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Effect of alkaline salts and whey protein isolate on the quality of rice-maize gluten-free pasta

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ABSTRACT

The objective of this study was to investigate the effect of the addition of alkaline salts (sodium + potassium carbonates) and/or whey protein isolate on the quality of rice-maize gluten-free (GF) pasta. Different levels of incorporation, i.e. 0.5 and 1% alkaline salts, 5% whey protein isolate (WPI) and 1% alkaline salts with 5% WPI, were evaluated. The GF pasta samples were tested for surface microstructure (by Scanning Electron Microscopy), pasting properties, colour, geometrical and cooking quality (weight gain and cooking loss) and textural properties (firmness and compression-extrusion energy). Compared to the control, the alkaline salts addition significantly ($p \leq 0.05$) increased viscosity, cooking loss, redness, yellowness and dimensions, and decreased weight gain, luminosity, firmness, and total compression-extrusion energy of cooked pasta. The WPI addition significantly ($p \leq 0.05$) increased cooking loss (13–18%), luminosity and yellowness of pasta while decreasing viscosity, firmness (22–27%) and total compression-extrusion energy (15%). However, the WPI addition enhanced the nutritional quality by increasing up to 48% the protein content and, in combination with alkaline salts, produced pasta with good cooking performance. The outcomes of this research can be useful in developing healthier GF pasta without extensively penalizing the cooking performance.

1. Introduction

Dry pasta is a popular food, thanks to its long shelf life, ease of storage and simple preparation for consumption (Gao et al., 2018; Šeregelj et al., 2022). Within this product category, gluten-free (GF) pasta has become a high-demand commercial product (Phongthai, D'Amico, Schoenlechner, Homthawornchoo, & Rawdkuen, 2017), a fact attributable to the relevant number of people (1% of Europe and North America population; Udachan & Sahoo, 2017) suffering from celiac disease (CD), a syndrome triggered by an immune reaction to gluten and related species that can damage the small intestine and impede the absorption of nutrients (Phongthai et al., 2017). CD patients must follow a strict lifelong GF diet as the only treatment to achieve a remission of the symptoms, carefully avoiding the ingestion of products made from gluten-containing cereals (*Triticum* spp., rye, barley, triticale and, possibly, oats). Unfortunately, the unique visco-elastic properties of gluten are the main reason for pasta structure. After cooking, the pasta prepared from durum wheat semolina maintains good texture, resists to surface disintegration, does not release in the cooking water an excessive amount of organic matter, retains a firm structure, and is not sticky on the surface (Larrosa, Lorenzo, Zaritzky, & Califano, 2016). In an effort to emulate gluten properties, various ingredients have been used for GF pasta-making, including flour from pseudo-cereals, legumes, vegetables and fruits; however the most common ones are rice and maize (Gao et al., 2018; Lucisano, Cappa, Fongaro, & Mariotti, 2012; Mariotti, Iametti, Cappa, Rasmussen, & Lucisano, 2011) because of their abundance, low cost and high expansion capacity (Padalino, Conte, & Del Nobile, 2016; Silva, Ascheri, & Ascheri, 2016). Rice flour is highly digestible, has white colour and bland taste, and displays hypoallergenic properties (Phongthai et al., 2017); unfortunately, it has a low protein

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content and a poor ability to develop a cohesive network, impairing its technological performance (Marengo et al., 2018; Marti & Pagani, 2013). Maize flour has lower protein and dietary fibre content than wheat and rice flours but higher polyphenols concentration (Hager, Wolter, Jacob, Zannini, & Arendt, 2012).

During cooking, the pasta undergoes complex modifications induced by heating and water uptake: the starch englobed in the gluten network gelatinizes and the amylose partially leaches into the cooking water, while protein coagulation improves the structuring of the product (De Noni & Pagani, 2010; Lucisano et al., 2012). When the gluten network is absent, as in GF pasta, an extensive leaking of starch may occur, and the pasta can disintegrate, or other defects can appear. For instance, Gao et al. (2018) reported a less cohesive and more extensible texture of GF pasta when compared with durum wheat pasta. Some progress has been achieved, but replacing the gluten network and attaining high quality gluten-free pasta is still a major technological challenge (Larrosa et al., 2016; Phongthai et al., 2017). To solve this conundrum, two main approaches are pursued: i) to choose appropriate processing conditions able to create a new and efficient arrangement of starch components in the final product (Mariotti et al., 2011), such as the extrusion-cooking technology (Bouasla, Wójtowicz, & Zidoune, 2017; Marti & Pagani, 2013); ii) to select appropriate ingredients and/or additives (e.g. modified starches, gums, emulsifiers, proteins, and enzymes) suitable for inducing a cohesive structure that overcomes the absence of gluten (Chillo, Laverse, Falcone, & Del Nobile, 2007; Mariotti et al., 2011; Sozer, 2009; Yalcin & Basman, 2008). For instance, the use of egg white or whey protein is an interesting alternative for rice-based GF (Marti et al., 2014).

Whey, an economically important by-product of cheese manufacturing, is a valuable ingredient in the food industry because of its good functional attributes and nutritional value, due to the high content of essential amino acid (especially leucine, lysine and methionine), vitamins and minerals (de Almeida Marques, de São José, Silva, & da Silva, 2016). A derivative, whey protein isolate (WPI), mainly prepared by ion-exchange processes, has 90% of the total proteins of whey, and minimal amounts of lactose, lipids and ash and its addition improves taste and texture of the foods (Blažić, Zavadlav, Kralj, & Šarić, 2018).

In noodles manufacturing, the alkaline reagents used are typically a mixture of sodium and potassium carbonates or sodium hydroxide, although sometimes calcium carbonate or sodium/potassium phosphates, with or without sodium chloride, are also employed (Wu, Beta, & Corke, 2006). The addition of alkali mixtures leads to competition between wheat starch and proteins for the available water, retarding the hydration of the proteins and the development of the matrix. Furthermore, growing salt concentration should compact the electrical double layer, decreasing proteins repulsion; this phenomenon, however, would be limited by drastic pH changes due to the alkali addition, that charge the proteins augmenting their repulsive forces (Wu et al., 2006). Alkaline-wheat flour interactions strengthen dough texture, reduce dough development time, improve firmness and chewiness of noodles, delay starch gelatinization, increase starch paste viscosity, inhibit enzyme activity and enzymatic browning and also contribute to improve yellow colour, aroma and flavour (Wang et al., 2018).

Therefore, the aim of this study was to investigate whether the addition of alkaline salts and/or whey protein isolate can positively affect the overall quality of GF raw and cooked pasta prepared from a rice-maize blend.

2. Materials and methods

2.1. Ingredients

Rice and maize flours were provided in 2020 by Bio Aglut company (Constantine, Algeria). According to label information, the flours were obtained by dry milling of Basmati long rice and yellow maize kernels, respectively. The alkaline salts mix (sodium carbonate and potassium carbonate, 9:1 w/w) was prepared from pure salts provided by INATAA (Brothers Mentouri Constantine 1 University, Constantine, Algeria), while the whey proteins isolate (WPI) was purchased from PINK SUN (Burn, UK).

2.2. Pasta production

GF pasta samples were prepared from a blend of rice and maize (2:1, w/w). In order to gelatinize starch and create an alternative protein-to-starch structure in the final dough, the flours underwent a thermal extrusion process. About 14 kg of basic formulation and enough water to reach 40% moisture were mixed and extruded with a single-screw PROGEL®plant (Braibanti, Milan, Italy), under the following parameters: extrusion pressure 10 bar, extrusion chamber temperature 130 °C, pellet temperature 85–90 °C, pellet length 5 mm. Afterward, the pasta (macaroni shape) was prepared using a small-scale industrial pilot plant (Mac30, Italpast, Parma, Italy) equipped with a pre-kneading tank, a kneading tank, a vacuum-pressurized extrusion cylinder (thermostated at 20 °C) and a Teflon die.

