaya & Kahveci (1990). The volumes of the GFP samples were measured before and after cooking, and the results were expressed as a percentage increase.

Determination of color properties of GFP

Color measurements were performed using a Minolta CR-410 (Minolta Co., Ltd., Tokyo, Japan). Measurements were taken from three different areas of the samples, and the color components Hunter lightness (L*), redness (a*), and yellowness (b*) were determined. The color scale was used with L* value [(0) black – (100) white], a* value [(+) red, (-) green], and b* value [(+) yellow, (-) blue] (Larrosa et al., 2016).

Determination of textural characteristics of dry-uncooked and cooked GFP

The textural characteristics of the dry uncooked GFP samples were assessed using a three-point bending test adapted from AACC Method 66-52.01 (AACC International, 2000). A TA.XT Plus texture analyzer (Stable Micro Systems Ltd., UK) fitted with a 50 N load cell was used. Two points supported each pasta strand, and a 0.8 mm blade probe was descended centrally at a constant speed of 1 mm/s until fracture occurred. The peak force of the resulting curve was reported as the breaking strength (N), and the distance to fracture was recorded as the deformation (mm).

The textural properties of the cooked pasta were evaluated using Texture Profile Analysis (TPA) based on the principles described by Acun et al. (2025). The pasta samples were cooked to their predetermined OCT. Immediately after cooking, the pasta was cooled for 1 min under cold running tap water and then drained using a Büchner funnel. Excess surface moisture was carefully removed by blotting the strands using paper towels. The TPA was performed using a texture analyzer (TA.XT Plus, Stable Micro Systems Ltd., UK) equipped with a 5 kg load cell. Three strands of cooked pasta were placed side by side on the instrument's platform and subjected to a two-cycle compression test using a 2 cm diameter flat cylindrical probe. The sample was compressed to 50% of its original height with the following test parameters: pre-test speed of 1 mm/s, test speed of 5 mm/s, and post-test speed of 5 mm/s. From the resulting force-time curve, the following textural parameters were calculated using the instrument's software: hardness (N), adhesiveness (N·s), springiness, cohesiveness (dimensionless), gumminess (N), chewiness (N), and resilience.

Sensory Evaluation of GFP

A panel of 10 semi-trained panelists, comprising students and staff of the Food Engineering Department, was formed. The evaluation took place in separate sensory booths with controlled environmental conditions (white light and a constant room temperature of 22°C) to reduce outside influences. The panelists were given warm (approximately 60°C) samples of GFP that had been cooked to their predetermined OCT, drained, and served. Each sample was placed in a white ceramic bowl and assigned a random three-digit code. To prevent order effects, the samples were presented to each panelist in a different order, and the panelists were instructed to drink room-temperature water and eat unsalted crackers between each sample to cleanse their palates. They examined the color, smell, general appearance, stickiness, taste, aroma, firmness, texture, and overall acceptability of the pasta. Each trait was given a score from 1 to 7, with 1 meaning "dislike very much" and 7 meaning "like very much." A distinct 7-point scale was employed to evaluate purchase intent (1: would not buy, 7: definitely would buy), which served as a proxy for marketability. The sensory analysis protocol was modified from the foundational principles of sensory evaluation outlined by Lawless & Heymann (2010).

Statistical Analysis

All analyses were conducted in triplicate, and the results are presented as mean ± standard deviation. Differences among means were evaluated using one-way ANOVA, followed by Duncan's post hoc test at a significance level of $p < 0.05$. Statistical analyses were performed using the SPSS software (version 24.0; SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSIONS

Proximate composition of GFP

The incorporation of LTF significantly enhanced the nutritional and chemical profiles of GFP. The results for moisture, water activity (aw), ash, protein, total dietary fiber, and titratable acidity are presented in Table 2.

Table 2. Proximate composition of GFP

GFP Sample	Moisture (%)	aw	Protein (%)	Ash (%)	Total Dietary fiber (%)	Titratable acidity
Control	13.32±1.15^{a}	0.350±0.00^{a}	10.44±0.00^{e}	0.45±0.05^{b}	15.49±0.30^{b}	0.24±0.03^{e}
GFP-2.5	9.57±1.04^{b}	0.279±0.00^{b}	11.77±0.00^{d}	0.63±0.01^{a}	16.84±0.80^{ab}	0.45±0.00^{d}
GFP-5	8.21±0.12^{bc}	0.272±0.00^{c}	13.63±0.00^{c}	0.70±0.08^{a}	17.45±0.21^{ab}	0.63±0.05^{c}
GFP-7.5	7.75±0.19^{c}	0.268±0.00^{d}	15.83±0.00^{b}	0.67±0.04^{a}	18.22±2.31^{ab}	0.84±0.06^{b}
GFP-10	8.03±0.66^{c}	0.264±0.00^{e}	17.54±0.00^{a}	0.67±0.03^{a}	18.53±0.29^{a}	1.02±0.05^{a}

GFP: Gluten-free pasta, LTF: Lyophilized Trout Flour, GFP-2.5: Gluten-free pasta containing 2.5% LTF, GFP-5: Gluten-free pasta containing 5% LTF, GFP-7.5: Gluten-free pasta containing 7.5% LTF, GFP-10: Gluten-free pasta containing 10%, aw: Water activity.

