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Effect of shape, gluten, and mastication effort on *in vitro* starch digestion and the predicted glycemic index of pasta

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Gluten-containing (GC) and gluten-free (GF) pasta consumption has been growing in recent years. The market offers a wide variety of pasta types, with differences in shape and formulation that influence the mastication process and, consequently, their nutritional behaviors (*i.e.* starch digestibility and glycemic response). This study investigated the effect of shape, gluten, and structural breakdown on *in vitro* starch digestibility and predicted the glycemic index (pGI) of GC and GF penne, spaghetti, and risoni. Pasta was cooked and minced to mimic short, intermediate, and long mastication efforts. Short mastication led to a higher number of big particles than intermediate and long mastications for all pasta samples, which was reflected in the different starch digestibility and pGI patterns. Multivariate analysis of variance showed that the three studied factors differently affected the *in vitro* starch digestion of pasta. Mastication effort, shape, and their interaction mainly affected the starch digestion rate and pGI. Gluten was the major factor in affecting the amount of digested starch. The results suggested that small shapes (*i.e.* risoni), the presence of gluten, and short mastication effort led to a lower pGI. The findings will be useful for the development of pasta products tailored to fulfill the needs of specific consumers following a rational food design approach.

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1. Introduction

The global pasta market size has been steadily growing in recent years (from USD 43.63 billion in 2021 to USD 46.84 billion in 2022) and is forecast to reach USD 77.83 billion by 2029, exhibiting a compound annual growth rate of 7.52% (2022–2029). The global COVID-19 pandemic significantly impacted the global pasta market, which experienced a 7.84% growth in 2020 as compared to 2019.[1] The great success of pasta is related to its affordable price, easiness to cook, and long shelf-life, and the rising adoption of Western food habits is expanding pasta consumption all over the world. The pasta market is dominated by durum wheat-based products but, in recent years, the gluten-free pasta segment, developed to respond to meet the needs of celiac individuals, has significantly expanded to represent about 2% of the global pasta market (US$ 1.1 billion in 2022 with a compound annual growth rate of 4.5% [2022–2032]).[2] Pasta is a staple food for a large part of the world's population. Pro capita pasta consumption varies in different parts of the world, reaching a maximum of 23 kg per year in Italy and around 8–11 kg in other European and American countries.[3] It plays a key role in the daily diet and, therefore, it is important to understand the factors involved with the release of its nutrients, mainly starch, during digestion.

During digestion, food matrix breakdown plays a pivotal role in how nutrients and bioactive compounds are made available for absorption in the human body, therefore regulating their concentration in the blood and their utilization in peripheral tissues.[4,5] Food matrix breakdown depends not only on the mastication effort (time of mastication) but is also related to the product's macrostructural and microstructural attributes, as indicated in previous studies.[6–8] Pasta macrostructural attributes are easily modulated by the use of different dies at the end of the extrusion process, which leads to the formation of pasta pieces with a large variety of shapes. At a microscopic scale, the presence or lack of a gluten network can importantly modify structural attributes in gluten-containing (semolina-based, GC) or gluten-free (*e.g.* maize- and rice-based, GF) products.

It was previously reported that pasta macrostructural attributes (the shape of the pasta) influenced both the mastication

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effort and the shape and size of the pieces present in boluses ready to be swallowed.[6,9,10] In particular, penne-shaped pasta is masticated for a longer time than spaghetti,[9,10] and further significantly longer than risoni in both GC and GF products.[9] The resulting boluses were also composed of residual pieces with markedly different particle size distributions. Risoni were characterized by more pieces of small particle size, while spaghetti and penne led to the generation of more pieces of large particle size in an actual oral processing experiment.[9,10] Furthermore, the size reduction of masticated pieces was found to increase the blood glucose release of cooked spaghetti compared to their intact strands.[11] A relatively lower glycemic response was found in consuming long pasta (spaghetti) compared with short pasta (penne) in an *in vivo* study, indicating a possible influence of different extrusion processes on the structural organization of the pasta matrix, which could influence starch digestibility.[10]

The presence/absence of a gluten network is often taken as the most important pasta microstructural attribute and it has been recognized to affect both the pasta quality and the nutrient bioavailability.[12–14] In a study of pasta oral processing, it was found that in pasta with the same macroscopic shape, gluten increased the chewing effort (chew number), but only marginally the particle size distribution of pieces present in ready-to-swallow boluses.[9]

It is here hypothesized that the macro- and micro-structural attributes of pasta, as well as the mastication effort, influence its structural breakdown, resulting in boluses composed of different sized pieces that, in turn, may play important roles in digestion and nutrient uptake. To the best of the authors' knowledge, the effect of pasta shape, gluten, and *in vitro* structural breakdown on starch digestion and predicted glycemic response has not yet been fully studied. This study aims, therefore, to investigate the effect of macro- (shape) and micro- (presence/absence of a gluten network) structure, as well as the degree of structural breakdown (mastication effort), on *in vitro* starch digestion and the predicted glycemic index of pasta.

