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(Article begins on next page)

High-amylose corn in gluten-free pasta: strategies to deliver nutritional benefits assuring the overall quality

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1 **Abstract:**

2 High-amylose corn alone or in combination (25% and 50%) with conventional corn was used to
3 produce gluten-free pasta. Flour pre-gelatinization in a tank (process A) or on a conveyor belt (process
4 B) were tested. Resistant starch (RS), soluble (SPAs) and cell-wall bound phenolic acids (CWBPAs)
5 and antioxidant capacity were significantly higher in high-amylose corn pasta. Cooked pasta from
6 process B showed a higher SPA concentration, likely due to the lower cooking loss. The structure of
7 pasta prepared with process B was more homogeneous, whereas it was more compact in the case of
8 process A, as shown by a lower starch susceptibility to α -amylase hydrolysis, higher beginning of
9 gelatinization temperature and lower water absorption. 25% HA represents a good compromise
10 between high RS (4.2%) and good cooking behavior. At higher HA levels, process B is more suitable
11 to obtain pasta with a better cooking quality.

12 **Keywords:** maize; pre-gelatinization; extrusion-cooking; resistant starch; phenolic acids; antioxidant
13 capacity.

14 **Abbreviations:**

15 25HA, blend 75%-25% of flour from conventional and high-amylose corn; 50HA, blend 50-50% of flour
16 from conventional and high-amylose corn; ABTS, 2,2'-Azino-bis(3-ethylbenzthiazoline-6-sulfonic acid);
17 ANOVA, Analysis of variance; CV, use of flour from conventional corn only; CWBPAs, cell wall-bound
18 phenolic acids; dw, dry weight; FRAP, ferric reducing antioxidant power; GF, gluten-free; GI,
19 glyceamic index; GL, glyceamic load; HA, use of flour from high-amylose corn only; REGW-F test,
20 Ryan/Einot and Gabriel/Welsch test; RP-HPLC/DAD, reverse phase high performance liquid
21 chromatograph coupled to a diode array detector; RS, resistant starch; SPAs, soluble phenolic acids.

22 **1. Introduction**

23 The consumption of gluten-free (GF) products is still growing due to the increased number of people
24 who adopt a GF diet for several reasons, including those who suffer from celiac disease and who wish
25 to reduce or eliminate the consumption of gluten-based foods from their diet because they're
26 considered less healthy than GF products.

27 Among cereal-based products, pasta is considered the easiest to redesign and make GF. This is
28 mainly due to its simplicity in terms of formulation, processing and structure, when compared to baked
29 goods (Marti & Pagani, 2013). In the case of GF pasta, the absence of a gluten network requires
30 starch to play a key role in creating a cohesive mass able to limit its solubilization during cooking
31 (Marti & Pagani 2013). This matrix is created by exploiting the tendency of starch to retrograde, in
32 other words to re-associate and interact after its gelatinization, resulting in newly organized structures.

33 The choice of both suitable raw materials (i.e., with high gelatinization and retrogradation capacity)
34 and processing conditions (i.e., thermal and mechanical stresses able to promote starch de-
35 structuration and its reorganization) are strategic for obtaining a product with good cooking behaviour
36 (Marti & Pagani, 2013). Positive results are obtained by the extrusion-cooking process that combines
37 thermal and mechanical stresses. Specifically, native flour is gelatinized with steam at high
38 temperatures and then pressed through a heated screw to obtain pellets; these are then extruded in a
39 conventional continuous press for making pasta (Marti & Pagani, 2013). The effects of the pasta-
40 making process on starch structure and pasta quality has been widely assessed in rice formulations
41 (Marti, Seetharaman, & Pagani, 2010; Marti, Pagani, & Seetharaman, 2011; Barbiroli, Bonomi,
42 Casiraghi, Iametti, Pagani, & Marti, 2013). On the contrary, little information is available on corn, which
43 is one of the most common ingredients in GF pasta (Morreale, Boukid, Carini, Federici, Vittadini, &
44 Pellegrini, 2019) as an interesting source of bioactive compounds such as polyphenols and
45 carotenoids (Nuss & Tanumihardjo, 2010).

46 As regards the raw materials, the role of amylose has been extensively studied in noodles: on one
47 hand, starches with low amylose content (i.e., waxy lines) leads to poor cooking quality (Dexter &
48 Matsuo, 1979); on the other hand, starches high in amylose (> 40%) do not seem to provide an
49 adequate degree of gelatinization during the thermal process, limiting the extent of further starch

50 retrogradation (Tam, Corke, Tan, Li, & Collado, 2004). However, to the best of our knowledge, most of
51 the studies on the relation between amylose content and product quality have been carried out on
52 noodles rather than on pasta which are different in formulation (starch vs flour), processing (sheeting
53 vs extrusion), shape and thus texture (Marti & Pagani, 2013). In addition, no solutions have been
54 proposed so far. Indeed, despite the technological issues related to high amylose content, starches
55 rich in amylose are interesting from a nutritional standpoint, since they are a good source of resistant
56 starch (RS). Many authors (Pellegrini & Agostoni, 2015; Berti, Riso, Monti, & Porrini, 2004) have
57 stressed that GF products result in a high glyceamic index (GI) and glyceamic load (GL). As far as GF
58 pasta is concerned, GI and GL values were higher in rice pasta compared to corn pasta or to pasta
59 containing both corn and rice as the main ingredients (Bacchetti, Saturni, Turco, & Ferretti, 2014).
60 Conversely, ingredients rich in RS make for products that are low in calories and GL (Sajilata, Singhal,
61 & Kulkarni, 2006).