The control pasta (P1) was made with 3 kg of pellet and sufficient water to reach a 40% dough moisture; after mixing for 12–15 min, the dough was extruded under vacuum (−0.9 bar) at 65–70 bar pressure. The other samples (P2–P4) were prepared by replacing, during dough mixing, part of the basic formulation with the alkaline salts mix and/or WPI to obtain pasta with 0.5% alkaline salts mix (P2), 1% alkaline salts mix (P3), or 5% WPI (P4). Instead, the GF pasta with 1% alkaline salts (P5) was prepared from pellets already containing the alkaline salts to investigate the potential effect of the different pasta making process. Finally, a combination of 1% alkaline salts in pellets and 5% WPI (P6) was also tested (Table 1). All the pasta samples were dried in an experimental drying chamber (Braibanti, Milan, Italy) for 18 h at 75% relative humidity and 60 °C maximum peak temperature. Finally, the samples were stored in plastic bags at room temperature until analysis.

For chemical analyses and visco-amylographic evaluation, the raw pasta samples were ground at 18000 rpm for 20 s with a Heavy-Duty Blender (Waring Commercial, Stamford, CT, USA) equipped with a blade for dusty materials, and sieved to exclude particles larger than 1 mm.

2.3. Chemical composition

The moisture (g/100 g) of ingredients and raw pasta samples was determined according to the gravimetric method 44–15.02 (AACC International, 1999), while their protein content (g/100) was evaluated following the Kjeldahl method 979.09 (AOAC International, 2000), using a nitrogen to protein conversion factor of 6.25 (992.23; AOAC International, 2000). To determine the thermal impact of the pasta making process, furosine content (mg furosine/100 g protein) of all the samples was evaluated by High Performance Liquid Chromatography (HPLC), as described by Hidalgo and Brandolini (2011). The analyses were performed in triplicate.

2.4. Pasting properties

To investigate the severity of the pasta making process, the pasting properties were assessed on ground raw pasta samples according to Mariotti et al. (2011) with a Rapid Visco Analyzer (RVA; Newport Scientific Pty. Ltd., Warriewood, Australia). The ground pasta (4 g adjusted to 14 g/100 g sample moisture) was dispersed in an aluminium canister with distilled water (25 g). With constant stirring, the sample-water suspension was held at 50 °C for 1 min, heated to 95 °C over 3 min 42 s, maintained at 95 °C for 2 min 30 s, progressively cooled to 50 °C over 3 min 48 s and held at 50 °C for 2 min. The parameters extrapolated from the resulting curves were peak viscosity (PV, cP), breakdown (BD, cP), final viscosity (FV, cP), setback (SB, cP), peak time (PT, s) and pasting temperature (°C). For each sample, at least two measurements

Table 1
Pasta ingredients at each production step, and sample code explanation. WPI: Whey Protein Isolate.

Flours pregelatinisation in PROGEL® plant				Pasta production in pilot plant				Pasta drying	
1st mixing and pellet extrusion				2nd mixing and pasta extrusion					
Rice/maize (kg)	Water (%)	Other	Output	Pellet (kg)	Water (%)	Other	Output	Output	Code
14	up to 40	none	Pellet	3	up to 40	none	Fresh pasta	Dry pasta	P1
14	up to 40	none	Pellet	3	up to 40	alkaline salts (0.5%)	Fresh pasta	Dry pasta	P2
14	up to 40	none	Pellet	3	up to 40	alkaline salts (1%)	Fresh pasta	Dry pasta	P3
14	up to 40	none	Pellet	3	up to 40	WPI (5%)	Fresh pasta	Dry pasta	P4
14	up to 40	alkaline salts (1%)	Pellet	3	up to 40	none	Fresh pasta	Dry pasta	P5
14	up to 40	alkaline salts (1%)	Pellet	3	up to 40	WPI (5%)	Fresh pasta	Dry pasta	P6

were performed.

2.5. Surface structure

The surface structure of the raw pasta was investigated by Scanning Electron Microscopy (SEM) with a Hitachi TM 3000 Benchtop SEM instrument operating at 15 kV acceleration voltage, equipped with a SwiftED3000 probe (Oxford Instruments) for Energy Dispersive X-ray (EDX) microanalysis. Small pieces of raw pasta were mounted on aluminium stubs and sputter-coated with gold. Images of longitudinal pasta surface and cross-section surface at low- and high-magnification (× 30, × 150 and × 1500) were acquired.

2.6. Cooking conditions

The samples were cooked at their optimum cooking time (OCT, 8 min) in boiling distilled water, without salt addition using a pasta to water ratio of 1:10 (w:w), following Lucisano et al. (2012): briefly, four trained panellists tasted the samples every 30 s covering a cooking time from 6 to 10 min. The cooking tests were independently performed at least in triplicate.

2.7. Colour measurements

The colour of raw and cooked pasta was measured by a Minolta Chroma Meter CR-210 (Minolta, Osaka, Japan) equipped with a standard illuminant C and calibrated with a standard-white reflector plate (Y = 87.7; x = 0.308; y = 0.315). For each sample, one reading was recorded on the central surface of ten randomly-selected macaroni. The results are expressed in the CIELAB space parameters L^* (lightness; 0 = black, 100 = white), a^* ($+a^*$ = redness, $-a^*$ = greenness) and b^* ($+b^*$ = yellowness, $-b^*$ = blueness) values. To evaluate the effect of alkaline salts and WPI on sample colour in comparison to the control, ΔE values were computed by the following equation (Eq. (1)):

$$\Delta E = \sqrt{\Delta L^2 + \Delta a^2 + \Delta b^2} \quad \text{(Eq. 1)}$$

2.8. Geometrical features

The geometrical features of raw and cooked pasta were determined by Image Analysis on ten randomly-selected macaroni. The samples (longitudinal- or cross-sections; Fig. 1) were placed on a flatbed scanner (Epson Perfection 3170 Photo, Seiko Epson Corp., Japan) and covered with a black box to amplify the contrast between objects and background. The images were captured at 600 dpi resolution, saved in TIFF format and processed with a dedicated software (Image Pro-Plus v. 4.5.1.29, Media Cybernetics Inc, Rockville, USA). The data recorded were longitudinal length (mm), width (mm) and surface area (mm^2), while the crown area (mm^2) was determined on the cross-section as the difference between total cross-section area and hole area (Fig. 1). The geometrical dimensions increase after cooking was expressed as percentage of the raw pasta dimensions.

2.9. Cooking quality

The weight gain (WG, %), due to water absorption of the pasta during cooking, was evaluated by weighing the samples before cooking, and after cooking and draining for 1.5 min. The results are the average of at least three determinations. Cooking loss (g/100 g DM) was evaluated as dry matter released in the cooking water (dried at 105 °C to constant weight) compared to the raw product (Method 66–50.01, AACC International, 1999). The results are the average of four measurements.