2. Materials and methods

2.1 Pasta samples

Commercial GF (90% corn flour, 10% rice flour, water; Massimo Zero, Merano, Italy) and GC (durum wheat semolina, water; Barilla, Parma, Italy) penne, spaghetti, and risoni were used in this study. All products were cooked in boiling deionized water (pasta : water = 1 : 10), without salt, for the cooking time suggested by producers as previously reported.[9] After cooking, pasta was quickly drained and cooled at room temperature for 3 min. The moisture content of the cooked pasta was measured (after drying at 105 °C to constant weight) to standardize the starch digestibility based on the dry matter. The water content was the following: 51.5% for GF penne, 48.5% for GC penne; 60.7% for GF spaghetti, 56.7% for GC spaghetti; 63.8% GF risoni, for 70% GC risoni.

2.2 Structural breakdown of cooked pasta

Cooked pasta samples were subjected to three *in vitro* structural breakdowns to represent short, intermediate, and long mastication efforts. The intermediate mastication effort aimed to reproduce the particle size distribution found in ready-to-swallow boluses produced by consumers measured in an oral processing experiment.[9]

Pasta structural breakdown was carried out *in vitro* in order to control and standardize the amylase content in ready-to-swallow boluses. Preliminary tests were carried out first to identify the chopping conditions that best replicated the particle size distribution of pieces present in ready-to-swallow boluses and set as intermediate mastication.[9] Cooked penne and spaghetti (100 g) were minced in a food processor bowl (1.2 L capacity; Magimix Cook Expert, 18900IT; MK2shop, Udine, Italy) for different times (5, 14, 15, 17, 19, 20, 22, 25, and 40 s for penne; 3, 5, 6, 8, 10, 12, and 16 s for spaghetti). Cooked risoni (5 g) were placed into a 10 mL beaker (2.5 cm diameter) and manually chopped (using a 2 cm blade knife) several times (0, 5, 6, 7, and 8 cuttings). Minced cooked pasta pieces were then characterized as previously described.[9] Briefly, 5 g of bolus was randomly taken and the constituent pieces were separated and placed on a flatbed scanner (Canon CanoScan Lide 400, USA) to obtain a TIFF image (RGB mode, 600 ppi resolution). Collected images were analyzed using Image J software (IJ 1.46r, NIH) by setting the scale and smoothing, and converting to 8-bit images, in order to count the number of fragments and to measure their particle sizes (area, mm^2). Three replicates of each pasta type were analyzed for each mincing time considered. Data were classified into 9-dimensional classes (1: ≤9.9; 2: 10.0–19.9; 3: 20.0–29.9; 4: 30.0–39.9; 5: 40.0–49.9; 6: 50.0–59.9; 7: 60.0–69.9; 8: 70.0–79.9; and 9: ≥80 mm^2). The particle size distributions for each pasta type were compared with those of oral processing to identify the most similar distribution to masticated bolus.[9] Intermediate mastication distributions were identified and set to correspond to 19 s chopping in a food processor for penne (GF and GC), 8 s and 6 s for GF and GC spaghetti, respectively, and 6 manual choppings for risoni (GF and GC).

Short mastication was arbitrarily set to be equal to 5 s and 3 s chopping in the food processor for, respectively, penne (GF and GC) and spaghetti (GF and GC), while risoni was not subjected to cutting. Particle size distributions of short mastication pasta samples were also measured.