Note: Differences between values shown with the same letter in the same column in the table are insignificant according to the 0.05 confidence limit.

The moisture content of the samples decreased progressively from 13.32% in the control to 8.03% in the 10% LTF formulation. This decline can be attributed to the inherently low moisture content of LTF and the partial replacement of the rice and corn flour blend with this animal-derived ingredient. Despite this decrease, all moisture levels remained within the 13% limit mandated by the Turkish Food Codex Pasta Regulation (Turkish Food Codex, 2021), indicating that the samples met commercially acceptable standards. Similar moisture reduction trends (from 34.05% to 32.08%) were reported by Çoban et al. (2026) for buckwheat-based gluten-free pasta enriched with lyophilized trout powder and by Pangestu et al. (2025), who noted that the moisture content in sago-based pasta was influenced by processing conditions rather than ingredients alone.

In parallel with moisture, aw values significantly decreased from 0.350 (control) to 0.264 (10% LTF). This reduction in aw is a critical finding, as lower aw levels typically enhance the microbial stability and shelf life of dried pasta products. Similar observations regarding the influence of fish-derived ingredients on the physicochemical stability of pasta were reported by Monteiro et al. (2019), who noted that such additions significantly affect the final product's quality.

One of the most notable impacts of LTF fortification was a significant increase in protein content, from 10.44% to 17.54% at the highest substitution level. This substantial increase ($p < 0.05$) was attributed to the high biological value of animal-derived proteins in trout, which contain essential amino acids necessary for human nutrition. Furthermore, this effectively compensates for the protein deficiency commonly observed in rice- and corn-based gluten-free products. This improvement is consistent with the findings of Monteiro et al. (2019), who reported a similar increase in protein content (from 8.44 to 18.38 g/100 g) in pasta enriched with tilapia waste flour. Furthermore, Çoban et al. (2026) observed even higher protein levels, reaching 20.98% at 10% inclusion, confirming that trout-derived proteins effectively address the nutritional gaps in rice- and corn-based gluten-free diets. Desai et al. (2018) similarly observed that fish powder supplementation significantly elevates protein, lipid, and ash contents in pasta formulations. Our results also align with previous studies that used legume flours for enrichment, such as Wood (2009), who reported a 17.4% increase in protein with 30% chickpea flour. However, LTF use achieved a similar protein level at a much lower substitution rate (10%), highlighting its efficiency as a concentrated protein source for growing pigs.

Similarly, the ash content increased from 0.45% in the control to 0.67–0.70% in the LTF-enriched samples. This increase reflected the high mineral content of trout flour, consistent with the findings of Çoban et al. (2026), who emphasized the effectiveness of trout-based ingredients in enhancing mineral density. A corresponding increase in ash levels was also observed in tilapia waste flour-enriched pasta (Monteiro et al., 2019), highlighting fish-derived flours as potent sources of inorganic matter for cereal-based products. This increase, caused by the high mineral content of trout flour, is consistent with other fortification studies using buckwheat or legume flours (Bilgiçli, 2009), where mineral-rich additives naturally elevated the total inorganic matter of the pasta.

Total dietary fiber levels also showed an upward trend, increasing from 15.49% to 18.53% with 10% LTF. Although fish is not a traditional fiber source, this increase may be attributed to the contribution of trout protein to a structural matrix that behaves similarly to fiber in analytical measurements, or to the synergy between fish flour and xanthan gum in the formulation.

A significant increase in acidity was observed with increasing LTF ratio. The titration acidity, which was 0.240% in the control sample, reached 1.015% in the GFP-10 sample. This can be attributed to the acidic nature of the natural free fatty acids and protein degradation products in trout flour (Jiang & Durance, 1985). Monteiro

et al. (2019) also identified that certain volatile compounds and fatty acids in fish flour contribute to the distinctive chemical and sensory profile of fortified pasta.

Cooking Characteristics of GFP Samples

Pasta cooking quality is a primary determinant of consumer acceptance and is typically evaluated using OCT, WAC, VI, and CL (Aínsa et al., 2021a; Mathew et al., 2022). The incorporation of LTF significantly influenced the cooking performance of rice- and corn-based GFP samples (Table 3).