2.10. Texture

Macaroni texture was assessed with a TA-HD plus Texture Analyzer (Stable Micro Systems, Surrey, UK) equipped with a 10-blade Kramer shear cell and a 250-kg load cell (Cappa et al., 2021). The Texture Exponent TEE32 V 3.0.4.0 software (Stable Micro System, Surrey, UK) was used to control the instrument and for data acquisition. The samples were cooked at their OCT and cooled in an airtight container for 25 min at room temperature, because the temperature can affect texture

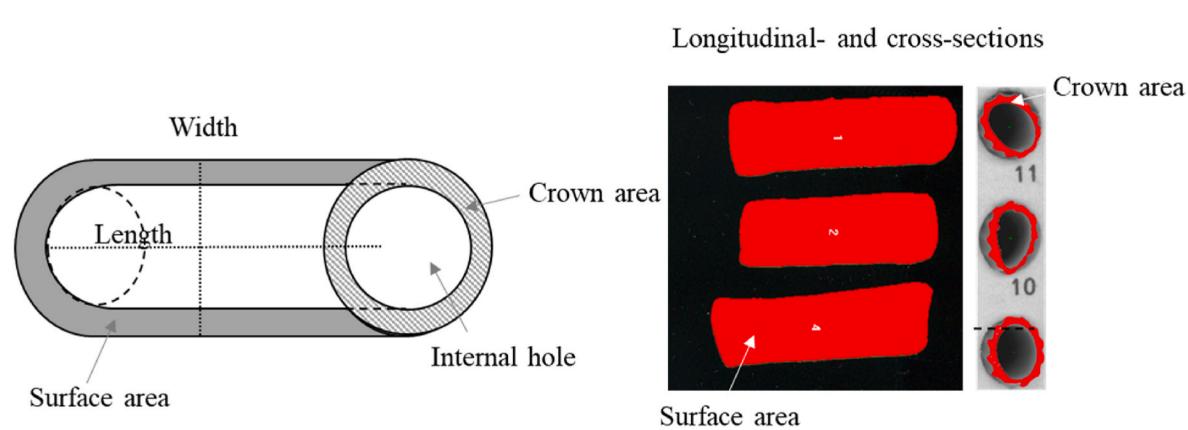

Fig. 1. Scheme of macaroni-shape pasta and geometric evaluation through image analysis.

properties. Aliquots of cooked pasta (30–35 g) were compressed, sheared, and extruded through the bottom openings of the Kramer cell by the blades moving at 1 mm/s speed, simulating chewing. Firmness (N; the maximum force necessary to shear and extrude the sample) and energy (mJ; the area under the compression/extrusion curve) were extrapolated from the stress–deformation curve as indices of the product hardness. Six replicates for each sample were performed.

2.11. Statistical analysis

The results are reported as mean ± standard deviation. To evaluate the effect of the different formulations, One-way Analysis of Variance (ANOVA) was performed considering the type of mixture as factor. When significant differences were found, Fisher's Least Significant Differences (LSD) test at 95% significance was computed. All analyses were performed using the STATGRAPHICS® Centurion statistical program (StatPoint Inc., Warrenton, USA).

3. Results and discussion

3.1. Chemical composition

The moisture, protein and furosine content of ingredients and GF pasta samples are shown in Table 2. The moisture content of the ingredients was inferior to 10.0 g/100 g, while that of the GF pastas ranged between 10.96 and 11.57 g/100 g; all the values were below the threshold (<12%) that ensures good preservation during storage at room temperature (Codex Alimentarius, 1995). The protein content of rice and maize flour was low, while that of WPI, as expected, was far higher (>10-fold). Accordingly, the protein concentration of GF pasta increased significantly ($p \leq 0.05$) after the addition of WPI, reaching 10.63% and 10.96% for pasta with 5% WPI and for pasta with 5% WPI and 1% of alkaline salts, respectively.

Furosine, a molecular marker of the damage caused by heat treatment (Brandolini, Lucisano, Mariotti, & Hidalgo, 2018), is produced by acid hydrolysis of the Amadori compounds formed during the Maillard reaction between reducing sugars and proteins (Gallegos-Infante et al., 2010). No furosine was detected in rice flour, as already observed by Betrouche et al. (2022), and only low values were found in maize flour (9.12 mg/100 g protein). On the other hand, the furosine content of WPI was 181.14 mg/100 g protein, a result attributable to the heat treatment applied during whey dehydration. Therefore, although the control and the alkaline salt enriched raw pastas showed furosine contents significantly superior ($p \leq 0.05$) to the WPI because of the Maillard reactions occurring during pasta drying, the GF pastas with WPI reached the highest levels ($p \leq 0.05$) for their superior content of proteins, one of the main reactants of the Maillard reaction (De Noni & Pagani, 2010). In fact, the control and alkaline salt enriched pastas values were within the variation reported in literature, and only the WPI-enriched pasta showed a furosine content superior to those reported for durum wheat and GF pasta (19–562 mg/100 g protein) (Betrouche et al., 2022; De Noni & Pagani, 2010; Gasparre, Betoret, & Rosell, 2019; Giannetti, Mariani, Mannino, & Testani, 2014; Hidalgo et al., 2020; Marti et al., 2014; Ćetković et al., 2022).

Table 2
Moisture, protein and furosine content (expressed on wet basis; mean ± standard deviation) of ingredients and raw pasta samples. P1, control pasta; P2, pasta with 0.5% alkaline salts; P3, pasta with 1% alkaline salts; P4, pasta with 5% whey protein isolate; P5, pasta from pellets containing 1% alkaline salts; P6, pasta from pellets containing 1% alkaline salts +5% whey protein isolate.

	Moisture (g/100 g)	Protein (g/100 g)	Furosine (mg/100 g protein)
Raw materials			
Rice flour	6.66 ± 0.11	7.57 ± 0.31	nd
Maize flour	8.98 ± 0.12	7.18 ± 0.74	9.1 ± 1.0
Whey protein isolate	5.53 ± 0.04	86.80 ± 2.47	181.1 ± 5.4
Pasta samples			
P1	11.10^{c} ± 0.01	7.40^{b} ± 0.06	264.7^{de} ± 12.5
P2	11.25^{b} ± 0.04	7.46^{b} ± 0.09	359.6^{d} ± 24.3
P3	11.57^{a} ± 0.04	7.00^{b} ± 0.07	523.9^{c} ± 23.3
P4	10.95^{d} ± 0.08	10.63^{a} ± 0.85	809.0^{b} ± 55.4
P5	10.96^{d} ± 0.05	7.70^{b} ± 0.03	221.7^{e} ± 20.9
P6	10.97^{d} ± 0.01	10.96^{a} ± 0.03	942.3^{a} ± 83.7

nd, not detectable (i.e., below the detection limit). Different letters in each column indicate significant differences ($p \leq 0.05$) among pasta samples.

3.2. Pasting properties

The pasting properties were evaluated on the flours obtained from the milling of the different GF pasta samples, because the starch gelatinization and retrogradation phenomena in the course of the heating and cooling cycles may reflect molecular changes during the technological process and are related to the ingredients used (Mariotti et al., 2011). The pasting properties of GF pasta are summarised in Table 3. The addition of alkaline salts significantly ($p \leq 0.05$) increased peak viscosity, final viscosity, breakdown and setback of GF pasta, probably because of changes in starch granules swelling and deformation during gelatinization (Rafiq, Singh, & Saxena, 2016; Tao et al., 2019). In particular, the higher peak viscosity suggests that the alkali salts promoted the swelling of the starch granules before their rupture, leading to the formation of a more viscous system; the high breakdown indicates that the alkaline conditions also favoured the collapse of the granular structure, leading to a general weakening (Lai, Karim, Norziah, & Seow, 2004; Wang et al., 2014). These results agree with those reported by Fan, Ai, Chen, Fu, and Bian (2018) and by Tao et al. (2019) for wheat-based yellow alkaline noodles and by Li, Sun, Han, Chen, and Tang (2018) for whole wheat-based alkaline noodles, suggesting that the alkaline salts modify the gelatinization behaviour of starch granules from different species (i.e., wheat, rice and maize). Furthermore, in porous polymers a portion of the solvent participates in saturating the porosity, while another portion permeates the inner structure of the polymer (Krasucka, Mergo, Wójcik, & Goworek, 2018); thus, the alkali salts may lead to the disruption of the amorphous regions within the starch granules, decreasing the inhibitory impact of amylose and enabling a more expansive swelling of the granules. Additionally, the existence of negative charges on starch molecules, arising from the ionization of the hydroxyl group, leads to repulsion, ultimately facilitating greater water infiltration within the granules and enhancing their swelling capacity (Rafiq et al., 2016).