62 Moreover, in the case of corn, high-amylose varieties exhibit an even better antioxidant capacity than
63 conventional and waxy genotypes (Alfieri, Bresciani, Zanoletti, Pagani, Marti, & Redaelli, 2020; Li,
64 Wei, White, & Beta, 2007; Bresciani, Giordano, Vanara, Blandino, & Marti 2020). In this context, some
65 actions should be taken in order to improve the quality of pasta from high-amylose starch. Thus, the
66 objective of the present study was to assess the role of the pasta-making process on the physico-
67 chemical properties of high-amylose corn and their impact on the nutritional features and cooking
68 behavior of GF pasta. Specifically, in order to improve the pasta-making performance of high-amylose
69 corn flour (alone or in combination with conventional corn flour), two pre-gelatinization systems were
70 adopted: in the first , steam is blown into a tank where dough (flour and water) are mixed; in the
71 second , steam is injected on a conveyor belt, where a thin layer of dough is placed. The effects of
72 different pre-gelatinization systems were investigated on starch features and pasta cooking quality,
73 without neglecting the effects on the content of bioactive compounds, such as phenolic acids and RS,
74 of which corn is a good source.

75 **2. Materials and methods**

76 *2.1 Corn flours*

77 Corn flour (particle size less than 150 μm) from a conventional hybrid (Pioneer P1547, amylose =
78 18%; CV) and a high-amylose hybrid (Amylor, amylose = 42%; HA) were obtained by means of
79 multiple-stream roller milling in an industrial mill (Molino Peila, Valperga, Italy). The chemical
80 composition of the hybrids was reported by Bresciani et al. (2020). Both hybrids were cultivated in the
81 2018 growing season in the same growing area in North West Italy. CV and HA were used alone, or
82 they were blended, and two HA substitution levels were considered. i.e., 25:75 (HA:CV) and 50:50
83 (HA:CV), namely 25HA and 50HA respectively.

84 *2.2 Pasta-making process*

85 Flours were mixed to mono- and di-glycerides of fatty acids (0.3%) and processed into pasta by
86 extrusion-cooking using two different pre-gelatinization systems. In process A, flour pre-gelatinization
87 was carried out in a pre-gelatinization tank (Braibanti, Milan, Italy). Specifically, flour and water (30%
88 final moisture content) were treated with steam at 130 °C for 15 min for CV, at 130 °C for 15 min for
89 25HA and 50HA, and at 130 °C for 30 min for HA. In process B, the flour-water mixture was treated
90 with steam (110 °C for 2 min) on a conveyor belt (Fava S.p.A., Cento, Italy) and fed into the extruder.

91 The pre-gelatinized mixture from either process A or B was extruded (screw temperature: 110 °C) into
92 small pellets (cylinder shape; 3 mm diameter), and then formed into a macaroni shape using a
93 continuous press for semolina pasta production (Braibanti, Milan, Italy). A jacket with cold water kept
94 dough temperature at about 50 °C at an extrusion pressure of 10^7 Pa. All samples were dried in an
95 experimental drying cell (Fava S.p.A., Cento, Italy) using a high-temperature drying cycle (70 °C for
96 3.5 h).

97 All samples were stored at room temperature until analyzed. For starch susceptibility to α -amylase,
98 pasting properties, phenolic acids and total antioxidant capacity samples were milled (particle size less
99 than 250 μm) using a laboratory mill (IKA Universalmühle M20; IKA Labortechnik, Staufen, Germany),
100 with a water-cooling system to avoid overheating.

101 The moisture content of both flours and pasta samples were determined by oven-drying at 105 °C for
102 24 h in order to express all the results as dry weight (dw).

103 *2.3 Pasta cooking procedure*

104 Pasta (25 g) was cooked in boiling distilled water (pasta:water ratio = 1:10) at the optimum cooking
105 time, which was determined by ten people after tasting the product at different cooking times. Pasta
106 cooking quality (section 2.7) was assessed directly after cooking. For the determination of phenolic
107 acids and total antioxidant capacity (section 2.5) and RS (section 2.6), the cooked pasta was treated
108 with liquid nitrogen, freeze-dried (-80°C for 72h; Alpha 1-2 LD plus; Delttek s.r.l., Naples, Italy), ground
109 (particle size < 500 µm) with a cyclotec 1093 sample mill (Foss, Padova, Italy), and stored at -25°C
110 until the beginning of the analyses.