Table 3. Cooking characteristics of GFP

GFP Sample	OCT (min)	WAC (%)	VI (%)	CL (%)
Control	7.55±0.01^{d}	114.96±1.20^{e}	12.72±0.29^{e}	2.52±0.11^{d}
GFP-2.5	7.51±0.01^{d}	129.92±0.23^{d}	14.55±0.28^{d}	3.22±0.01^{bc}
GFP-5	10.41±0.07^{c}	140.40±0.91^{c}	15.45±0.29^{c}	3.11±0.24^{c}
GFP-7.5	11.09±0.10^{b}	173.44±1.10^{b}	16.36±1.20^{b}	3.52±0.00^{ab}
GFP-10	11.51±0.04^{a}	188.48±1.13^{a}	18.18±1.29^{a}	3.76±0.11^{a}

GFP: Gluten-free pasta, OCT:Optimal cooking time, WAC: Water absorption capacity, VI:Volume increase, CL: Cooking loss, LTF: Lyophilized Trout Flour, GFP-2.5: Gluten-free pasta containing 2.5% LTF, GFP-5: Gluten-free pasta containing 5% LTF, GFP-7.5: Gluten-free pasta containing 7.5% LTF, GFP-10: Gluten-free pasta containing 10% LTF.

Note: Differences between values shown with the same letter in the same column in the table are insignificant according to the 0.05 confidence limit.

The OCT significantly increased ($p < 0.05$) as the LTF substitution level increased, extending from 7.55 min (control) to 11.51 min (10% LTF). This trend is consistent with the findings of Çoban et al. (2026), who noted that higher levels of trout powder delayed starch gelatinization by acting as a physical barrier to water penetration. Similar findings have been reported in the literature, where hydrocolloid incorporation significantly prolonged cooking time due to enhanced structural stability (Sriwichai et al., 2024; Mostafa et al., 2025). The observed increase can be attributed to the formation of a denser protein–starch network, in which the addition of trout flour enhances the matrix's compactness, thereby restricting water diffusion. In addition, xanthan gum contributes to this effect by forming a highly viscous, stable structure that limits water mobility, consistent with previous reports highlighting its structure-strengthening role. While some studies suggest that fish supplementation can shorten cooking times in wheat-based systems due to the disruption of the gluten network (Desai et al., 2018; Rathlavath et al., 2024), the opposite effect observed here suggests that in a gluten-free rice/corn matrix, animal proteins and lipids likely form a denser structural complex that requires longer hydration times. Similar observations were made by Ramya et al. (2015), who reported prolonged OCT in pasta fortified with high-protein shrimp.

A notable significant increase was observed in both WAC and VI with LTF addition. At the 10% substitution level, WAC increased from 114.96% to 188.48 %, and VI increased from 12.72% to 18.18%. This increase in WAC suggests a high water-holding capacity of the trout protein within the starch matrix, potentially enhanced by the presence of xanthan gum in the formulation (Rathlavath et al., 2024; Pangestu et al., 2025). This finding aligns with that of Devi et al. (2013), who documented higher WAC in pasta containing fish mince owing to the protein's ability to retain water within the structural network. In contrast, some researchers have reported a decrease in the WAC of fish-fortified pasta, attributing it to competition between protein and starch for available water (Desai et al., 2018; Monteiro et al., 2019). The divergent result of this study (higher WAC) indicates a highly functional starch–protein interaction that facilitates superior hydration during cooking (Rathlavath et al., 2024).

CL, which reflects the amount of soluble material released during cooking, is an important indicator of the structural integrity. CL is a critical indicator of the integrity of the pasta protein matrix; high losses (>8%) generally indicate a weak structure and poor quality (Desai et al., 2018; Mathew et al., 2022). In the current study, CL increased significantly from 2.52% (control) to 3.76% (10% LTF). This rise is attributed to the inclusion of non-gluten proteins (LTF), which dilute the starch-protein network and cause the release of soluble solids into the cooking water (Monteiro et al., 2019; Rathlavath et al., 2024). This trend is widely supported by the literature;

Monteiro et al. (2019) and Desai et al. (2018) reported increased cooking losses in pasta enriched with tilapia and red cod powders, respectively. However, it is important to note that all samples remained well below the 8% acceptability limit, indicating that the LTF-enriched GFP maintains excellent structural stability during cooking. Xanthan gum also stabilizes by forming a cohesive network that reduces the diffusion of soluble components, thereby contributing to structural integrity.

The findings in Table 3 demonstrate that incorporating up to 10% LTF enhances GFP's hydration properties without compromising its structural integrity. While the increase in OCT and CL must be considered for industrial standardization, the low CL values and high WAC suggest that LTF is a technologically viable and superior ingredient for improving the functional quality of gluten-free products.