Long mastication was set to correspond to the complete mashing of the pasta structure and this was achieved by passing 50 g of cooked product through a meat grinder (ARTUS T25, Reber Srl, Italy), as is commonly done in the determination of *in vitro* starch digestion.[15–18]

2.3 *In vitro* starch digestion and predicted glycaemic index

Pasta samples with particle size distributions corresponding to short, intermediate, and long mastication were subjected to the *in vitro* starch digestion procedure, as previously reported with slight modifications, to determine the amount of released

glucose.[19] Pepsin–guar gum solution (pH = 3) was prepared to simulate gastric phase as described in the method including pepsin (5 mg mL^{-1}; P-7000, 444 U mg^{-1}, Sigma) and guar gum (5 mg mL^{-1}; G-4129, Sigma). The enzyme mixture was prepared from pancreatin (P-7545, 8× USP, Sigma, trypsin activity 6 U mg^{-1}), amyloglucosidase (A7095, Sigma), and invertase (I4504, Sigma). Pancreatin was suspended in water at 150 mg mL^{-1} and, after 10 min of magnetic stirring, was centrifuged at 1500*g* at 4 °C for 5 min. Afterward, three-fourths of the cloudy supernatant were taken out to further mix with amyloglucosidase (0.04 mL mL^{-1} in the final mixture) and invertase (0.562 mg mL^{-1} in the final mixture) to achieve the final enzyme mixed suspension. All enzyme solutions were freshly prepared.

Five grams of pasta of each mastication effort were statically incubated for 30 min with 10 mL of pepsin–guar gum solution in a water bath at 37 °C. Later, 10 mL of 0.25 M sodium acetate and 5 glass marbles (∅ 15 mm) were added, and then 5 mL of the enzyme mixture was added for further incubation with shaking (37 °C, 160 strokes per min, 120 min). Every 15 min from 0 to 120 min, 0.1 mL of each sample was collected, immediately mixed with 0.4 mL of absolute ethanol to stop the hydrolysis reaction, and diluted with water (1 : 10). The diluted samples were vortexed and centrifuged (3000*g*, 10 min) to obtain the supernatant for glucose measurement, which was determined using a D-glucose assay kit (K-GLUC, Megazyme, Bray, Ireland). A blank, containing water rather than all the enzymes used for digestion, was also carried out. The starch digested at each time interval was calculated based on the amount of glucose multiplied by the conversion factor of 0.9 and was expressed in g per 100 g dry cooked pasta for the evaluation of starch digestibility and g per 100 g cooked pasta to calculate the predicted glycemic index (pGI). The pGI was calculated using white bread digestion as a reference. The *in vitro* digestion of each sample was conducted in three replicates.

The digested starch values of each mastication level of each pasta sample (g per 100 g dry cooked pasta) were fitted to the Box–Lucas model (eqn (1)),[20] using OriginPro 9.1 (OriginLab Corporation, Northampton, MA, USA):

$$C_t = C_\infty\ (1 - e^{-kt}) \quad (1)$$

where C_t and C_∞ are the amounts of digested starch (g per 100 g dry cooked pasta) at a given (t; 90 and 120 min) and infinite (∞) time and k is the digestibility rate constant. The terms C_{90} and C_{120} represent the extent of starch digested after 90 and 120 min and were obtained from the starch digestibility data. These specific time points were selected based on previous data on starch digestibility.[21,22]

Digested starch values of pasta (g per 100 g cooked pasta) and white bread (g per 100 g bread) were used for calculating the hydrolysis index (HI). The HI was derived from the ratio between the area under the hydrolysis curve (0–120 min) and the corresponding area of white bread expressed as a percentage over the same digestion time. The pGI for each mastication level of each pasta sample was calculated based on the following formula: pGI = 8.198 + 0.862 HI.[22]

2.4 Statistical analyses

Data were analyzed using IBM SPSS Statistics 22 (IBM Corporation, New York, USA). All the results were normally distributed as verified with the Shapiro–Wilk test ($p < 0.05$) and expressed as mean ± standard deviation (SD) of three replicates. Significant differences of starch digested at 90 min (C_{90}), 120 min (C_{120}), infinite time (C_∞) and the digestibility rate constant (k), and the predicted glycemic index (pGI) between samples were identified through one-way analysis of variance (ANOVA, $p < 0.05$) followed by Duncan's test. A t-test ($p < 0.05$) was used to verify the significant difference of C_{90}, C_{120}, C_∞, k, and pGI between GF and GC pasta with the same shape. Variance homogeneity was previously verified according to the Levene test ($p < 0.05$). To better understand the correlation between the pGI and C values, Pearson's correlation coefficient was run. A multivariate analysis of variance (MANOVA) based on Pillai's Trace test was run with shape, gluten, and mastication level as fixed factors (at a significance level of $\alpha = 0.05$) to assess the influence of pasta shape, gluten, and mastication level on C_{90}, k, and pGI.