The presence of 5% WPI did not significantly ($p > 0.05$) affect peak viscosity but limited final viscosity and setback, probably because the WPI may have slowed the water uptake of individual starch granules, and consequently their gelatinization, due to a competition for the water available (Mariotti et al., 2011); a further reason may be the small dilution of starch concentration due to the protein addition. The breakdown, linked to starch ability to withstand high-temperature heating and shear stresses, was slightly superior in pasta samples with WPI compared to the control, but was much higher in the alkaline salts enriched samples due to the weakening effect discussed above. The addition of 1% alkaline salts and 5% WPI had no significant effect ($p > 0.05$) on peak viscosity, whereas it increased breakdown and decreased final viscosity, setback and pasting temperature compared to the control. Finally, the peak time was shortened after the addition of alkaline salt or/and WPI, probably because of water competition (Mariotti et al., 2011).

3.3. Surface structure

Representative images of the pasta surface and of the pasta cross-section surface are reported in Figs. 2 and 3, respectively, while

Table 3
RVA characteristics (mean ± standard deviation) of raw pasta samples. P1, control pasta; P2, pasta with 0.5% alkaline salts; P3, pasta with 1% alkaline salts; P4, pasta with 5% whey protein isolate; P5, pasta from pellets containing 1% alkaline salts; P6, pasta from pellets containing 1% alkaline salts +5% whey protein isolate.

Sample	Peak viscosity (cP)	Breakdown (cP)	Final viscosity (cP)	Setback (cP)	Peak time (s)	Pasting temperature (°C)
P1	$382.5^{d} \pm 3.5$	$47.5^{d} \pm 0.7$	$1096.5^{d} \pm 7.8$	$761.5^{d} \pm 10.6$	$6.4^{a} \pm 0.1$	$95.0^{a} \pm 0.3$
P2	$784.0^{b} \pm 29.7$	$173.5^{a} \pm 4.9$	$2084.0^{b} \pm 35.4$	$1473.5^{b} \pm 10.6$	$5.7^{c} \pm 0.1$	$84.9^{c} \pm 1.5$
P3	$887.5^{a} \pm 27.6$	$189.5^{a} \pm 16.3$	$2347.5^{a} \pm 36.1$	$1649.5^{a} \pm 24.7$	$5.6^{c} \pm 0.1$	$78.7^{d} \pm 0.6$
P4	$356.5^{d} \pm 14.8$	$61.5^{cd} \pm 6.4$	$949.0^{f} \pm 24.0$	$654.0^{f} \pm 15.6$	$5.7^{c} \pm 0.1$	$92.4^{a} \pm 0.6$
P5	$530.0^{c} \pm 5.7$	$107.5^{b} \pm 2.1$	$1594.0^{c} \pm 2.8$	$1171.5^{c} \pm 4.9$	$5.9^{b} \pm 0.1$	$92.0^{ab} \pm 2.4$
P6	$389.5^{d} \pm 2.1$	$77.5^{c} \pm 9.2$	$1032.0^{e} \pm 12.7$	$720.0^{e} \pm 24.0$	$5.6^{c} \pm 0.1$	$89.2^{b} \pm 0.6$

cP: centipoise. Different letters indicate significant differences ($p \leq 0.05$) among pasta samples within the column.

number and size of surface fractures and holes are reported in Table 4. In accordance with Li et al. (2018), that focused on alkaline wheat-based noodles, rice-maize pasta samples showed visible holes on the cross-section surface. This may be caused by the rapid formation of a protein network, resulting in a less connected surface that can contribute to the increased cooking loss of pasta containing alkaline salts (Li et al., 2018). In particular, with an increase in salt content (from P1 to P3), the size of the holes decreased by 80%. The P5 showed significant ($p \leq 0.05$) smaller hole sizes compared to the control, but significant ($p \leq 0.05$) larger sizes compared to P3, which has the same salt content: therefore, not only the presence of alkaline salts but also the moment of their addition affects pasta surface porosity. The addition of proteins increased the number of holes, especially in P4, and decreased their dimensions (Table 4); additionally, the distribution of the holes was more uneven compared to the other samples. The outer surface of the pasta (Fig. 2) showed numerous fractures in all the samples because of the pasta making process, but no significant ($p > 0.05$) differences were observed among samples (Table 4). At low magnifications, the surface appeared smooth in all samples except in those containing proteins (P4 and P6), which were very rough and characterized by many fractures (Fig. 3). At higher magnifications (Fig. 2), the addition of salt from P1 to P3 led to the appearance of material separated from the rest of the matrix; the P3 surface seemed more eroded than those of P1 and P2. In P4, the presence of proteins made the pasta less homogeneous, while the addition of salt in P6 led to starch granules that were incorporated in P4 to resurface. P5 continued to resemble P1 and P2.

3.4. Colour

Pasta colour is an important quality attribute that influences consumers and that depends on ingredients, phenolic substrates, polyphenol oxidase activity, and environmental parameters such as pH and ionic strength (Cappa et al., 2021; Kumar et al., 2019; Mariotti et al., 2011), as well as processing conditions. The colour characteristics of GF raw and cooked pasta are reported in Table 5. The incorporation of alkaline salts increased a^* and b^* compared to the control pasta. The yellow hue of the alkali-containing pasta may be due to the maize flour pigments, which undergo chromophore change at alkaline pH (Fu, 2008). Contrary to the expectation, the incorporation of WPI in GF pasta significantly ($p \leq 0.05$) increased luminosity and yellowness indices of the raw pasta, but did not change a^*, compared to the control pasta. Furthermore, no relation between colour coordinates and furosine levels was observed; thus it can be assumed that the pasta samples enriched with WPI (P4 and P6) showed higher luminosity as a consequence of the intrinsic lighter colour of the WPI and because the heat damage was limited, due to the low drying temperature; indeed, the higher furosine values describe only the initial steps of the Maillard reaction, but do not lead to the darkening of the final product observed in oven products (e.g. bread) under more drastic thermal treatments (Hidalgo & Brandolini, 2011).

In general, the addition of alkaline salts or WPI significantly ($p \leq 0.05$) affected the colour of the product, as evidenced by the ΔE values (2.3–6.5), suggesting that colour differences from the control may be perceptible by the human eye (De Souza & Fernández, 2011). These results are similar to those reported for maize-based GF pasta enriched with whey powder (Ungureanu-Iuga, Dimian, & Mironeasa, 2020).

In cooked pasta, the luminosity decreased significantly ($p \leq 0.05$) with increasing alkaline salt incorporation, while a^* and b^* increased; the pre-incorporation of 1% alkaline salts, instead, decreased L^* and a^* but not b^*. On the other hand, the addition of 5% WPI led to a significant ($p \leq 0.05$) decrease in brightness (L^*) and yellowness (b^*), but not in a^*. The incorporation of 1% alkaline salts in combination with 5% WPI, instead, had no significant effect ($p > 0.05$), except for a decrease of a^*. The high ΔE values (>13; Table 5) in comparison to the raw samples indicate that cooking affected the colour of the product, mainly causing a 25–36% increase of lightness, attributable to the leaching of solid material into cooking water and the thermal damage of pigmented compounds.