Color Characteristics of GFP Samples

Color is one of the most critical quality attributes influencing consumer acceptance and perceived “naturalness” of enriched food products (Çoban et al., 2026). The instrumental color parameters (L∗, a∗, and b∗) of the rice- and corn-based GFP samples were significantly affected by the level of LTF incorporation (Table 4). Images of GFP samples containing increasing levels of LTF are shown in Figure 2.

Table 4. Color characteristics of gluten-free pasta samples

GFP Sample	L*	a*	b*
Control	98.17±0.38^{c}	0.36±0.08^{c}	29.32±0.52^{c}
GFP-2.5	98.90±0.27^{ab}	0.53±0.08^{c}	29.77±0.15^{bc}
GFP-5	98.51±0.29^{bc}	0.80±0.18^{b}	30.04±0.84^{abc}
GFP-7.5	98.70±0.12^{b}	0.98±0.06^{ab}	30.54±0.49^{ab}
GFP-10	99.25±0.08^{a}	1.05±0.08^{a}	30.93±0.29^{a}

GFP: Gluten-free pasta, L*: lightness, a*: redness, b*: yellowness, LTF: Lyophilized Trout Flour, GFP-2.5: Gluten-free pasta containing 2.5% LTF, GFP-5: Gluten-free pasta containing 5% LTF, GFP-7.5: Gluten-free pasta containing 7.5% LTF, GFP-10: Gluten-free pasta containing 10% LTF.

Note: Differences between values shown with the same letter in the same column in the table are insignificant according to the 0.05 confidence limit.



Figure 2. Images of GFP samples containing increasing levels (0-2.5-5-7.5 and 10%) of LTF.

The L* of the GFP samples showed a significant upward trend ($p < 0.05$) with increasing LTF levels, rising from 98.17 in the control to 99.25 in the 10% LTF formulation. This brightening effect is a noteworthy finding, as it mirrors the results of Çoban et al. (2026), who also reported a lightening effect (L∗ increase) at 10% trout inclusion, attributed to the stabilizing effect of lyophilization on fish pigments and the high whiteness of the base starch matrix. This result contrasts with that of several other studies involving fish enrichment. For instance, Monteiro et al. (2019), Desai et al. (2018), and Pangestu et al. (2025) reported a darkening effect (a decrease in L) when using tilapia, red cod, and tuna flour, respectively. The high L∗ values in the current study suggest that -and-corn base combined with the lyophilization process effectively maintains a bright, appealing appearance, avoiding the grayish or brownish hues often associated with fish-derived ingredients. This phenomenon can be explained by the more homogeneous distribution of pigments at higher concentrations and the stabilizing effect of lyophilization, which helps preserve the natural color of the ingredients. Interestingly, the

impact of lyophilization on lightness appears to be highly dependent on the food matrix and its physical state. In a study on avocado pulp, Dal-Bó & Freire (2022) observed that, while sliced samples became darker (L* decreased) due to the Maillard reaction at higher heating temperatures, paste samples showed a significant increase in L* values, appearing lighter than the fresh fruit. The brightening observed in our GFP samples (which were processed as a homogeneous dough matrix) aligns with the behavior of avocado paste, suggesting that the homogenization and lyophilization of the trout flour matrix effectively distributed pigments and maintained a high reflectance, thereby avoiding the darkening often observed in non-lyophilized fish enrichment studies (Desai et al., 2018; Monteiro et al., 2019). Furthermore, Aínsa et al. (2021a) noted that the inclusion of yellow corn flour can give gluten-free pasta a color similar to that of traditional, commercial pasta.

A significant increase ($p < 0.05$) was observed in both a * and b * values as LTF substitution increased. The $a*$ values increased from 0.36 to 1.05, whereas the $b*$ values increased from 29.32 to 30.93 at the 10% substitution level. The increase in redness is directly linked to the natural pigments (such as myoglobin and hemoglobin) found in the fish muscle, which are transferred to the pasta matrix during mixing. This trend is consistent with the findings of Monteiro et al. (2019) and Pangestu et al. (2025), who reported that fish flour supplementation typically shifts the color profile toward higher a* and b* values. The increase in $b*$ is particularly desirable in pasta products, as it is traditionally associated with the carotenoid content of high-quality durum wheat semolina (Monteiro et al., 2019; Pangestu et al., 2025). In gluten-free systems, which often lack these pigments, the addition of LTF helps to achieve a vivid yellow-amber hue. This outcome aligns with Aínsa et al. (2021a), who emphasized that selecting ingredients that enhance yellowness can make the GFP more visually similar to conventional commercial pasta.