3. Results

3.1 Particle size distribution of the chopped pasta

Particle size distribution analysis was possible for short and intermediate mastication of pasta but not for long mastication as these samples were completely mashed at the end of the long oral processing simulation. In general, short mastication led to a higher number ($p < 0.05$) of big particles than the intermediate mastication for all pasta shapes (Fig. 1). Sixty-nine percent of GF penne, 90% of GC penne, 48% of GF spaghetti, and 40% of GC spaghetti pieces had an area larger than 80 mm^2 when short mastication was simulated. In contrast, short mastication of GF and GC risoni generated more than three-quarters of pieces in the ranges 20.0–29.9 mm^2 and 30.0–39.9 mm^2, respectively. Intermediate mastication of all samples generated a prevalence of particle size pieces between the first and fourth classes, which means a range between 0 and 39.9 mm^2. For GF and GC penne, a reduction in both length and width was observed, producing heterogeneously shaped fragments, while GF and GC spaghetti were shortened in length resulting in short spaghetti strands.

3.2 *In vitro* starch digestion of minced pasta

In vitro starch digestion of different GF and GC pasta shapes was examined by fitting the digestion curves to the Box–Lucas model ($R^2 \geq 0.97$ for all samples, Table 1 and Fig. 2). The rate of starch digestion (k) revealed a different pattern as a function of the mastication effort ($F = 61.8$, $p < 0.001$; Table 2), the shape of pasta ($F = 81.9$, $p < 0.001$), and their interaction ($F = 7.6$, $p < 0.001$). k increased with the extent of mastication with a steep increase in the starch digestion curves in the first