3.5. Geometrical features

The geometric characteristics of raw and cooked GF pasta are shown in Table 6. Together with colour, they are an important quality indicator for the consumers, can influence the mechanical properties of raw pasta and may affect its behaviour during cooking (Cappa & Alamprese, 2017; Mariotti et al., 2011). As evident from Fig. 4, the raw samples did not show defects (e.g., cracking, white stripes and irregular shape, etc.), suggesting that the formulation and extrusion conditions applied were suitable for GF pasta. In raw pasta, the surface area varied from 330.8 mm^2 to 400.2 mm^2 and the length varied from 31.3 mm to 39.1 mm, while the width was similar. The highest values were recorded for pasta with 5% WPI (P4) and for pasta with 1% alkaline salts (P5). These geometrical differences may be partially attributable to changes in dough viscosity during the extrusion of the pasta. Changing the formulation (i.e., amount of WPI and alkaline salt) the rheological properties of the dough and shear stress inside the extruder change: the higher the viscosity of the dough inside the extruder barrel, the higher the friction and shear forces (Cappa, Masseroni, Ng, & Alamprese, 2020; Köksel, Ryu, Basman, Demiralp, & Ng, 2004).

Cooking increased the surface area from 25.3% (P5) to 46.7% (P4), while less variation was noticed for length (11.5–18.8%) and width (12.2–19.4%). The sample with WPI (P4) showed the highest length increase, that could be associate with a less-structured GF pasta (Lucisano et al., 2012). On the other hands, crown area increments, calculated as the difference among cross-section area and hole area of cooked pasta vs. raw pasta, ranged from 85% (P4) to 105% (P2), showing that WPI partially contributes to limit the pasta cross-sectional increase, somehow structuring the sample. In general, the combination of WPI and alkaline salts (P6) resulted in geometrical features not significantly different ($p > 0.05$) from the control (P1), both on raw and cooked pasta.

3.6. Cooking quality

3.6.1. Weight gain

Pasta water absorption during cooking reflects the swelling capacity of starch and protein when heated (Larrosa et al., 2016). The weight gain varied from 63.6% for pasta with 1% of alkaline salts (P5) to 75.6% for pasta with 1% of alkaline salts and 5% WPI (P6; Fig. 5). The weight gain decreased significantly ($p \leq 0.05$) with increasing rate of added alkaline

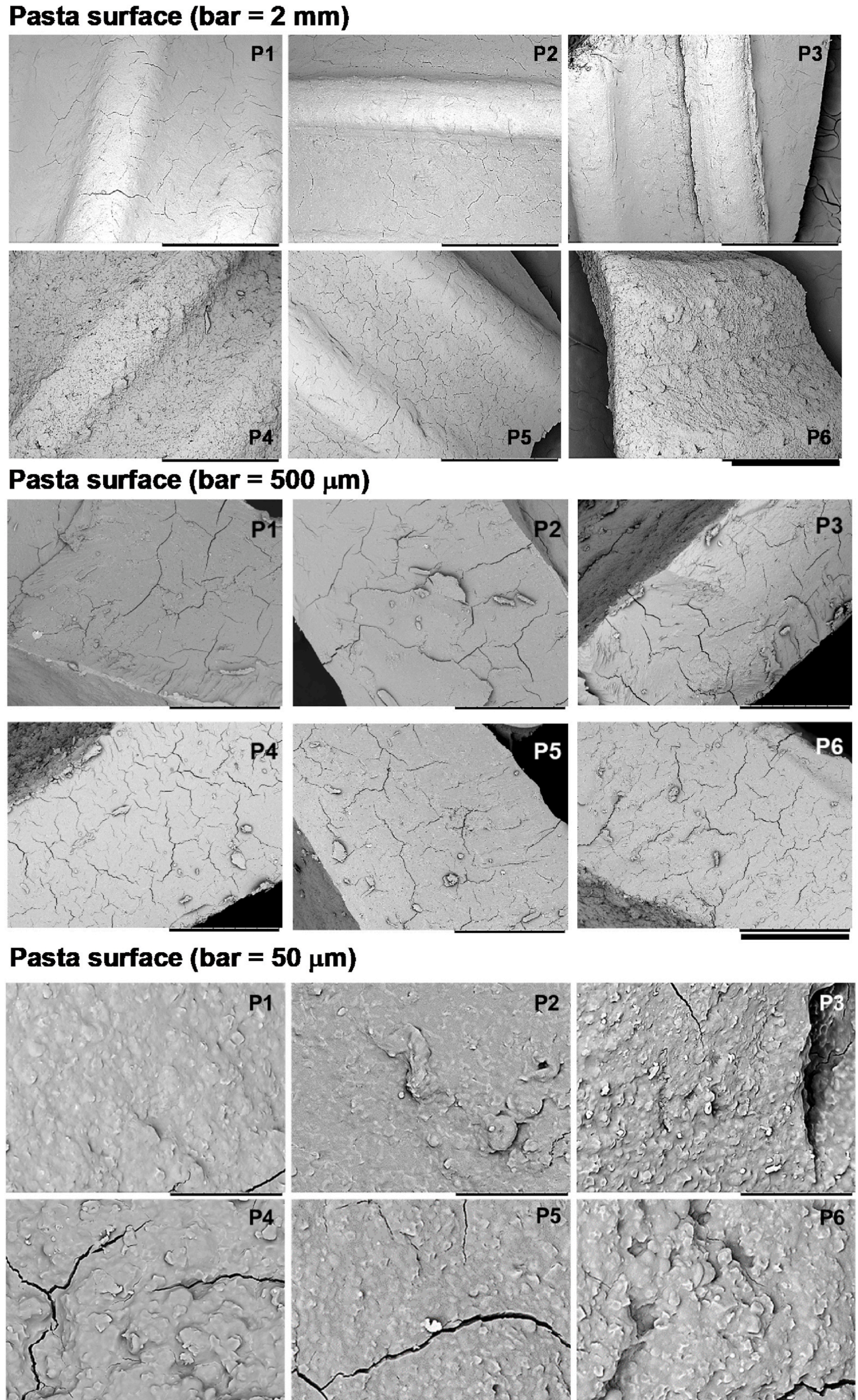

Fig. 2. Scanning Electron Microscopy images of pasta surface. P1, control pasta (rice:maize 2:1); P2, pasta with 0.5% alkaline salts; P3, pasta with 1.0% alkaline salts; P4, pasta with 5% whey protein isolate; P5, pasta from pellets containing 1% alkaline salts; P6, pasta from pellets containing 1% alkaline salts + 5.0% whey protein isolate.