Overall, the results demonstrate that incorporating trout flour decisively influences the color development of gluten-free pasta, increasing lightness and yellowness while moderately enhancing redness. The total color difference (ΔE) resulting from these changes (calculated from the control) confirmed that the modifications were visually perceptible to consumers. However, unlike the grayish or “whole-wheat” appearance reported in other fish-enriched pasta studies, the LTF-enriched GFP maintained an extremely high lightness and a controlled yellowish tone. This indicates that lyophilized trout flour is a superior ingredient for nutritional fortification, as it enhances the protein profile without the common sensory barrier of an unattractive dark color.

Textural Characteristics of Dried-Uncooked GFP Samples

The hardness and brittleness values of the GFP samples are listed in Table 5. The textural integrity of dried pasta is a critical factor in determining its resistance to mechanical stress during packaging, transportation, and storage. The instrumental evaluation of the hardness and brittleness of dried, uncooked rice- and corn-based GFP samples revealed distinct trends influenced by LTF incorporation level.

Table 5. Hardness and brittleness of dried-uncooked GFP samples

GFP Sample	Hardness (g)	Brittleness (mm)
Control	77.11±0.05^{a}	33.75±0.30^{a}
GFP-2.5	74.08±0.08^{b}	33.87±0.82^{a}
GFP-5	66.79±0.06^{c}	33.96±0.11^{a}
GFP-7.5	65.47±0.02^{d}	34.48±0.74^{a}
GFP-10	62.29±0.03^{e}	34.53±0.10^{a}

GFP: Gluten-free pasta, LTF: Lyophilized Trout Flour, GFP-2.5: Gluten-free pasta containing 2.5% LTF, GFP-5: Gluten-free pasta containing 5% LTF, GFP-7.5: Gluten-free pasta containing 7.5% LTF, GFP-10: Gluten-free pasta containing 10% LTF.

Note: Differences between values shown with the same letter in the same column in the table are insignificant according to the 0.05 confidence limit.

The hardness of the dried uncooked GFP samples decreased significantly ($p < 0.05$) as the LTF substitution level increased, decreasing from 77.11 g in the control to 62.29 g in the 10% LTF formulation. This downward trend indicates that replacing starchy components (rice and corn flour) with animal-derived protein (LTF) resulted in a less compact, softer dry matrix. This finding is consistent with the observations of Çoban et al. (2026), who noted that supplementation with trout powder in gluten-free systems tends to yield a softer protein matrix than in starch-heavy controls. In gluten-free formulations, the absence of a strong gluten network means

that the structural density relies heavily on starch-hydrocolloid interactions. The inclusion of LTF, which contains myofibrillar proteins and lipids, likely interferes with the continuity of the starch-xanthan gum matrix, thereby reducing the force required to break the dry strands (Aínsa et al., 2021a). These results contrast with those of studies on wheat-based pasta, where the addition of fish flour typically increases hardness by reinforcing the gluten-starch network. For instance, Desai et al. (2018) and Monteiro et al. (2019) reported higher firmness and hardness in durum wheat pasta enriched with red cod and tilapia powder, respectively. The divergence in the current study highlights that animal protein interacts differently with gluten-free starches, often leading to a dilution effect rather than a reinforcing effect.

Interestingly, despite the significant changes in hardness, the brittleness (the distance the sample deforms before breaking) of the GFP samples showed no significant difference ($p > 0.05$) among all treatments, with values ranging from 33.75 to 34.53 mm. This suggests that while the LTF-enriched samples are “softer” (less resistant to the initial force), their structural flexibility and point of fracture remain stable. This stability in brittleness can be attributed to the functional role of xanthan gum used in the formulation. As noted by Aínsa et al. (2021a) and Pangestu et al. (2025), hydrocolloids are essential in gluten-free systems to mimic gluten's viscoelastic properties, providing the elasticity needed to maintain pasta integrity during drying. The results in Table 5 indicate that xanthan gum effectively stabilized the structural framework, preventing the pasta from becoming overly fragile or brittle despite the high inclusion of non-starchy LTF protein.

The decrease in dry hardness, combined with stable brittleness, indicates that LTF can be successfully integrated into GFP at 10% without causing excessive fragility. Although the samples become easier to break (lower hardness), they do not become more “brittle” in a way that would lead to increased breakage during industrial handling. This confirms that the combination of rice/corn starch, xanthan gum, and the stabilizing effect of lyophilized trout protein provides a technologically viable matrix for functional gluten-free products.

Textural Characteristics of Cooked GFP Samples

The textural profile of cooked pasta, including hardness, adhesiveness, springiness, cohesiveness, gumminess, chewiness, and resilience, is a primary quality indicator that dictates mouthfeel and consumer satisfaction. The incorporation of LTF into rice- and corn-based GFP significantly altered their cooked texture (Table 6).

