

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

Andrea Bresciani<sup>1</sup>, Debora Giordano<sup>2</sup>, Francesca Vanara<sup>2</sup>, Massimo Blandino<sup>2,\*</sup>, Alessandra Marti<sup>1,\*</sup>

<sup>1</sup> Department of Food, Environmental and Nutritional Sciences (DeFENS), Università degli Studi di Milano, via G. Celoria 2, 20133 Milan, Italy

<sup>2</sup> Department of Agricultural, Forest and Food Sciences (DISAFA), Università degli Studi di Torino, Largo Braccini 2, 10095, Grugliasco (TO), Italy

\* corresponding author: A.M.: [alessandra.marti@unimi.it](mailto:alessandra.marti@unimi.it); M.B.: [massimo.blandino@unito.it](mailto:massimo.blandino@unito.it)

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  $\alpha$ -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  $\mu\text{m}$ ) 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  $\alpha$ -amylase,  
98 pasting properties, phenolic acids and total antioxidant capacity samples were milled (particle size less  
99 than 250  $\mu\text{m}$ ) 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  $\alpha$ -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  $\alpha$ -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 $\alpha$ -amylase (or damaged starch)

205 The susceptibility of starch to the  $\alpha$ -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  $\alpha$ -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.

Table 1. Starch susceptibility to  $\alpha$ -amylase of pasta samples (g/100 g uncooked pasta)

Pasta	Process A	Process B
CV	14.33 $\pm$ 0.67 b	16.42 $\pm$ 0.06 a
25HA	12.99 $\pm$ 0.18 bc	14.50 $\pm$ 0.54 b
50HA	11.79 $\pm$ 0.54 c	13.48 $\pm$ 0.24 bc
HA	8.86 $\pm$ 0.96 d	12.27 $\pm$ 0.25 c

CV, use of flour from conventional corn only; 25HA, blend 75%-25% of flour from conventional and high amylose corn; 50HA, blend 50-50% of flour from conventional and high amylose corn; HA, use of flour from high amylose corn only.

Pasta making-process: A, with flour pre-gelatinization in tank; B, with flour pre-gelatinization on a conveyor belt.

Values with different letters are significantly different ( $P < 0.001$ ), according to the REGW-F test. Reported values are based on two replications.