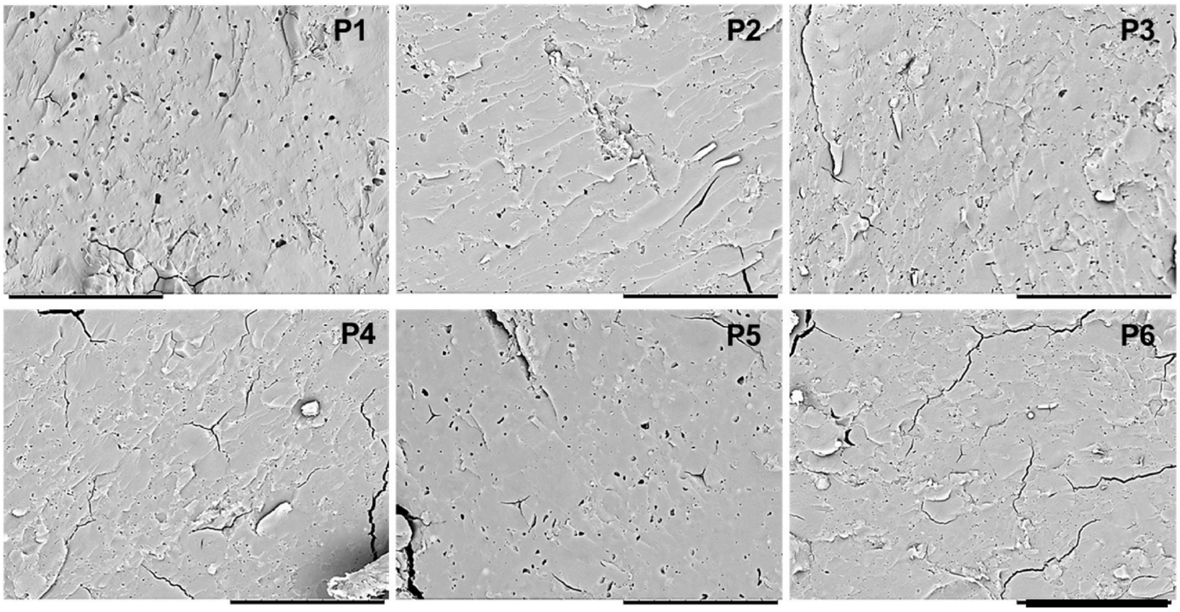

Fig. 3. Scanning Electron Microscopy images of the surface of pasta cross-section. P1, control pasta (rice:maize 2:1); P2, pasta with 0.5% alkaline salts; P3, pasta with 1.0% alkaline salts; P4, pasta with 5% whey protein isolate; P5, pasta from pellets containing 1% alkaline salts; P6, pasta from pellets containing 1% alkaline salts + 5.0% whey protein isolate.

Table 4
Number and size (mean ± standard deviation) of surface fractures (magnification x150) and of surface holes (magnification x1500). P1, control pasta; P2, pasta with 0.5% alkaline salts; P3, pasta with 1% alkaline salts; P4, pasta with 5% whey protein isolate; P5, pasta from pellets containing 1% alkaline salts; P6, pasta from pellets containing 1% alkaline salts +5% whey protein isolate.

Sample	Number of fractures	Fractures area (μm^2)	Number of holes	Holes area (μm^2)
P1	$281^{a} \pm 136$	$124^{a} \pm 40$	$182^{c} \pm 53$	$1.04^{a} \pm 0.16$
P2	$92^{a} \pm 13$	$210^{a} \pm 38$	$496^{abc} \pm 269$	$0.34^{bc} \pm 0.20$
P3	$155^{a} \pm 116$	$129^{a} \pm 68$	$529^{abc} \pm 368$	$0.21^{cd} \pm 0.05$
P4	$337^{a} \pm 88$	$79^{a} \pm 24$	$644^{ab} \pm 344$	$0.14^{d} \pm 0.03$
P5	$206^{a} \pm 132$	$124^{a} \pm 57$	$376^{bc} \pm 202$	$0.40^{b} \pm 0.13$
P6	$255^{a} \pm 159$	$191^{a} \pm 159$	$821^{a} \pm 446$	$0.15^{d} \pm 0.02$

Different letters in each column indicate significant differences ($p \leq 0.05$) among pasta samples.

salts compared to the control GF pasta, possibly because of superior cooking loss. These results partially agree with those reported by Fan et al. (2018), who found that water absorption increased up to 0.2% alkaline salts addition and then decreased, while Guo, Wei, and Zhu (2017) reported a water absorption increase with 0.5% of the alkaline salts and decrease with 1.0% alkaline salts. The addition of 5% WPI (P4) or of 1% alkaline salts and 5% WPI (P6) did not significantly ($p > 0.05$) change the weight gain compared to the control pasta, as already noticed by Marti et al. (2014) after incorporating 6% whey protein in rice-based GF pasta. Conversely, Kumar et al. (2019) observed that the addition of whey protein concentrate significantly ($p \leq 0.05$) decreased the water absorption capacity of millet flour-based GF pasta, possibly because the milk proteins competitively reduced the water required for starch swelling during gelatinization.

3.6.2. *Cooking loss*

Cooking loss is an indicator of pasta quality because it reveals the extent of material leaching into the cooking water (Guo et al., 2017), therefore, a low residue indicates high pasta quality (Del Nobile, Baiano, Conte, & Mocci, 2005). It has to be remembered that the absence of gluten in GF pasta leads to less efficient retention of starch polymers in the dough matrix, resulting in higher cooking loss (Marti et al., 2014). Hence, in GF pasta production, ingredients having structuring properties, proteins able to create three-dimensional network mimicking the viscoelastic properties of gluten, and technological processes aimed at gelatinising starch and inducing its retrogradation are employed (Mariotti et al., 2011).

Cooking losses of GF pasta increased significantly ($p \leq 0.05$) adding alkaline salts or WPI alone (Fig. 5). This result is in line with the microstructure observation that showed the presence of holes in all pasta samples, indicating a less cohesive surface that can contribute to the higher cooking loss. Fan et al. (2018), Li et al. (2018) and Rombouts, Jansens, Lagrain, Delcour, and Zhu (2014) evidenced higher cooking loss in wheat samples and buckwheat samples (Guo et al., 2017) containing alkaline salts. Apparently, the alkaline salts addition raises the

Table 5
Colour coordinates (mean ± standard deviation) of gluten-free pasta. P1, control pasta; P2, pasta with 0.5% alkaline salts; P3, pasta with 1% alkaline salts; P4, pasta with 5% whey protein isolate; P5, pasta from pellets containing 1% alkaline salts; P6, pasta from pellets containing 1% alkaline salts +5% whey protein isolate.

Sample	Raw pasta				Cooked pasta			
	*L**	*a**	*b**	ΔE vs. P1	*L**	*a**	*b**	ΔE vs. raw
P1	$51.9^{c} \pm 2.1$	$-4.3^{b} \pm 0.3$	$17.8^{d} \pm 0.7$		$70.8^{a} \pm 1.8$	$-4.8^{c} \pm 0.3$	$20.5^{c} \pm 1.2$	19.1
P2	$51.0^{c} \pm 1.6$	$-2.5^{a} \pm 0.4$	$19.0^{c} \pm 2.0$	2.3	$68.2^{b} \pm 2.5$	$-2.8^{a} \pm 0.3$	$24.7^{b} \pm 1.6$	18.1
P3	$49.0^{d} \pm 0.9$	$-2.4^{a} \pm 0.3$	$22.8^{a} \pm 0.7$	6.1	$62.2^{c} \pm 1.1$	$-3.3^{b} \pm 0.5$	$27.2^{a} \pm 0.7$	13.9
P4	$54.8^{b} \pm 1.6$	$-4.5^{b} \pm 0.6$	$20.9^{b} \pm 0.8$	4.2	$69.3^{b} \pm 1.9$	$-5.0^{cd} \pm 0.4$	$19.0^{d} \pm 1.3$	14.6
P5	$51.4^{c} \pm 1.4$	$-4.9^{c} \pm 0.4$	$20.4^{b} \pm 0.9$	2.7	$69.1^{b} \pm 0.6$	$-5.6^{d} \pm 0.5$	$20.7^{c} \pm 1.4$	17.7
P6	$56.7^{a} \pm 1.3$	$-5.0^{c} \pm 0.2$	$22.2^{a} \pm 0.6$	6.5	$71.1^{a} \pm 1.3$	$-5.2^{e} \pm 0.2$	$20.6^{c} \pm 0.8$	14.5

Different letters in each column indicate significant differences ($p \leq 0.05$) among pasta samples.