111 *2.4 Pasta colour*

112 The color of uncooked pasta was measured using a reflectance color meter (CR 210, Minolta Co.,
113 Osaka, Japan) to measure the lightness and saturation of the color intensity. Results were expressed
114 in the CIE L* a* b* colour space.

115 *2.5 Phenolic acids and total antioxidant capacity*

116 Phenolic acids and antioxidant capacity were analyzed in corn flour, uncooked and cooked pasta.
117 Extraction and quantification of soluble (free and conjugated, SPAs) and cell wall-bound phenolic
118 acids (CWBPAs) by means of reverse phase high performance liquid chromatograph coupled to a
119 diode array detector (RP-HPLC/DAD) were performed as reported in Giordano, Reyneri, Locatelli,
120 Coïsson, & Blandino (2019). Total antioxidant capacity (AC) was determined by means of FRAP
121 (Ferric Reducing Antioxidant Power) and the ABTS [2,2'-Azino-bis(3-ethylbenzthiazoline-6-sulfonic
122 acid)] assays adapted into QUENCHER method as described by Serpen, Gökmen, & Fogliano (2012).

123 *2.6 Starch properties*

124 Starch susceptibility to α -amylase (or damaged starch) was assessed on the uncooked pasta
125 according to the standard method AACC 76-31.01 (AACCI, 2001).

126 Pasting properties of flours and uncooked pasta were evaluated using a Micro Visco-Amylo-Graph,
127 MVAG, (Brabender GmbH., Duisburg, Germany), according to the procedure described by Bresciani
128 et al. (2020). Twelve grams of flour were dispersed in 100 ml of distilled water, scaling both sample
129 and water weight on a 14% flour moisture basis. The suspensions were subjected to the following
130 temperature profile: heating from 30° up to 95°C, holding at 95°C for 20 minutes and cooling from 95°
131 to 30°C with a heat/cooling rate of 3°C/min. One representative curve for each sample was reported.
132 The amount of resistant starch was measured in corn flour, uncooked and cooked pasta according to
133 the standard method AACC 32-40.01 (AACCI, 2001).

134 *2.7 Cooking quality*

135 Cooking loss was evaluated by determining the amount of solids lost in cooking water according to the
136 standard method AACC 66-50.01 (AACCI, 2001). After cooking, the level of water was brought to the
137 initial volume. Dry matter was determined on 40 ml of cooking water, dried to a constant weight at 105
138 °C. Results are expressed as grams of matter loss/100 g pasta dry basis.

139 Pasta weight increase due to cooking was expressed as the ratio percentage between the weight
140 increase and the weight of uncooked pasta.

141 Texture properties of cooked pasta were determined by a compression-extrusion test using a texture
142 analyzer (Z005, Zwick Roell, Ulm, Germany), equipped with a 10-blade Kramer cell and a 5 kN load
143 cell. 25 g of pasta were cooked and then compressed and extruded with a 0.67 mm/s crosshead
144 speed. Results are expressed as average values of firmness, i. e. the maximum compression force
145 (N).

146 *2.8 Statistics*

147 Pasta color was determined on ten pieces of pasta. Starch susceptibility and pasting properties were
148 measured in duplicate. All the chemical analyses were performed in triplicate. Four independent

149 cooking trials were carried out for pasta weight increase, cooking loss and texture analysis. For
150 cooking loss determination, two subsamples from each cooking trial were assessed.

151 One-way analysis of variance (ANOVA) was performed with the SPSS for Windows statistical package
152 version 24 (SPSS Inc., Chicago, Illinois, US) on AC, RS and phenolic acid content measured in corn
153 flour, uncooked and cooked pasta obtained from both pasta-making processes. ANOVA was
154 performed on starch properties susceptibility to α -amylase, color and cooking behavior of pasta, in
155 which the combination of the level of HA corn flour substitution and the pasta-making process was set
156 as an independent variable. The Ryan/Einot and Gabriel/Welsch F (REGW-F) test on treatment
157 means was performed for multiple comparison purposes.

158 **3. Results and discussion**

159 *3.1 Pasta color*

160 The images of the pasta samples and the related color indices are reported in Supplementary Figure
161 1. As the HA level increased, the pasta became darker, with an increase in redness and a decrease in
162 yellowness, suggesting the occurrence of the Maillard reaction to different degrees in the products.
163 The worst change was found in pasta from HA, due to the prolonged steaming phase, together with
164 the high temperature, applied to the raw material in order to promote starch gelatinization. The
165 different gelatinization systems in Process A and Process B might account for the differences between
166 the related pasta. Specifically, pasta from Process A exhibited a significantly greater redness at each
167 HA substitution level.