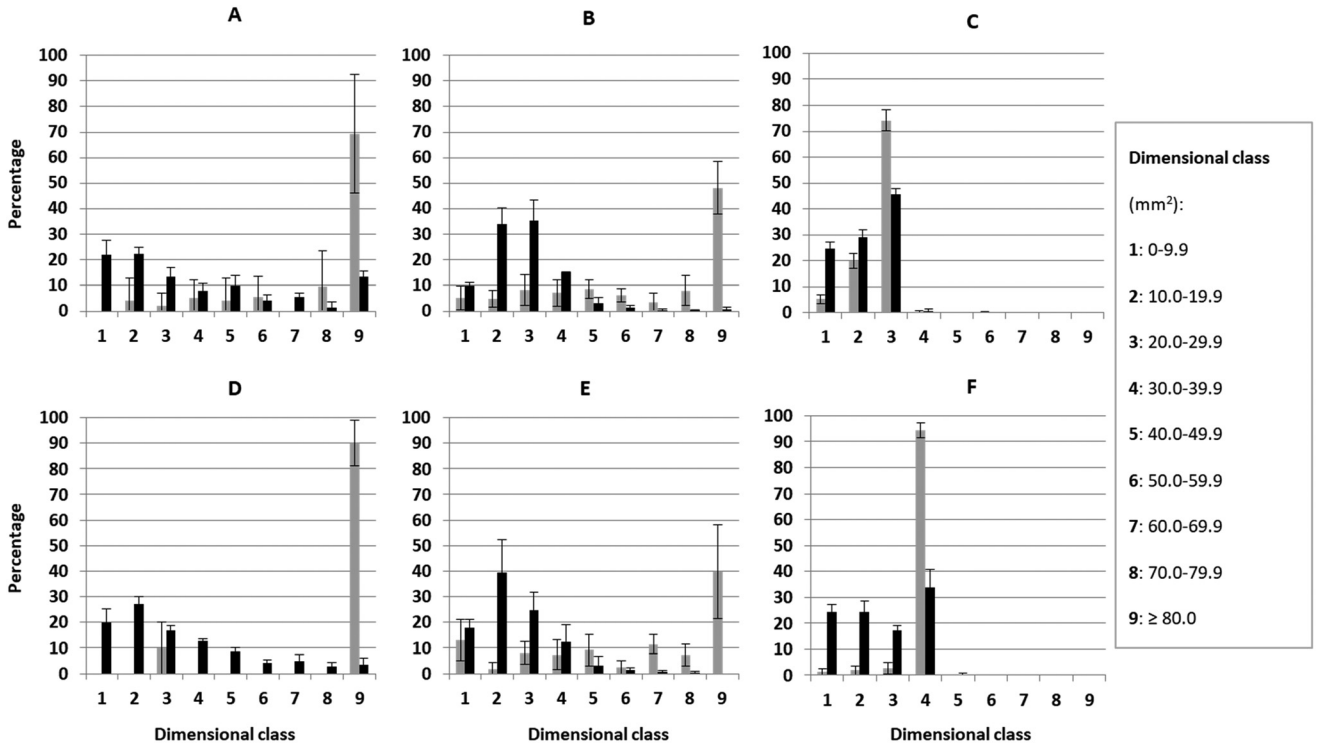

Fig. 1 The particle size distribution (mean ± SD, n = 3) of GF (A: penne; B: spaghetti; and C: risoni) and GC (D: penne; E: spaghetti; and F: risoni) cooked pasta with short (gray bars) and intermediate (black bars) mastication.

Table 1 Amount of starch (g per 100 g dry cooked pasta) digested after 90 (C_{90}) and 120 (C_{120}) minutes, and at an infinite time (C_∞); the digestibility rate constant (k) of pasta calculated by the Box–Lucas model fitting at short, intermediate, and long mastication times

	Shape	Mastication effort	C_{90}	C_{120}	C_∞	k
GF	Penne	Short	66.6 ± 4.7 a ^{b}	70.7 ± 3.3 b ^{b}	78.1 ± 2.6 a ^{a}	0.020 ± 0.005 b^{*b}
		Intermediate	71.5 ± 1.9 b ^{ab}	74.7 ± 1.7 b ^{ab}	76.5 ± 2.3 b ^{a}	0.029 ± 0.002 b ^{a}
		Long	75.3 ± 1.9 b^{*a}	78.8 ± 2.6 ab^{**a}	79.4 ± 2.3 b* ^{a}	0.033 ± 0.005 b^{*a}
	Spaghetti	Short	73.2 ± 2.0 a^{***c}	79.6 ± 3.3 a^{*a}	79.5 ± 3.6 a^{**a}	0.030 ± 0.004 a ^{b}
		Intermediate	78.6 ± 1.1 a^{***b}	83.4 ± 3.6 a^{**a}	83.2 ± 2.4 a^{**a}	0.032 ± 0.003 b ^{b}
		Long	83.3 ± 2.7 a^{***a}	85.2 ± 2.3 a^{***a}	83.9 ± 2.4 a^{***a}	0.039 ± 0.002 ab ^{a}
	Risoni	Short	64.3 ± 5.6 a ^{b}	70.3 ± 1.3 b ^{a}	67.9 ± 2.5 b^{*b}	0.038 ± 0.002 a ^{b}
		Intermediate	67.2 ± 2.2 c ^{ab}	72.8 ± 4.4 b ^{a}	71.2 ± 3.5 b ^{ab}	0.039 ± 0.004 a ^{b}
		Long	73.8 ± 0.1 b^{***a}	75.6 ± 1.2 b ^{a}	74.4 ± 1.6 c^{*a}	0.047 ± 0.005 a ^{a}
GC	Penne	Short	62.4 ± 4.6 B ^{B}	68.0 ± 4.8 A ^{A}	87.9 ± 9.0 A ^{A}	0.012 ± 0.001 C ^{C}
		Intermediate	66.9 ± 4.0 A ^{AB}	72.0 ± 0.5 A ^{A}	76.7 ± 6.7 A ^{AB}	0.023 ± 0.006 B ^{B}
		Long	70.0 ± 1.7A ^{A}	71.2 ± 1.1 A ^{A}	71.3 ± 0.5 A ^{B}	0.046 ± 0.006 A ^{A}
	Spaghetti	Short	61.7 ± 0.7 B ^{B}	67.6 ± 0.9 A ^{A}	69.1 ± 1.3 B ^{A}	0.026 ± 0.002 B ^{B}
		Intermediate	61.4 ± 0.1 B ^{B}	67.9 ± 1.4 B ^{A}	67.4 ± 1.4 B ^{AB}	0.028 ± 0.002 B ^{B}
		Long	63.6 ± 0.7 B ^{A}	66.3 ± 0.5 A ^{A}	66.7 ± 0.5 B ^{B}	0.035 ± 0.001 B ^{A}
	Risoni	Short	72.0 ± 3.6 A ^{A}	72.7 ± 1.4 A ^{A}	74.0 ± 2.6 B ^{A}	0.042 ± 0.003 A ^{A}
		Intermediate	70.4 ± 2.3 A ^{A}	72.8 ± 1.8 A ^{A}	72.1 ± 0.3 AB ^{A}	0.043 ± 0.002 A ^{A}
		Long	69.8 ± 0.7 A ^{A}	71.0 ± 4.0 A ^{A}	71.2 ± 1.0 A ^{A}	0.051 ± 0.007 A ^{A}