Table 6
Geometrical characteristics (mean ± standard deviation) of gluten-free pasta. P1, control pasta; P2, pasta with 0.5% alkaline salts; P3, pasta with 1% alkaline salts; P4, pasta with 5% whey protein isolate; P5, pasta from pellets containing 1% alkaline salts; P6, pasta from pellets containing 1% alkaline salts +5% whey protein isolate.

Sample	Raw				Cooked			
	Area (mm^2)	Length (mm)	Width (mm)	Crown area (mm^2)*	Area (mm^2)	Length (mm)	Width (mm)	Crown area (mm^2)*
P1	352.9^{cd} ± 25.2	34.8^{bc} ± 3.2	12.1^{b} ± 0.5	32.6^{b} ± 3.1	479.1^{d} ± 43.2	39.3^{c} ± 3.8	14.0^{d} ± 0.5	65.4^{a}±1.3
P2	330.8^{d} ± 23.3	31.3^{d} ± 1.9	12.5^{ab} ± 0.8	28.1^{c}±2.6	443.1^{e} ± 38.4	35.9^{d} ± 2.3	14.6^{bc}±1.0	57.8^{c}±3.6
P3	377.0^{bc} ± 18.2	36.1^{b} ± 1.4	12.4^{b} ± 0.8	31.1^{b} ± 1.9	540.1^{b} ± 29.0	42.5^{b} ± 1.4	14.8^{b} ± 0.9	58.7^{bc}±3.4
P4	400.2^{ab} ± 36.4	38.4^{a} ± 3.6	13.1^{a}±0.9	35.4^{a}±3.0	586.9^{a} ± 46.5	45.6^{a} ± 4.0	15.6^{a}±1.0	65.6^{a}±6.7
P5	414.2^{a} ± 49.9	39.1^{a} ± 2.4	12.3^{b} ± 0.5	30.8^{b} ± 3.3	519.0^{bc} ± 44.3	43.6^{ab} ± 2.8	13.8^{cd} ± 0.8	59.0^{bc}±3.5
P6	347.9^{d} ± 27.6	33.0^{cd} ± 2.6	12.2^{b} ± 0.7	31.1^{b} ± 2.9	494.6^{cd} ± 31.7	38.4^{c} ± 2.5	15.1^{ab} ± 0.9	62.3^{ab} ± 3.5

Different letters in each column indicate significant differences ($p \leq 0.05$) among pasta samples; *evaluated on pasta cross-section.

boiling water pH, favouring starch release (Moss, Miskelly, & Moss, 1986); in addition, the very same alkaline salts or salt-soluble proteins rapidly leach out. Coherently, sample P5, even if performed well in terms of geometrical increase, had an intermediate cooking loss value (12.4 g/100 g) significantly ($p \leq 0.05$) higher than those of the control pasta. The pasta samples with WPI (P4 and P6) performed better than the alkaline salts pasta, although still showed a slight increase (13–18%) in cooking loss (8.4 and 8.0%, respectively) compared to the control pasta (7.1%). However, the difference between P6 and the control pasta was not significant ($p > 0.05$). These results are in line with those obtained by Marti et al. (2014), Phongthai et al. (2017), and Prabhasankar, Rajiv, Indrani, and Rao (2007), although Kumar et al. (2019) and Ungureanu-Iuga et al. (2020) reported minor cooking loss in whey protein-containing pasta. The RVA final viscosity and the cooking loss values were positively correlated ($r = 0.950$, $p \leq 0.005$), suggesting that starch organization was the main contributor to the structure of the product. In general, pasta with cooking loss ≤3.0% is considered very good, up to 6.0% fairly good, up to 8.0% regular, and more than 10% poor (Phongthai et al., 2107; Silva et al., 2016), therefore, all pasta samples except P2 and P3 can be considered acceptable. However, it is worth noting that even though the cooking loss of P2 and P3 slightly exceeded the limit of 10%, these pasta sample still maintained their structure without disintegrating (Fig. 4).

3.6.3. *Texture*

The textural properties are critical for evaluating quality and consumer acceptability of pasta (Fan et al., 2018). Generally Italian consumers preferred "*pasta al dente*", a performance associated with a not completely hydrated and cooked pasta (Lucisano et al., 2012): in fact, the optimal cooking time, when possible, is determined as the time required for the disappearance of at least 95% of the white, opaque core of the samples. Consequently, there are not standard values for discriminating "good" or "poor" pasta texture; this is also due to the fact that pasta firmness is affected by the cooking conditions applied (i.e., water-to-pasta ratio, cooking time, etc.) and the test employed (i.e., compression test, compression-extrusion, cut test, probe speed, etc.).

The firmness and total compression-extrusion energy of GF pasta are shown in Table 7. Coherently with the high cooking loss values, compared to the control pasta the addition of alkaline salts and/or WPI resulted in a significant ($p \leq 0.05$) weakening of the pasta structure, i.e. 20-28% lower firmness and 12–16% lower compression-extrusion energy. These results are in contrast with Guo et al. (2017) and Fan et al. (2018), who found that the presence of alkaline salts increased the firmness of noodles by promoting protein cross-linking and the formation of a tight protein network. The discrepancy may be due to the different cereals used (i.e., wheat, buckwheat, rice, etc.) and their protein characteristics (i.e., amino acid composition, protein solubility, etc.) as well as the pasta making conditions applied (Lucisano et al., 2012; Mariotti et al., 2011). Concerning whey proteins, Ungureanu-Iuga et al. (2020) reported likewise results for GF pasta, while Marti et al. (2014) and Prabhasankar et al. (2007) found that their addition decreased the firmness of GF pasta and wheat vermicelli, respectively, due to a decrease in gluten protein strength and integrity. Whey proteins may even be responsible for the formation of discrete entities instead of a protein gel structure, leading to lower firmness (Kumar et al., 2019).

4. Conclusion

The addition of alkaline salts and/or WPI affected the GF pasta cooking performance differently than what reported in literature for noodles and rice-based GF pasta, indicating that the matrix to which they are added plays a key role together with the process conditions applied. The technological properties (i.e., cooking loss and pasta firmness) of the rice-maize control sample were not enhanced by the two strategies evaluated. However, the addition of 1% alkaline salt in pellets (P5) limited the longitudinal dimensional increases of the pasta during cooking and gave the highest firmness, confirming that a combination of formulation and processing conditions (viz. adding the alkaline in pellets instead of directly during mixing) led to a better cooking quality. On the other hand, the addition of 5% WPI (P4 and P6) enhanced the nutritional quality of the pasta by increasing up to 48% its protein content. The combination of alkaline salts and WPI (P6) gave pasta with good cooking performance along with higher better nutritive value.

The outcomes of this research can be useful to enlarge the knowledge of the structuring capabilities of specific ingredients (i.e., alkaline salts and WPI) in a rice-maize matrix without penalizing too much their cooking performance and can help the industries interested in developing healthier GF pasta.