168 *3.2 Phenolic acids and antioxidant capacity*

169 HA corn flour showed a significantly higher content of CWBPAs and AC (FRAP assay) than CV, while
170 SPA content did not differ among the raw materials (Figure 1 and 2). The higher concentration of
171 CWBPAs in HA flour referred to a high content in ferulic (+41% than CV), *p*-coumaric (+33%) and
172 sinapic (+31%) acids, which are the main CWBPAs, and in other minor compounds such as
173 protocatechuic, hydroxybenzoic, caffeic and syringic acids (on average +40% than CV).

174 As is well known for different food matrices, extrusion cooking could lead to changes in phenolic acid
175 content (Hu, Zhang, Hu, Yu, Zho, & Sao, 2018; Zeng, Liu, Luo, Chen, & Gong, 2016), that are strictly
176 related to extrusion conditions (temperature, pressure, time, moisture) (Brennan, Brennan,
177 Derbyshire, & Tiwari, 2011), as well as to the type of food matrix. In a previous work on the dry-
178 extrusion process (Bresciani et al., 2020), the decrease in SPAs detected in CV and HA corn snacks
179 was -63% and -51%, respectively, while no change occurred for CWBPAs. In the present study, wet-
180 extrusion for pasta-making significantly affected the content of phenolic acids, emphasizing the
181 differences among the HA corn substitution levels. SPA concentration was significantly reduced by the
182 pasta-making process: on average Process B resulted in a greater loss (-69%) than Process A (-59%).
183 The decrease in SPAs was significantly higher in CV (-82%, average of process A and B) than HA (-
184 39%), while, as expected, their blends showed an intermediate behaviour (-77% for 25HA and -62%
185 for 50HA). SPA content increased after cooking, with cooked pasta from Process B resulting in a
186 significantly higher SPA concentration than pasta from Process A (+42%). The concentration of
187 CWBPAs significantly increased with pasta-making for 50HA (Process A, +61%) and HA (Processes A
188 and B, +104% and +52%, respectively), while no differences were observed between flour and
189 uncooked pasta when CV or 25HA were used. Furthermore, cooking significantly reduced CWBPA
190 concentration by 13% for 50HA and HA obtained through Process A, while no significant changes
191 were detected in other raw materials X pasta-making process combinations. These results are in
192 accordance with a previous study on black rice pasta, whose cooking promoted a decrease in total
193 bound phenolics and an increase in total free phenolics (Rocchetti et al., 2017). The effect of cooking
194 on phenolic acids may depend on several factors including the cooking procedure, the degree of
195 heating, the leaching into the cooking water, and the surface area exposed to water and oxygen
196 (Rocchetti et al., 2017).

197 Differences in AC among the raw materials were highlighted by pasta-making (Figure 2). In both
198 processes, HA uncooked pasta resulted in a significantly higher AC than CV (+63% and 151% for
199 ABTS and FRAP assays, respectively), while 25HA and 50HA showed intermediate values. As far as
200 the ABTS method is concerned, pasta-making resulted in a significant decrease in AC, followed by an

201 increase in cooked pasta only for HA (Processes A and B) and 25HA (Process A). Similarly, the FRAP
202 assay showed an increase in AC after pasta cooking only for HA (+23%).

203 *3.3 Starch properties*

204 *3.3.1 Starch susceptibility to α -amylase (or damaged starch)*

205 The susceptibility of starch to the α -amylase hydrolysis of pasta products is shown in Table 1.
206 Whenever applied to flours, the test provides information about the starch damage during milling (i.e.,
207 damaged starch). In the case of pasta, the index is related to starch modification (i.e., starch
208 swelling/gelatinization or retrogradation) occurring during processing (Marti et al., 2010). Since
209 gelatinized granules present high contact surface to the enzyme, the higher the value, the higher the
210 gelatinization degree of gelatinization. On the other hand, low values might be a consequence of
211 starch retrogradation, if the heating treatment is followed by cooling (Marti et al., 2010). The damaged
212 starch of raw materials ranged from 4.68 to 5.56 g/100 g for CV and HA, respectively (Bresciani et al.,
213 2020). The pasta-making process promoted the increases in the index, due to the combination of both
214 thermal and mechanical stresses that led to starch gelatinization. As the level of HA increased, starch
215 susceptibility decreased, reaching the lowest value in the pasta from HA flour, likely due to the high
216 amylose content and its difficulty to gelatinize during the process. Findings agreed with those reported
217 on snacks made by the dry-extrusion process (Bresciani et al., 2020). As regards the type of pasta-
218 making process, pasta samples from process A showed lower susceptibility to hydrolysis compared to
219 Process B, likely suggesting that a part of the starch was organized in a more compact structure, at
220 least in the regions easily accessible to hydrolysis.