Table 6. TPA properties of GFP samples

GFP Sample	Hardness (g)	Adhesiveness (g)	Springiness	Gumminess	Chewiness	Cohesiveness
Control	775.86±0.26^{a}	-1.00±0.14^{a}	0.98±0.00^{a}	51.55±1.06^{a}	20.52±0.84^{b}	0.07±0.00^{b}
GFP-2.5	626.20±0.68^{b}	-2.28±0.23^{b}	0.97±0.00^{a}	48.80±0.01^{b}	37.85±1.23^{a}	0.06±0.00^{c}
GFP-5	607.88±0.58^{c}	-5.23±1.17^{c}	0.97±0.01^{a}	41.37±1.10^{c}	38.86±0.92^{a}	0.05±0.00^{d}
GFP-7.5	596.94±0.67^{d}	-8.56±0.30^{d}	0.88±0.01^{b}	39.37±0.60^{c}	38.93±0.36^{a}	0.10±0.00^{a}
GFP-10	544.13±0.60^{e}	-10.08±0.85^{e}	0.86±0.01^{c}	35.63±1.72^{d}	39.32±0.46^{a}	0.10±0.00^{a}

GFP: Gluten-free pasta, LTF: Lyophilized Trout Flour, GFP-2.5: Gluten-free pasta containing 2.5% LTF, GFP-5: Gluten-free pasta containing 5% LTF, GFP-7.5: Gluten-free pasta containing 7.5% LTF, GFP-10: Gluten-free pasta containing 10% LTF.

Note: Differences between values shown with the same letter in the same column in the table are insignificant according to the 0.05 confidence limit.

A statistically significant decrease in the hardness of the green-flesh peach (GFP) was observed with the addition of LTF ($p<0.05$). The highest hardness value was 775.86 g in the control sample, while the lowest was 544.13 g in the GFP-10 sample. This trend indicates a softening of the pasta matrix upon inclusion of fish protein. LTF cannot form a gluten-like elastic network structure despite its high protein content, and it weakens the starch-protein interaction. This finding is consistent with that of Çoban et al. (2026), who reported that supplementation with trout powder in gluten-free systems yielded a softer protein matrix than in starch-only controls. Similarly, Aínsa et al. (2021a) observed that gluten-free pasta typically exhibits lower hardness than traditional wheat-based pasta. In gluten-free systems, the absence of a strong gluten network means that the structural integrity relies on the starch-hydrocolloid matrix. The addition of LTF likely interferes with the continuity of the starch-xanthan gum network, leading to a dilution effect rather than reinforcement. This behavior differs from that observed in wheat-based systems, where Desai et al. (2018) and Monteiro et al. (2019) found that fish

flour often increases the cooked hardness by reinforcing the gluten-starch complex. The divergence observed in the current study highlights that animal proteins interact differently with gluten-free starches, often acting as physical barriers that weaken the overall structure.

Adhesiveness (the work required to overcome attractive forces between the pasta and probe) increased significantly ($p < 0.05$), becoming more negative from -1.00 g (control) to -10.08 g (10% LTF). This indicates that the pasta became stickier as the LTF content increased. This situation is attributed to the soluble protein and lipid components in ltf, which form a sticky structure on the surface of GFP. Increased adhesiveness in fish-enriched GFP was also documented by Aínsa et al. (2021a), who attributed this change to the release of soluble solids and the presence of surface-active proteins and lipids from the fish concentrate that may have adhered to the probe during compression. Similar trends were reported by Dib et al. (2018), who found that ingredient enrichment increased adhesiveness values.

Springiness, which reflects a food sample's ability to recover its height after the first compression cycle, is a vital indicator of the protein-starch matrix's elasticity and structural integrity. The springiness values of the cooked GFP samples showed no significant difference at low . However, at 7.5% and 10% substitution levels, a significant reduction in springiness was observed ($p < 0.05$). This suggests that at low concentrations, trout proteins and lipids can be integrated into the rice and corn starch matrices without disrupting the elastic properties provided by xanthan gum. In contrast, the decrease in springiness at high addition levels is consistent with the findings of Aínsa et al. (2021a), who reported that enriched gluten-free pasta is generally characterized by lower springiness than traditional wheat-based pasta due to the lack of a cohesive gluten network. Similarly, Monteiro et al. (2019) observed that the addition of tilapia waste flour reduced springiness, cohesiveness, and resilience. The reduction in elasticity at higher LTF levels can be attributed to the interference of myofibrillar proteins and fish lipids with the formation of a continuous starch-hydrocolloid network. In gluten-free systems, the structural framework relies heavily on starch gelatinization and the binding effect of gum. High concentrations of animal-derived ingredients may act as physical barriers, weakening the three-dimensional network and leading to a subsequent loss of elastic recovery (Aínsa et al., 2021a). This trend contrasts with some studies on buckwheat-based systems (Çoban et al., 2026), where fish protein was reported to slightly increase springiness, highlighting that the impact of fish flour is highly dependent on the base flour matrix (rice/corn vs. buckwheat) and the specific interactions between ingredients.