Data are expressed as mean ± SD. For each formulation and mastication effort, different letters mean significant differences of the same digestibility parameter between different pasta shapes (GF: lowercase; GC: uppercase) (p < 0.05, Duncan's test, n = 3). For each pasta shape and mastication level, asterisks mean significant differences between the same digestibility parameter of the GF and GC counterparts (*: p < 0.05; **: p < 0.01; ***: p < 0.001, t-test, n = 3). For each pasta and digestibility parameter, different superscript letters mean significant differences between mastication efforts (GF: lowercase; GC: uppercase) (p < 0.05, Duncan's test, n = 3).

15 min for all pasta samples (Fig. 2). Moreover, at the same mastication effort, k differed among the three pasta shapes (Table 1). Following the digestion, the amount of digested starch increased progressively until 120 min following a first-order kinetic except for the curve of short masticated GC penne, which had a very low k (0.012 ± 0.001) and an unreli-

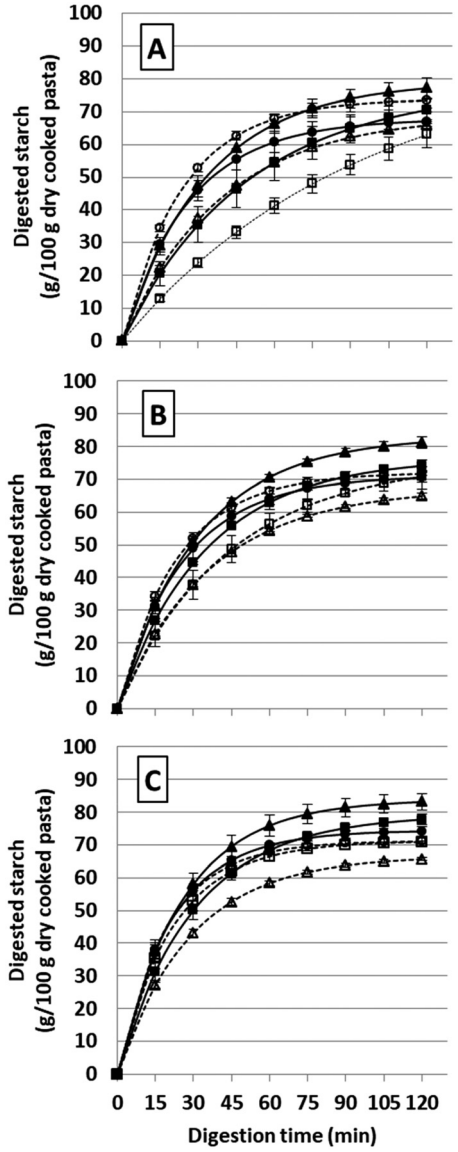

Fig. 2 Pasta starch (g per100 g dry cooked pasta) digested at different mastication efforts (A: short; B: intermediate; and C: long). Black squares: GF penne; white squares: GC penne; black triangles: GF spaghetti; white triangles: GC spaghetti; black circles: GF risoni; and white circles: GC risoni. Lines represent the fitting of the Box–Lucas model. Data are expressed as mean ± SD (n = 3).

able calculated C_∞ (87.9 ± 9.0 g per 100 g dry cooked pasta). In general, the trend showed that starch digested at C_{90} was lower than that at C_{120}, which, in turn, was similar to that at C_∞. This was not true for GC penne at short mastication effort meaning that the starch digestion cannot be considered near completion at 120 min. Except for risoni at short and intermediate mastication, the amount of starch digested at C_{90}, C_{120}, and C_∞ was higher in GF pasta than that in their GC counterparts (Table 1), suggesting the main role of gluten on the starch digestibility as a single factor (F = 76.0, p < 0.001; Table 2) and as interaction with pasta shape (F = 63.4, p < 0.001) and mastication (F = 9.2, p < 0.001). The amount of digested starch was also influenced by mastication (F = 27.0, p < 0.001), which positively contributed to starch digestion when the time of mincing was increased.