221 *3.3.2 Pasting properties*

222 The effects of the pasta-making process on starch pasting properties is shown in Figure 3. CV flour
223 showed a pasting profile typical for corn flour, with a pasting temperature around 67 °C, a high
224 tendency to gelatinization (peak viscosity: 560 BU) and retrogradation (final viscosity: 925 BU;
225 setback: 620 BU). On the other hand, HA flour did not present a gelatinization profile, and
226 consequently no retrogradation tendency. Differences in pasting profiles between the two flours might
227 be related to the amylose content and, thus, to starch structure. Indeed, in HA flour, amylose is

228 packed in a more compact structure in the starch granules, limiting their gelatinization (Liu, Yuan,
229 Wang, Reimer, Isaak, & Ai, 2019). Consequently, replacing CV flour with 25% and 50% of HA flour led
230 to a gradual increase in pasting temperature (72 and 76 °C for 25HA and 50HA blends, respectively),
231 decrease in both peak (350 and 190 BU for 25HA and 50HA blends, respectively) and final (665 and
232 300 BU for 25HA and 50HA blends, respectively) viscosity values. As the HA flour level increased, the
233 breakdown value decreased (CV: 248 BU; 25HA: 130 BU; 50HA: 45 BU), suggesting that the starch
234 granules are more stable with regards to both thermal and shear actions. Once again, this behavior is
235 related to the low gelatinization properties of HA flour, which resisted the gelatinization phenomenon
236 maintaining its structure even at high temperatures, so that the breakdown value was not detectable.
237 Regardless of the type of process, all pasta samples showed lower gelatinization properties than the
238 related flours (Figure 3). Specifically, the pasting temperature and the maximum viscosity decreased,
239 showing a plateau during the holding period at 95 °C and suggesting the formation of a new
240 macromolecular organization during the pasta-making process. Indeed, the new structure showed
241 reduced viscosity values, probably due to either a relevant compactness or the contribution of those
242 starch granules that did not undergo gelatinization and reorganization during the process. Similar
243 results were found when either rice (Marti, Caramanico, Bottega, & Pagani, 2013) or durum wheat
244 semolina (Marti, Seetharaman, & Pagani, 2013) were processed into pasta.

245 As regards the type of process, pasta samples from either process A or B showed similar profiles of
246 gelatinization and retrogradation indicating that the process has a similar effect in modifying the starch
247 pasting properties. It should also be considered that the use of a high-temperature drying cycle might
248 have lowered the effect of the type of process on starch pasting properties. Despite that, some
249 differences between process A and B were observed. Specifically, all pasta samples made using
250 process A showed a higher viscosity at the beginning of the test (30 °C). This could be due to the
251 presence of gelatinized starch granules that are able to absorb water even at low temperatures.

252 In addition, pasta from process A required higher temperature for gelatinization to begin (+ 15.3 °C for
253 the pasta from CV) and for reaching maximum viscosity (+ 2.5 °C for the pasta from CV), which is the
254 peak of gelatinization, suggesting that a part of the starch was organized in a more compact structure.
255 These results agreed with the data on starch susceptibility in Table 1. Finally, pasta from process A

256 showed higher final viscosity (+ 60 UB for the pasta from CV) indicating that the gelatinized starch
257 granules were more prone to retrograde during the cooling step. Previous studies compared the
258 processing conditions used in our study for Process A to a conventional extrusion, using parboiled rice
259 as raw material (Marti et al., 2010; Marti et al., 2011). Applying a multidisciplinary approach, the
260 authors stated that extrusion- cooking (i.e. Process A) was able to create a structure with an external
261 region characterized by an amorphous structure and a core characterized by a crystalline structure.
262 On the other hand, data about Process B might suggest the formation of a more homogeneous
263 structure, thanks to the flour steaming treatment on a conveyor belt that could promote homogenous
264 gelatinization, in agreement with data in Table 1.

265 In addition to the differences stated above, pasta samples from process B exhibited a shoulder at
266 about 58 and 60 °C for CV and 25HA pasta samples respectively, before the beginning of
267 gelatinization (63 and 72°C, for CV and HA pasta samples, respectively), suggesting the presence of
268 two populations of starch granules that start to absorb water, swell and gelatinized at two different
269 temperatures. There are no differences for the other higher HA substitution levels. The first shoulder?
270 might be due to starch granules which have already been partially modified during the pasta-making
271 process; whereas the others - that were less gelatinized during the process - started to gelatinize at
272 higher temperatures. Differences between the two processes were evident in pasta samples from CV
273 and 25HA; as expected, a higher percentage of HA reduced the overall pasting profile and therefore
274 the differences were less notable.

275 3.3.3 *Resistant starch*

276 The RS content was measured in flours with the purpose of assessing the effect of the pasta-making
277 process on starch organization. RS content in flours ranged from 0.5% to 18.4%, for CV and HA corn
278 respectively, confirming previous data showing a RS content of 4.5% and 20.5% in conventional and
279 high amylose corn, respectively (Zhang et al., 2016).

280 In the case of the CV sample, neither the pasta-making or the cooking process affect the RS content,
281 which remained very low (< 1%) for all processes. Conversely, pasta-making significantly decreased
282 the RS content in HA-enriched pasta, resulting on average in a drop of -41% and -48% for process A
283 and B, respectively, due to starch gelatinization during processing. Similar results were obtained by

284 Zhang et al. (2016), while assessing the effect of dry-extrusion on RS. Specifically, dry-extrusion in a
285 co-rotating twin extruder significantly reduces RS by 60% for high amylose samples.