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CRediT authorship contribution statement

Meryem Bouziane: Conceptualization, Formal analysis, Investigation, Resources, Writing – original draft, Writing – review & editing. **Carola Cappa:** Data curation, Investigation, Software, Supervision, Writing – original draft, Writing – review & editing. **Abdallah Bouasla:** Investigation, Visualization, Supervision, Writing – review & editing. **Agostina Moles:** Formal analysis. **Antonio Barabba Terno:** Formal analysis. **Cristina D'Arrigo:** Formal analysis, Methodology, Writing – original draft, Writing – review & editing. **Andrea Brandolini:** Data curation, Formal analysis, Investigation, Supervision, Writing – original draft, Writing – review & editing. **Alyssa Hidalgo:** Conceptualization, Investigation, Resources, Supervision, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial

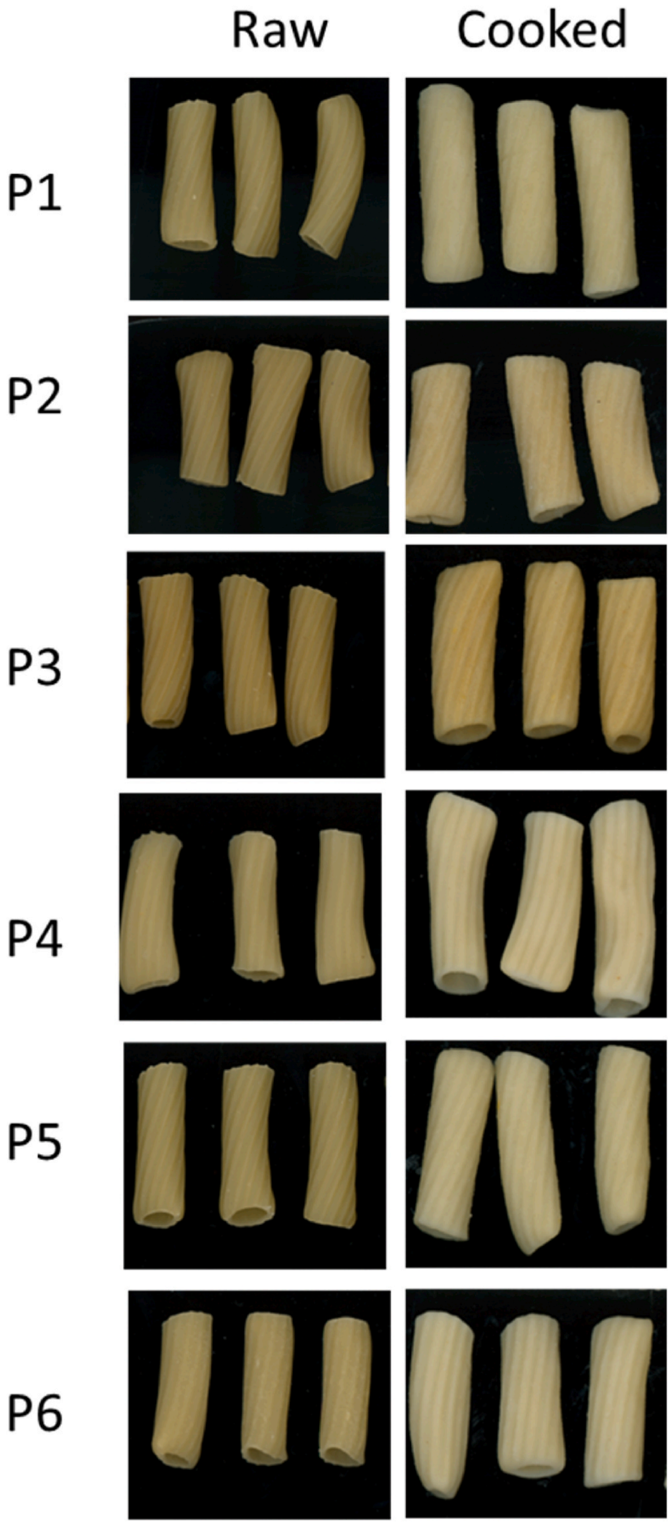

Fig. 4. Raw and cooked macaroni (randomly selected out of 10) with code samples indication. P1, control pasta (rice:maize 2:1); P2, pasta with 0.5% alkaline salts; P3, pasta with 1.0% alkaline salts; P4, pasta with 5% whey protein isolate; P5, pasta from pellets containing 1% alkaline salts; P6, pasta from pellets containing 1% alkaline salts + 5.0% whey protein isolate.

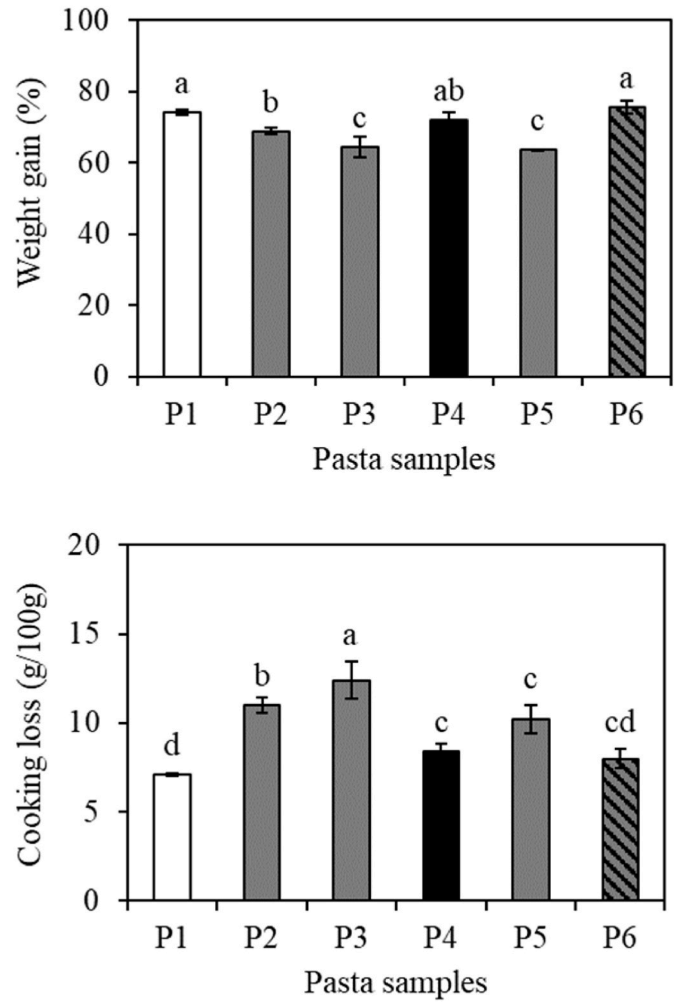

Fig. 5. Weight gain and cooking loss of macaroni samples. Different letters in each column indicate significant differences (p ≤ 0.05) among samples. P1, control pasta; P2, pasta with 0.5% alkaline salts; P3, pasta with 1% alkaline salts; P4, pasta with 5% whey protein isolate; P5, pasta from pellets containing 1% alkaline salts; P6, pasta from pellets containing 1% alkaline salts +5% whey protein isolate.

Table 7
Compression-extrusion energy and firmness (mean ± standard deviation) of cooked gluten-free pasta samples. P1, control pasta; P2, pasta with 0.5% alkaline salts; P3, pasta with 1% alkaline salts; P4, pasta with 5% whey protein isolate; P5, pasta from pellets containing 1% alkaline salts; P6, pasta from pellets containing 1% alkaline salts +5% whey protein isolate.

Sample	Firmness (N)	Compression-extrusion energy (mJ)
P1	336.2^{a} ±19.3	2830.9^{a} ± 181.9
P2	241.8^{d} ± 16.2	2395.7^{b} ± 131.3
P3	261.3^{bc} ± 5.0	2491.8^{b} ± 132.0
P4	244.9^{cd} ± 12.2	2395.2^{b} ± 178.9
P5	267.4^{b} ± 13.9	2388.3^{b} ± 263.3
P6	262.7^{bc} ± 17.1	2405.7^{b} ± 210.9

Different letters in each column indicate significant differences (p ≤ 0.05) among pasta samples.

interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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