3.3 Predicted glycemic index

The pGI progressively increased by increasing mastication effort (F = 85.5, p < 0.001; Table 2), especially for penne, increasing from below 55 with short mastication to nearly 70 with long mastication (Fig. 3). GF spaghetti showed low pGI (<55) at short and intermediate mastication and medium pGI (55 < GI < 69) at long mastication time, while the pGI of GC spaghetti and risoni remained low at all mastication efforts. Pasta shape was found to be the main factor influencing the pGI (F = 337.9, p < 0.001; Table 2) as, independently from the mastication time, the penne pGI was higher than the spaghetti pGI, which, in turn, was higher than the risoni pGI. Moreover, the pGI of GC pasta was generally lower than its GF counterparts, suggesting an important role of gluten in influencing the predicted glycaemic index (F = 93.2, p < 0.001).

4. Discussion

Food oral processing is the first step of digestion. It is characterized by a large inter-individual variability that produces boluses with pieces of different particle sizes that may affect the digestion rate of nutrients (*i.e.* the starch digestion rate and glycemic response).[5,6,9,23–25] As pasta is a staple food widely consumed in different shapes and formulations, the effects of its macroscopic (shape) and microscopic (gluten network) structure and the degree of structural breakdown related to different mastication efforts on starch digestion were studied using an *in vitro* digestion protocol and the predicted GI. The three studied factors affected differently the *in vitro* starch digestion of pasta. Mastication effort, shape, and their interaction mainly affected the starch digestion rate (*i.e.*, k) and the pGI. Gluten was the major factor in affecting the amount of digested starch.

The different composition and structural organization of GF and GC pasta were expected to affect water uptake by the pasta matrix, leading to a different moisture content of the cooked products. Therefore, to highlight the effect of structure (macroscopic and microscopic) and mastication effort on the starch digestion rate of pasta with different shapes, with or without gluten, and differently masticated, it was necessary to dry the cooked pasta samples to remove the effect of different water content gained during cooking between GF and GC pasta. In contrast, the pGI was calculated on the wet weight of cooked pasta, because GI *in vivo* is measured on food as eaten, and is correlated with C_{90}. This result was in agreement with the findings of Edwards *et al.*,[21] who studied the validity of the Box–Lucas model to fit the starch digestion of starch-rich foods concluding that the extrapolated C_∞ might not be appropriate to describe the behavior of some samples.

Regarding the effect of microscopic structure (*i.e.*, gluten), the amount of digested starch (C_{90}) was higher in GF penne

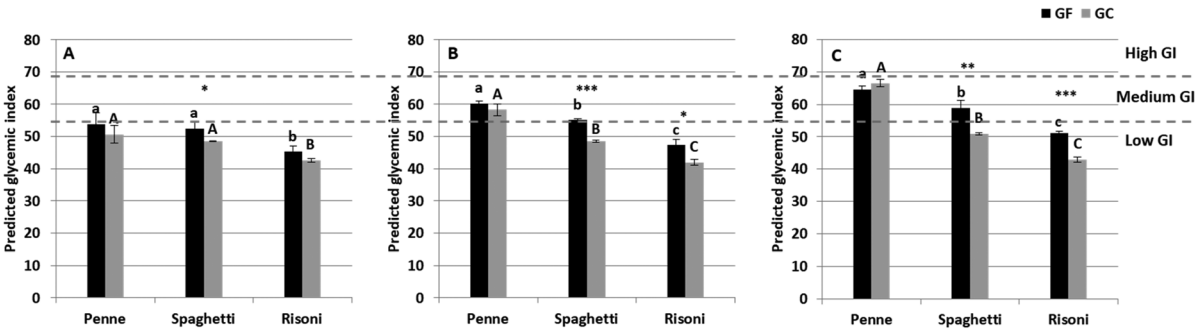

Fig. 3 Predicted glycemic index (mean ± SD) of GF (black) and GC (gray) pasta for short (A), intermediate (B), and long (C) mastication times. Different letters in each subfigure mean significant differences between pasta with different shapes and the same formulation (lowercase for GF, uppercase for GC) ($p < 0.05$, Duncan's test, $n = 3$). Asterisk means significant differences between GF and GC pasta with the same shape at the same mastication level (* = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$, and t-test, $n = 3$).