286 The cooking process did not affect the RS content for any HA substitution levels or pasta-making
287 processes. Interestingly, even after cooking, 50HA and HA pasta samples exhibited the highest RS
288 content (> 6 g/100g). In addition, as expected, RS values for HA-enriched pasta were higher
289 compared to those measured in commercial pasta from conventional corn (i.e., about 3 g/100g as
290 reported by Marti, Abbasi Parizad, Marengo, Erba, Pagani, & Casiraghi, 2017).

291 To the best of our knowledge there are no studies reporting the RS content in HA pasta. Indeed, the
292 available studies propose the addition of RS as an ingredient instead. Specifically, uncooked rice
293 pasta enriched in 20 g/100g of a RS ingredient (a high-amylose corn pure starch) showed 7.9 g/100g
294 of RS (Foschia, Beraldo, & Peressini, 2017). The authors attributed the 30% loss in RS to the steam
295 treatment (10 min at 130 °C) carried out to induce starch gelatinization during the pasta-making
296 process. Indeed, when the same ingredient was used in durum wheat pasta – whose process does
297 not require the steam treatment – Gelencsér, Gál, & Salgó (2010) a significant decrease in the RS
298 content was not observed. Otherwise, the drop of RS by 50% with cooking has been attributed to the
299 greater impact of thermal treatment and/or higher solid lost into cooking water for this matrix
300 (Gelencsér et al., 2010). According to this hypothesis, the use of corn flour naturally rich in RS could
301 determine lower RS loss during cooking.

302 *3.4 Cooking behaviour*

303 Adding HA hybrid to CV flour led to a decrease in cooking time (Table 2). In the case of HA and 50HA
304 pasta samples, the indicated cooking time did not represent the optimal cooking time but the time
305 within which the pasta maintained its structure before breaking up in the cooking water. Indeed,
306 samples produced with more than 25% of HA flour tended to break easily during cooking indicating a
307 less compact structure, unable to withstand cooking stresses. As regards water absorption, the value
308 decreased as the percentage of HA flour increased, due to the packed structure of HA starch that
309 limited gelatinization during the pasta-making process and therefore pasta water absorption during
310 cooking. Comparing the processes, process B produced a pasta with higher water absorption. This

311 result agrees with the data related to starch susceptibility to α -amylase hydrolysis (Table 1). Indeed,
312 the higher starch susceptibility to hydrolysis of pasta from Process B might suggest the presence of
313 external layers able to absorb more water during cooking.

314 The cooking loss represents - together with pasta firmness - one of the main criteria for defining pasta
315 quality. Regardless of the type of process, pasta from CV flour showed values for both cooking loss
316 and firmness similar to those measured for a commercial corn pasta (data not shown). As the level of
317 HA flour increased, an increase in cooking loss and a decrease in firmness were observed, suggesting
318 the presence of a less compact structure and reaching unacceptable values in the case of HA pasta.
319 In the case of Process A, the increase in cooking loss seemed to be proportional with the level of HA
320 flour. The effect of the process was not evident in pasta from CV or 25HA; conversely, lower cooking
321 losses were found when process B was applied to high HA blends. The particular starch organization
322 coming from the pasting profile would account for the differences in cooking loss. Moreover, the lower
323 cooking loss in pasta from process B might account for the higher SPA content found in the related
324 cooked samples, compared to cooked pasta from process A (Fig. 1). On the other hand, the Kramer-
325 shear cell test was not able to highlight the effect of the process, except for 50HA pasta where
326 applying process B decreased the firmness of the cooked product. However, such data should be
327 confirmed by future studies focused on sensory analysis.

328 **4. Conclusion**

329 Overall, the results of this study highlighted that HA corn flour can be a suitable ingredient to produce
330 GF pasta with high RS and phenolic compounds. Pasta using 25HA resulted in 4.3 g of RS per 100g
331 for both processes even after cooking, so this pasta can be considered as a “source of fibre”
332 according to the nutritional requisite of Reg.1924/2006. Samples using 50HA, with 6.1 g of RS per
333 100g, could therefore be considered “higher in fibre”. Specifically, blending HA with CV at 25% level
334 represents an optimum compromise between health benefits and cooking behavior. At higher HA
335 substitution levels (i.e., 50%), some process level measures should be taken, such as flour pre-
336 gelatinization on a conveyor belt (process B) rather than in a tank (process A). Indeed, starch
337 gelatinization of HA seemed to be more homogeneous when thin layers of the material were placed on
338 the conveyor belt and subjected to steam treatment for a few minutes. Thus, process B produced HA

339 pasta with lower cooking loss and higher SPAs after cooking, while maintaining its high level of
340 resistant starch.