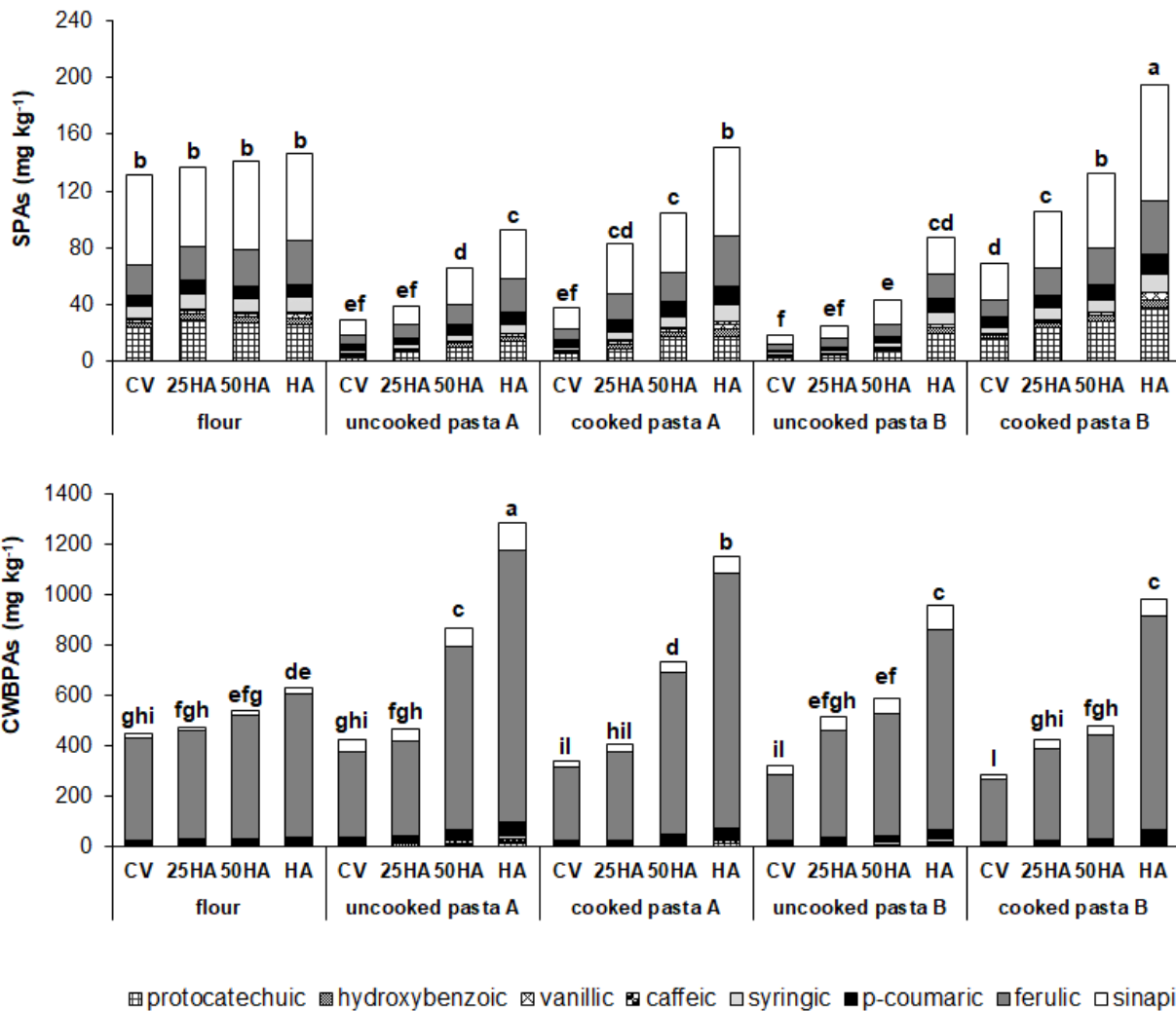
Table 2. Cooking behavior of pasta samples

Pasta	Optimal cooking time (min)		Weight increase (g/100 g pasta)		Cooking loss (g/100 g pasta)		Firmness (N)	
	Process A	Process B	Process A	Process B	Process A	Process B	Process A	Process B
CV	13	14	89.8 ± 2.8 ab	91.6 ± 1.9 a	2.63 ± 0.19 e	2.95 ± 0.32 e	685.5 ± 38.2 a	693.5 ± 16.9 a
			80.7 ± 1.2 c	88.2 ± 0.3 b	4.38 ± 0.18 cd	2.92 ± 0.23 e	591.9 ± 27.2 b	632.5 ± 29.8 b
25HA	12	12	81.3 ± 1.2 c	89.4 ± 0.6 ab	5.30 ± 0.39 c	4.18 ± 0.51 d	524.5 ± 32.3 c	450.9 ± 25.4 d
			17.1 ± 0.9 e	65.4 ± 3.6 d	42.76 ± 0.85 a	15.49 ± 1.60 b	325.2 ± 17.6 e	373.0 ± 25.4 e
50HA	13	13	89.8 ± 2.8 ab	91.6 ± 1.9 a	2.63 ± 0.19 e	2.95 ± 0.32 e	685.5 ± 38.2 a	693.5 ± 16.9 a
			80.7 ± 1.2 c	88.2 ± 0.3 b	4.38 ± 0.18 cd	2.92 ± 0.23 e	591.9 ± 27.2 b	632.5 ± 29.8 b
HA	9	11	81.3 ± 1.2 c	89.4 ± 0.6 ab	5.30 ± 0.39 c	4.18 ± 0.51 d	524.5 ± 32.3 c	450.9 ± 25.4 d
			17.1 ± 0.9 e	65.4 ± 3.6 d	42.76 ± 0.85 a	15.49 ± 1.60 b	325.2 ± 17.6 e	373.0 ± 25.4 e

CV, use of flour from conventional corn only; 25HA, blend 75%-25% of flour from conventional and high amylose corn; 50HA, blend 50-50% of flour from conventional and high amylose corn; HA, use of flour from high amylose corn only.