Table 2 Multivariate analysis of variance of starch (g per 100 g dry cooked pasta) digested at 90 min (C_{90}), the digestibility rate constant (k), and the predicted glycemic index (pGI) after 120 minutes

		C_{90}	k	pGI
Shape	F	1.8	81.9	337.9
	Sig.	ns	***	***
Gluten	F	76.0	0.2	93.2
	Sig.	***	ns	***
Mastication	F	27.0	61.8	85.5
	Sig.	***	***	***
Shape × Gluten	F	63.4	5.5	13.2
	Sig.	***	**	***
Gluten × Mastication	F	9.2	4.6	1.2
	Sig.	***	*	ns
Shape × Mastication	F	2.0	7.6	18.6
	Sig.	ns	***	***
Shape × Gluten × Mastication	F	2.5	4.6	4.9
	Sig.	ns	**	**

*: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$; and ns: not significant.

and spaghetti than that in their GC counterparts at each mastication level. These findings underlined a very strong impact of gluten microstructure on starch digestion, supporting the extensively reported hypothesis that the three-dimensional gluten network formed during kneading of semolina-based pasta entraps gelatinized starch, and thereby, acts as a physical barrier to limit the enzyme hydrolysis of starch, contributing to a lower starch digestibility.[13,14] Moreover, the GF pasta analyzed in our study was formulated with corn and rice, which are cereals known to have a higher starch digestibility than wheat.[22,26]

Shape had a major effect on the pGI independently from the gluten presence. Penne had the highest pGI, followed by spaghetti and risoni. The higher pGI of penne compared to spaghetti agreed with the results of the post-prandial *in vivo* glucose response measured by Vanhatalo *et al.*[10] Besides the macroscopic and microscopic structure, the C_{90} and pGI were affected by the mastication level as the values progressively increased by increasing the mincing time for the three types of pasta, as expected. From short to intermediate to long mastication, the number of small particles progressively increased at the expense of larger particles, leading to a higher contact surface for the enzymatic activity and, consequently, to a higher amount of digested starch that caused, in turn, the rise of the pGI. The particle size was already reported to affect the starch digestibility and pGI of bread having the same formulation but different matrixes, such as roll bread and loaf bread.[25] In particular, more extensively simulated bread mastication led to the formation of smaller particle sizes than a less fractured bolus.[25] This result agrees with our finding, emphasizing that food structure and the oral mastication phase significantly affect the bolus particle size distribution. Previous studies on rice also revealed a positive correlation between the mastication degree, starch digestibility, and pGI, demonstrating that the dimension of rice particles affected the *in vitro* digestibility of starch with the smaller particles digested at a higher rate.[24] Moreover, they proved by an *in vivo* study that the glycemic index of rice chewed 15 times was significantly lower than when masticated 30 times (GI 68 and 88, respectively).[27] In contrast, risoni contradicted these results having the highest number of small particles but the lowest pGI. However, as previously reported, risoni were swallowed as almost not masticated pieces.[9] This behavior might be related to the harder structure of risoni compared to penne and spaghetti, which might have limited the enzyme penetration and, consequently, the amount of digested starch. Moreover, risoni gained more water during cooking than penne and spaghetti, resulting in lower total starch content and, consequently, a lower pGI.

5. Conclusion

In this study, the starch digestibility and pGI of pasta having different shapes and formulations was investigated through three levels of mastication. Pasta shape was found to be the predominant factor in affecting the starch digestion rate and pGI. Moreover, the presence of an organized microstructure (*i.e.* the continuous gluten network with embedded gelatinized

starch) reduced the starch digestion and the predicted glycemic response. A more extensive mastication effort, with consequent more extensive physical structural breakdown, facilitated starch digestion and induced a higher expected glycemic index. The present results suggest that small pasta like risoni, gluten-containing products, or less mastication effort can be a strategy to have a relatively lower expected glycemic index. The findings of this investigation will be useful for the development of pasta products tailored to fulfill the needs of specific consumers following a rational food design approach.

Author contributions

Xinying Suo: conceptualization – equal, data curation – lead, formal analysis – lead, investigation – lead, visualization – equal, and writing – original draft – equal. Anna Baggio: writing – original draft – equal, data curation – equal, and formal analysis – equal. Nicoletta Pellegrini: conceptualization – equal, supervision – equal, and writing – review & editing – equal. Silvia Vincenzetti: supervision – equal, and writing – review & editing – equal. Elena Vittadini: conceptualization – equal, funding acquisition – lead, project administration – lead, supervision – equal, writing – review & editing – equal, and visualization – equal.

Conflicts of interest

There are no conflicts to declare.

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