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346 **References**

347 AACC Approved Methods of Analysis, 2001. Cereals & Grains Association, St. Paul, MN, U.S.A.

348 Alfieri, M., Bresciani, A., Zanoletti, M., Pagani, M. A., Marti, A., & Redaelli, R. (2020). Physical,
349 chemical and pasting features of maize Italian inbred lines. *European Food Research and*
350 *Technology*, 246, 2205–2214. <https://doi.org/10.1007/s00217-020-03565-1>.

351 Bacchetti, T., Saturni, L., Turco, I., & Ferretti, G. (2014). The postprandial glucose response to some
352 varieties of commercially available gluten-free pasta: a comparison between healthy and celiac
353 subjects. *Food & Function*, 5, 3014-3017. <https://doi.org/10.1039/c4fo00745j>.

354 Barbiroli, A., Bonomi, F., Casiraghi, M. C., Iametti, S., Pagani, M. A., & Marti, A. (2013). Process
355 conditions affect starch structure and its interactions with proteins in rice pasta. *Carbohydrate*
356 *Polymers*, 92, 1865-1872. <https://doi.org/10.1016/j.carbpol.2012.11.047>

357 Berti, C., Riso, P., Monti, L.D., & Porrini, M. (2004). In vitro starch digestibility and in vivo glucose
358 response of gluten-free foods and their gluten counterparts. *European Journal of Nutrition*, 43, 198-
359 204. <https://doi.org/10.1007/s00394-004-0459-1>.

360 Brennan, C., Brennan, M., Derbyshire, E., & Tiwari, B. K. (2011). Effects of extrusion on the
361 polyphenols, vitamins and antioxidant activity of foods. *Trends in Food Science & Technology*, 22,
362 570-575. <https://doi.org/10.1016/j.tifs.2011.05.007>.

363 Bresciani, A., Giordano, D., Vanara, F., Blandino, M., & Marti, A. (2020). The effect of the amylose
364 content and milling fractions on the physico-chemical features of co-extruded snacks from corn. *Food*
365 *Chemistry*. <https://doi.org/10.1016/j.foodchem.2020.128503>.

366 Dexter, J. E., & Matsuo, R. R. (1979). Effect of starch on pasta dough rheology and spaghetti cooking
367 quality. *Cereal Chemistry*, 56, 190-195.

368 European Commission (2006). Commission Regulation (EC) No. 1924/2006, of 20 December 2006 on
369 nutrition and health claims made on foods. *Official Journal of European Union*, 404, 9-25.

370 Foschia, M., Beraldo, P., & Peressini, D. (2017). Evaluation of the physicochemical properties of
371 gluten-free pasta enriched with resistant starch. *Journal of Science and Food Agriculture*, 97, 572-577.
372 <https://doi.org/10.1002/jsfa.7766>.

373 Gelencsér, T., Gál, V., & Salgó, A. (2010). Effects of applied process on the in vitro digestibility and
374 resistant starch content of pasta products. *Food and Bioprocess Technology*, 3, 491-497.
375 <https://doi.org/10.1007/s11947-008-0105-7>.

376 Giordano, D., Reyneri, A., Locatelli, M., Coïsson, J. D., & Blandino, M. (2019). Distribution of bioactive
377 compounds in pearled fractions of tritordeum. *Food Chemistry*, 301, 125228.
378 <https://doi.org/10.1016/j.foodchem.2019.125228>.

379 Hu, Z., Tang, X., Zhang, M., Hu, X., Yu, C., Zhu, Z., & Shao, Y. (2018). Effects of different extrusion
380 temperatures on extrusion behavior, phenolic acids, antioxidant activity, anthocyanins and
381 phytosterols of black rice. *RSC Advances*, 8, 7123-7132. <http://dx.doi.org/10.1039/c7ra13329d>.

382 Li, W., Wei, C., White, P. J., & Beta, T. (2007). High-amylose corn exhibits better antioxidant activity
383 than typical and waxy genotypes. *Journal of Agricultural and Food Chemistry*, 55, 291-298.
384 <https://doi.org/10.1021/jf0622432>.

385 Liu, S., Yuan, T. Z., Wang, X., Reimer, M., Isaak, C., & Ai, Y. (2019). Behaviors of starches evaluated
386 at high heating temperatures using a new model of Rapid Visco Analyzer–RVA 4800. *Food*
387 *Hydrocolloids*, *94*, 217-228. <https://doi.org/10.1016/j.foodhyd.2019.03.015>.

388 Marti, A., Seetharaman, K., & Pagani, M. A. (2010). Rice-based pasta: A comparison between
389 conventional pasta-making and extrusion-cooking. *Journal of Cereal Science*, *52*, 404-409.
390 <https://doi.org/10.1016/j.jcs.2010.07.002>.

391 Marti, A., Pagani, M. A., & Seetharaman, K. (2011). Understanding starch organisation in gluten-free
392 pasta from rice flour. *Carbohydrate Polymers*, *84*, 1069-1074.
393 <https://doi.org/10.1016/j.carbpol.2010.12.070>.