Pasta making-process: A, with flour pre-gelatinization in tank; B, with flour pre-gelatinization on a conveyor belt.

Values with different letters are significantly different ( $P < 0.001$ ), according to the REGW-F test. Reported values are based on four independent cooking trials. For cooking loss determination, two subsamples from each cooking trial were considered.



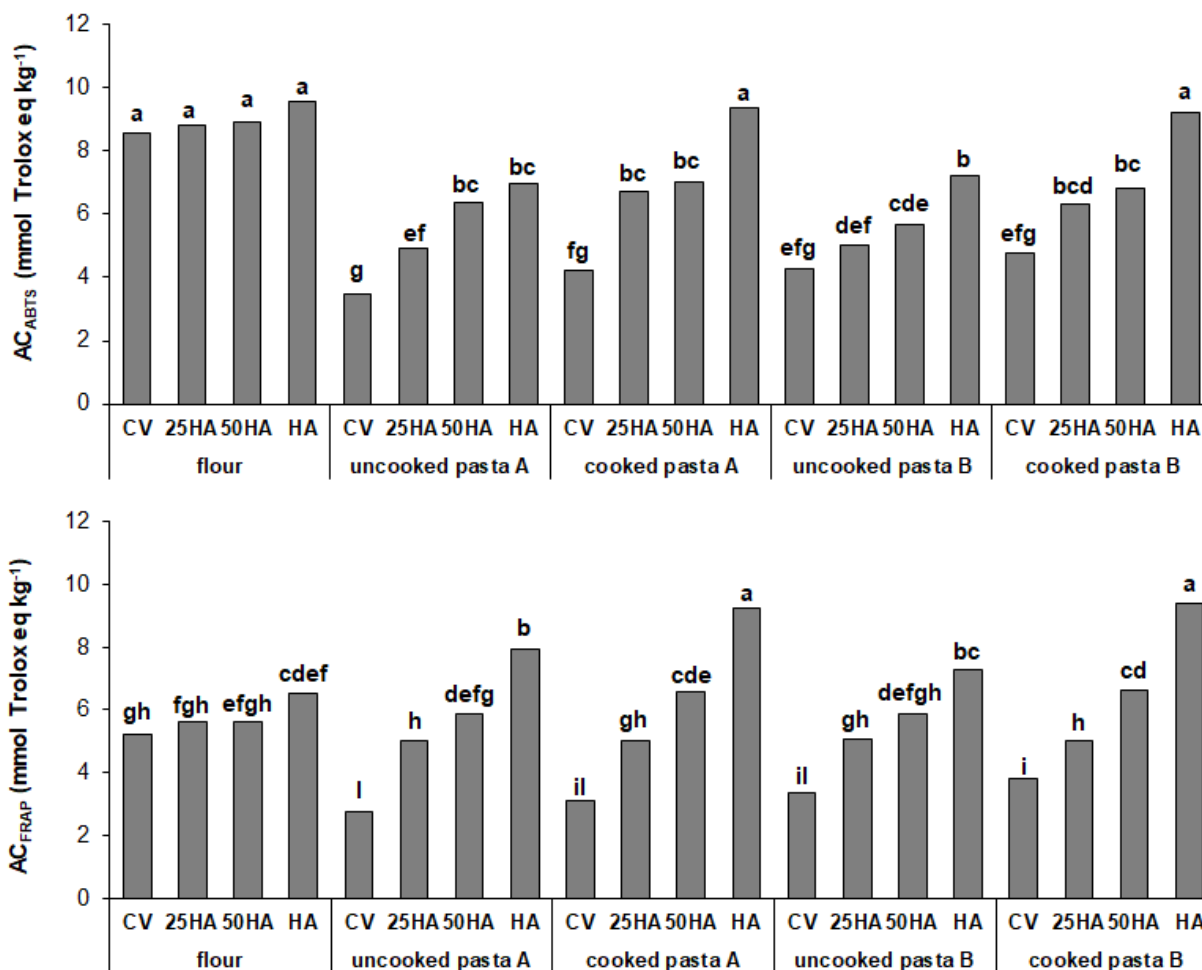
**Figure 1.** Soluble (SPAs) and cell-wall bound (CWBPAs) phenolic acid content in corn flour, uncooked and cooked pasta, made with different substitution level of high amylose corn and pasta-making processes.