The gumminess values decreased significantly from 51.55 to 35.63, reflecting an overall reduction in hardness and structural density. In contrast, chewiness exhibited a significant upward trend, increasing from 20.52 in the control to 39.32 in the 10% LTF sample. This suggests that while pasta is easier to compress initially (lower hardness), the interaction between trout proteins and the xanthan-stabilized starch matrix requires more energy to disintegrate before swallowing. This finding is unique because Aínsa et al. (2021a) reported lower chewiness in their fish-enriched GF samples. However, the presence of xanthan gum in our formulation likely provided a stabilizing effect, maintaining a certain level of masticatory resistance even as the peak force (hardness) decreased. Furthermore, cohesiveness showed slight but significant increases at 7.5% and 10% LTF.

The TPA results demonstrated that LTF effectively modified the cooked texture of GFP, producing a softer, more adhesive, and chewier product. While the reduction in hardness and increase in stickiness are common challenges in fish-enriched products, the low inclusion levels (2.5% to 5%) maintained a textural profile relatively close to that of the control. The successful application of lyophilization and xanthan gum helps maintain structural resilience, making LTF-enriched GFP a technologically viable alternative for providing high-quality animal proteins in a gluten-free format.

Sensory Characteristics of GFP Samples

Sensory quality is the ultimate determinant of consumer acceptance of fortified food products. The sensory profiles of the rice- and corn-based GFP samples, including color, odor, appearance, stickiness, taste, and overall acceptability, were evaluated to determine the optimal level of LTF incorporation (Figure 3).

A general decrease in sensory parameters of the GFP samples was observed compared with the control group. The sensory scores for characteristic color and homogeneity in Figure 3 align with the instrumental color data (L*, a*, and b*) discussed previously. The panelists preferred the bright, slightly yellow-amber appearance of the LTF-enriched samples. This preference is consistent with the observations of Aínsa et al. (2021a), who noted that consumer acceptance is higher when gluten-free pasta visually mimics the golden-yellow color of traditional durum wheat pasta. Unlike tuna flour, which can impart grayish or brownish hues (Monteiro et al., 2019; Pangestu et al., 2025), the pigment stabilization achieved through lyophilization ensured a vibrant, homogeneous visual profile (Çoban et al., 2026).

The “fishy odor” is widely recognized in the literature as the most significant barrier to the acceptance of fish-enriched pasta (Rathlavath et al., 2024; Pangestu et al., 2025;Çoban et al., 2026). In the current study, as the LTF ratio increased, the fishy smell, taste, and aroma became more pronounced, particularly at the 7.5% and 10% addition levels, negatively affecting consumer preference. Similarly, Monteiro et al. (2018) and Aínsa et al.

(2021b) reported that the typical seafood aroma in bread and pasta containing fish protein reduced overall acceptability of the products. Furthermore, Hayıt et al. (2023) reported relatively low sensory scores for gluten-free pasta, suggesting inherent limitations in consumer acceptance of such products. In contrast, Akın (2024) demonstrated that formulations containing xanthan gum can improve sensory characteristics, particularly structure-related attributes such as texture and chewiness. Recently, Musika et al. (2024) reported relatively high sensory scores but emphasized that increasing hydrocolloid levels may adversely affect overall acceptability, suggesting the importance of formulation balance. Çoban et al. (2026) reported that while color and structural attributes remain relatively stable, higher inclusion levels may lead to increased perception of fish odor, thereby reducing sensory acceptance. This finding supports the general observation that aroma-related attributes are the most sensitive parameters in protein-enriched, gluten-free systems.