394 Marti, A., & Pagani, M. A. (2013). What can play the role of gluten in gluten free pasta? *Trends in*
395 *Food Science & Technology*, *31*, 63-71. <https://doi.org/10.1016/j.tifs.2013.03.001>.

396 Marti, A., Caramanico, R., Bottega, G., & Pagani, M. A. (2013). Cooking behavior of rice pasta: Effect
397 of thermal treatments and extrusion conditions. *LWT-Food Science and Technology*, *54*, 229-235.
398 <https://doi.org/10.1016/j.lwt.2013.05.008>.

399 Marti, A., Seetharaman, K., & Pagani, M. A. (2013). Rheological approaches suitable for investigating
400 starch and protein properties related to cooking quality of durum wheat pasta. *Journal of Food Quality*,
401 *36*, 133-138. <https://doi.org/10.1111/jfq.12015>.

402 Marti, A., Abbasi Parizad, P., Marengo, M., Erba, D., Pagani, M. A., & Casiraghi, M. C. (2017). In vitro
403 starch digestibility of commercial gluten-free pasta: the role of ingredients and origin. *Journal of Food*
404 *Science*, *82*, 1012-1019. <https://doi.org/10.1111/1750-3841.13673>.

405 Morreale, F., Boukid, F., Carini, E., Federici, E., Vittadini, E., & Pellegrini, N. (2019). An overview of
406 the Italian market for 2015: cooking quality and nutritional value of gluten-free pasta. *International*
407 *Journal of Food Science & Technology*, *54*, 780-786. <https://doi.org/10.1111/ijfs.13995>.

408 Nuss, E. T., & Tanumihardjo, S. A. (2010). Maize: a paramount staple crop in the context of global
409 nutrition. *Comprehensive Reviews in Food Science and Food Safety*, 9, 417-436.
410 <https://doi.org/10.1111/j.1541-4337.2010.00117.x>.

411 Pellegrini, N., & Agostoni, C. (2015). Nutritional aspects of gluten free products. *Journal of Science
412 and Food Agriculture*, 95, 2380–2385. <https://doi.org/10.1002/jsfa.7101>.

413 Rocchetti, G., Lucini L. Chiodelli, G., Giuberti G., Montesano, D., Masoero, F., & Trevisan, M. (2017).
414 Impact of boiling on free and bound phenolic profile and antioxidant activity of commercial gluten-free
415 pasta. *Food Research International*, 100, 69-77. <http://dx.doi.org/10.1016/j.foodres.2017.08.031>

416 Sajilata, M.G., Singhal, R.S., & Kulkarni P.R. (2006). Resistant Starch, A Review. *Comprehensive
417 Reviews in Food Science and Food Safety*, 5, 1-17. [https://doi.org/10.1111/j.1541-
418 4337.2006.tb00076.x](https://doi.org/10.1111/j.1541-4337.2006.tb00076.x).

419 Serpen, A., Gökmen, V., & Fogliano V. (2012). Solvent effects on total antioxidant capacity of foods
420 measured by direct QUENCHER procedure. *Journal of Food Composition and Analysis*, 26, 52-57.
421 <https://doi.org/10.1016/j.jfca.2012.02.005>.

422 Tam, L. M., Corke, H., Tan, W. T., Li, J., & Collado, L. S. (2004). Production of bihon-type noodles
423 from maize starch differing in amylose content. *Cereal Chemistry*, 81, 475-480.
424 <https://doi.org/10.1094/CCHEM.2004.81.4.475>.

425 Zeng, Z., Liu, C., Luo, S., Chen, J., & Gong, E. (2016). The profile and bioaccessibility of phenolic
426 compounds in cereals influenced by improved extrusion cooking treatment. *PloS one*, 11(8),
427 e0161086. <https://doi.org/10.1371/journal.pone.0161086>.

428 Zhang, X., Chen, Y., Zangh, R., Zhong, Y., Luo, Y., Xu, S., Liu, J., Xue, J., & Guo, D. (2016). Effects
429 of extrusion treatment on physicochemical properties and in vitro digestion of pregelatinized high
430 amylose maize flour. *Journal of cereal Science*, 68, 108-115. <https://doi.org/10.1016/j.jcs.2016.01.005>.

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Figure 1. Soluble (SPAs) and cell-wall bound (CWBPAs) phenolic acids content in corn flour, uncooked and cooked pasta, made with different substitution level of high amylose corn and pasta-making processes.

Figure 2. Antioxidant capacity (AC) measured by means of the ABTS and FRAP assays in corn flour, uncooked and cooked pasta, made with different substitution levels of high amylose corn and pasta-making processes.

Figure 3. Effect of pasta-making process on starch pasting properties.

Figure 4. Resistant starch (RS) in corn flour, uncooked and cooked pasta, made with different substitution levels of high amylose corn and pasta-making processes.

Supplementary Figure 1. Images of pasta samples and color indices.