Data are expressed on a dw basis.

CV, use of flour from conventional corn only; 25HA, blend 75%-25% of flour from conventional and high amylose corn; 50HA, blend 50-50% of flour from conventional and high amylose corn; HA, use of flour from high amylose corn only.

Pasta making-process: A, with flour pre-gelatinization in tank; B, with flour pre-gelatinization on a conveyor belt.

Bars with different letters are significantly different ( $P < 0.001$ ), according to the REGW-F test. Reported values are based on 3 replications.



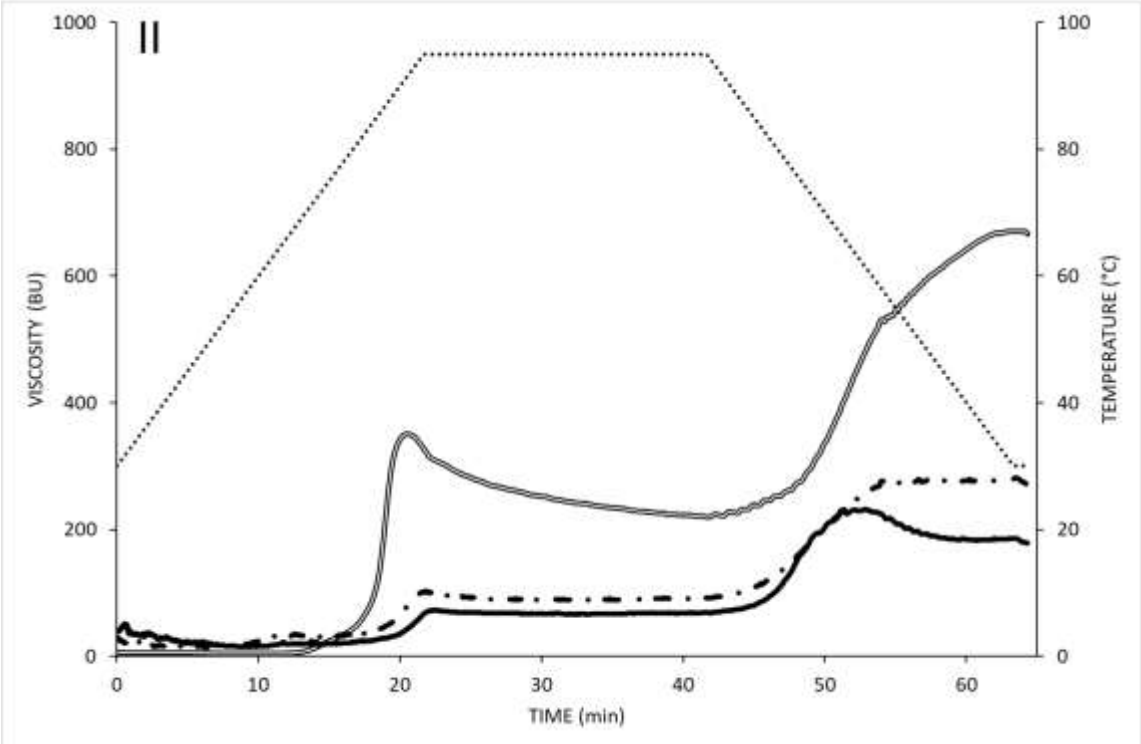
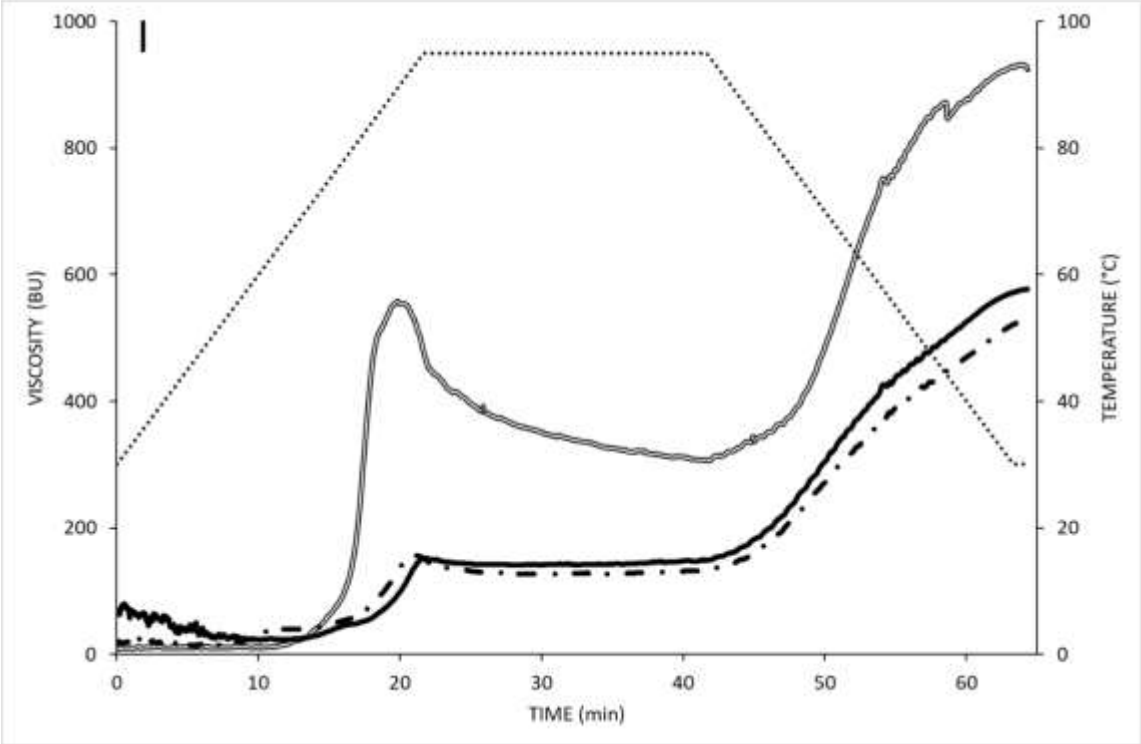
**Figure 2.** Antioxidant capacity (AC) measured by means of the ABTS and FRAP assays in corn flour, uncooked and cooked pasta, made with different substitution levels of high amylose corn and pasta-making processes.

Data are expressed on a dw basis.