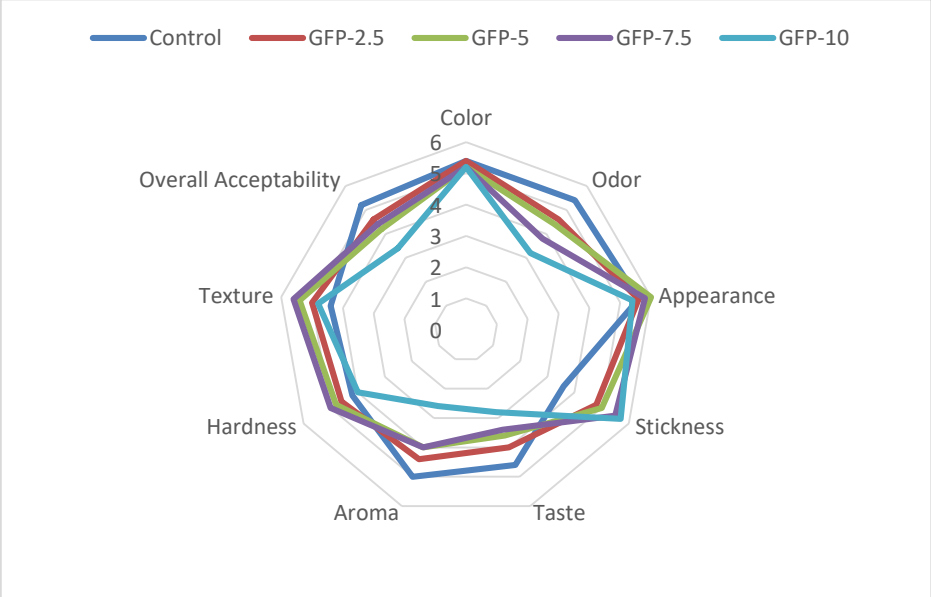


Figure 3. Sensory characteristics of GFP samples.

GFP: Gluten-free pasta, LTF: Lyophilized Trout Flour, GFP-2.5: Gluten-free pasta containing 2.5% LTF, GFP-5: Gluten-free pasta containing 5% LTF, GFP-7.5: Gluten-free pasta containing 7.5% LTF, GFP-10: Gluten-free pasta containing 10% LTF.

As observed in the instrumental TPA results, the stickiness of the GFPs increased with increasing LTF content. Sensory perception of hardness and texture decreased as LTF content increased to 10%. This sensory feedback aligns with instrumental TPA findings, which show that higher fish protein inclusion resulted in a softer, less springy matrix. Aínsa et al. (2021a) and Rathlavath et al. (2024) observed that the dilution of the starch-hydrocolloid network by animal proteins typically results in a softer mouthfeel. However, the scores in Figure 3 indicate that the mouthfeel remained within the “acceptable” range, likely due to the stabilizing effect of xanthan gum, which helps maintain structural integrity during mastication.

The primary barrier to fish-enriched products is the “fishy odor.” In this study, vinegar pretreatment and lyophilization were used to neutralize these components. This methodology is strongly supported by Çoban et al. (2026), who demonstrated that vinegar-based deodorization effectively traps volatile amines. While Damat et al. (2024) and Pangestu et al. (2025) observed lower sensory scores for aroma and taste in products without such intensive pretreatment, the results in Table 2 and Figure 3 confirm that LTF levels of up to 7.5% remain highly acceptable.

In conclusion, LTF-enriched GFP is a nutritionally superior and sensory-acceptable functional food, offering a viable dietary alternative for individuals with gluten sensitivity.

CONCLUSIONS

This study developed rice- and corn flour–based GFP fortified with xanthan gum and LTF to address technological weaknesses (loss of gluten structure) and nutritional limitations (low protein) common in gluten-free products. LTF significantly improved nutritional/chemical attributes: moisture decreased and aw fell, supporting better shelf stability, while protein, ash, and total dietary fiber increased. Technologically, LTF increased OCT, and strongly increased hydration-related metrics (WAC 114.96% to 188.48%; VI 12.72% to 18.18%), indicating enhanced water holding within the starch–protein–xanthan matrix; CL also increased but remained far below the commonly cited 8% quality threshold, indicating preserved cooking integrity.

Instrumental color shifted in a consumer-relevant direction: L* increased, with modest rises in a* and b*, yielding a brighter, more yellow-amber appearance without the darkening often associated with fish ingredients, attributed to lyophilization and matrix effects. Texture results showed progressive softening: dry pasta breaking strength decreased while brittleness (deformation at fracture) did not change significantly, suggesting reduced rigidity without added fragility, likely stabilized by xanthan gum. In cooked pasta, hardness decreased and gumminess declined, whereas adhesiveness increased markedly; springiness dropped at higher inclusions (7.5–10%); cohesiveness slightly increased at 7.5–10%; and chewiness increased, producing a softer yet stickier and more masticatory product. Sensory evaluation broadly mirrored instrumental findings: panelists favored the bright, slightly yellow appearance of LTF samples, but fishy odor/taste/aroma intensified with LTF level and became the principal acceptability constraint, particularly at 7.5–10%; with results indicating that formulations up to ~7.5% LTF remain highly acceptable while delivering substantial protein enrichment. Overall, the study demonstrates that combining 1% xanthan gum with controlled LTF addition can produce nutritionally enhanced, technologically stable gluten-free pasta, offering a practical route to higher-protein gluten-free staples while highlighting aroma management and inclusion level as key determinants of consumer acceptance.

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Conflicts of Interest:

The authors declare that they do not have any conflict of interest.

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