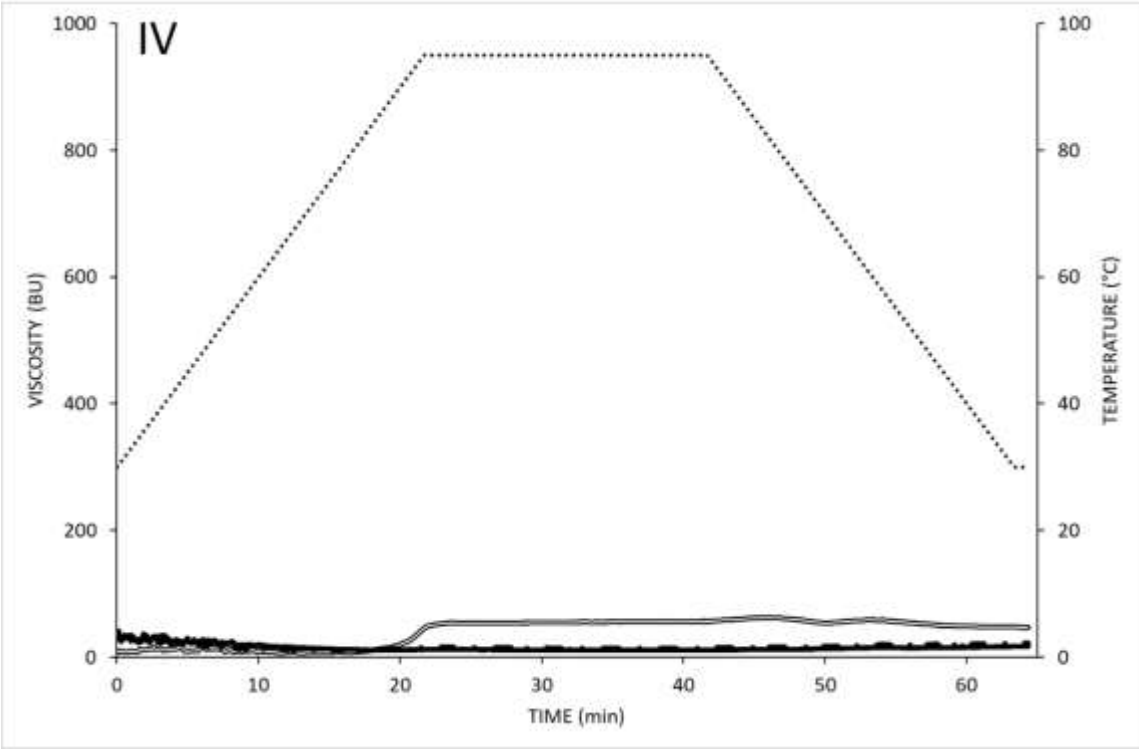
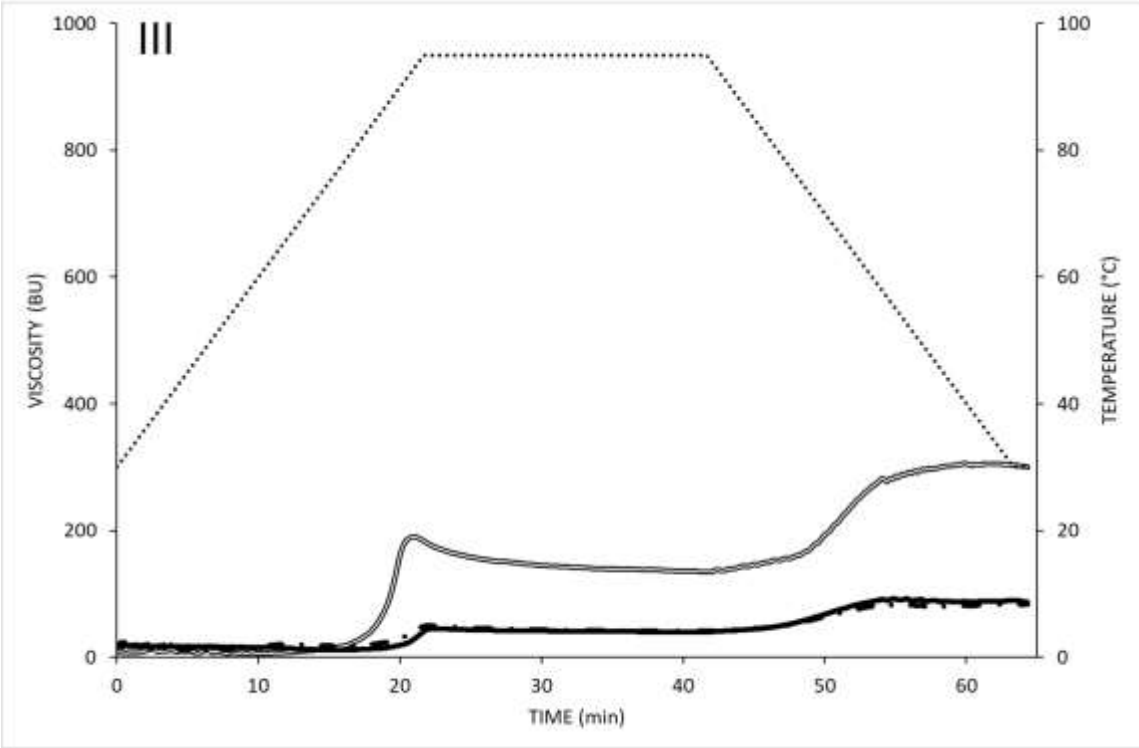
CV, use of flour from conventional corn only; 25HA, blend 75%-25% of flour from conventional and high amylose corn; 50HA, blend 50-50% of flour from conventional and high amylose corn; HA, use of flour from high amylose corn only.

Pasta making-process: A, with flour pre-gelatinization in tank; B, with flour pre-gelatinization on a conveyor belt.

Bars with different letters are significantly different (P < 0.001), according to the REGW-F test. Reported values are based on 3 replications.



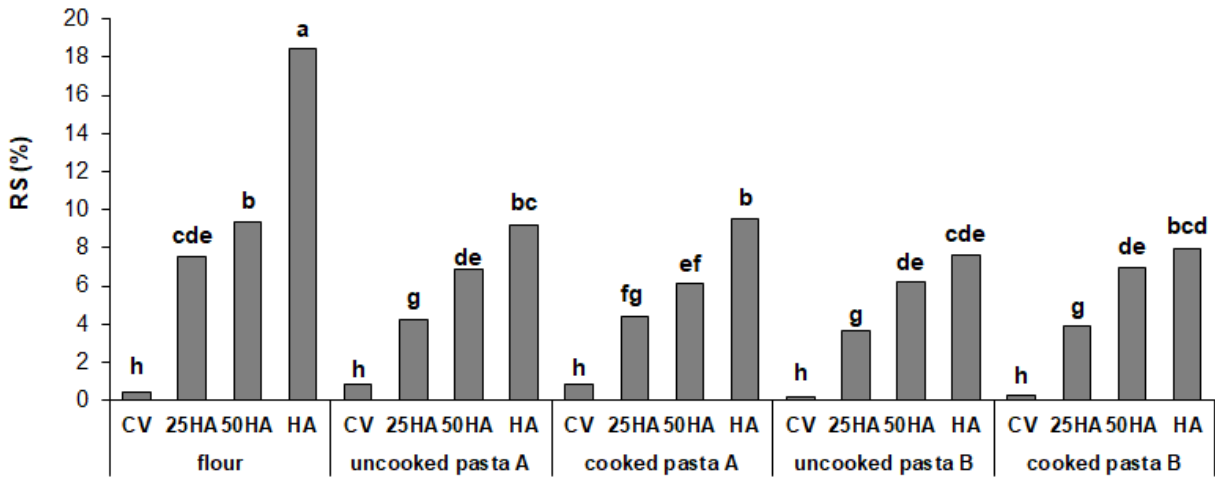




**Figure 3.** Pasting profile of pasta samples from (I) CV, (II) 25HA, (III) 50HA and (IV) HA flours.

CV, use of flour from conventional corn only; 25HA, blend 75%-25% of flour from conventional and high amylose corn; 50HA, blend 50-50% of flour from conventional and high amylose corn; HA, use of flour from high amylose corn only.

Flour (grey line); Process A (black solid line), pasta-making process with flour pre-gelatinization in tank  
Process B (black dotted line), pasta-making process with flour pre-gelatinization on a conveyor belt.



**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.

Data are expressed on a dw basis.

CV, use of flour from conventional corn only; 25HA, blend 75%-25% of flour from conventional and high amylose corn; 50HA, blend 50-50% of flour from conventional and high amylose corn; HA, use of flour from high amylose corn only.

Pasta making-process: A, with flour pre-gelatinization in tank; B, with flour pre-gelatinization on a conveyor belt.

Bars with different letters are significantly different ( $P < 0.001$ ), according to the REGW-F test. Reported values are based on 3 replications.