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

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

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

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

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

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

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

As regards the raw materials, the role of amylose has been extensively studied in noodles: on one hand, starches with low amylose content (i.e., waxy lines) leads to poor cooking quality (Dexter & Matsuo, 1979); on the other hand, starches high in amylose (> 40%) do not seem to provide an adequate degree of gelatinization during the thermal process, limiting the extent of further starch
retrogradation (Tam, Corke, Tan, Li, & Collado, 2004). However, to the best of our knowledge, most of the studies on the relation between amylose content and product quality have been carried out on noodles rather than on pasta which are different in formulation (starch vs flour), processing (sheeting vs extrusion), shape and thus texture (Marti & Pagani, 2013). In addition, no solutions have been proposed so far. Indeed, despite the technological issues related to high amylose content, starches rich in amylose are interesting from a nutritional standpoint, since they are a good source of resistant starch (RS). Many authors (Pellegrini & Agostoni, 2015; Berti, Riso, Monti, & Porrini, 2004) have stressed that GF products result in a high glyceamic index (GI) and glyceamic load (GL). As far as GF pasta is concerned, GI and GL values were higher in rice pasta compared to corn pasta or to pasta containing both corn and rice as the main ingredients (Bacchetti, Saturni, Turco, & Ferretti, 2014). Conversely, ingredients rich in RS make for products that are low in calories and GL (Sajilata, Singhal, & Kulkarni, 2006).

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

2.1 Corn flours

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

2.2 Pasta-making process

Flours were mixed to mono- and di-glycerides of fatty acids (0.3%) and processed into pasta by extrusion-cooking using two different pre-gelatinization systems. In process A, flour pre-gelatinization was carried out in a pre-gelatinization tank (Braibanti, Milan, Italy). Specifically, flour and water (30% final moisture content) were treated with steam at 130 °C for 15 min for CV, at 130 °C for 15 min for 25HA and 50HA, and at 130 °C for 30 min for HA. In process B, the flour-water mixture was treated with steam (110 °C for 2 min) on a conveyor belt (Fava S.p.A., Cento, Italy) and fed into the extruder. The pre-gelatinized mixture from either process A or B was extruded (screw temperature: 110 °C) into small pellets (cylinder shape; 3 mm diameter), and then formed into a macaroni shape using a continuous press for semolina pasta production (Braibanti, Milan, Italy). A jacket with cold water kept dough temperature at about 50 °C at an extrusion pressure of $10^7$ Pa. All samples were dried in an experimental drying cell (Fava S.p.A., Cento, Italy) using a high-temperature drying cycle (70 °C for 3.5 h).

All samples were stored at room temperature until analyzed. For starch susceptibility to α-amylase, pasting properties, phenolic acids and total antioxidant capacity samples were milled (particle size less than 250 µm) using a laboratory mill (IKA Universalmühle M20; IKA Laborteknic, Staufen, Germany), with a water-cooling system to avoid overheating.
The moisture content of both flours and pasta samples were determined by oven-drying at 105 °C for 24 h in order to express all the results as dry weight (dw).

2.3 Pasta cooking procedure

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

2.4 Pasta colour

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

2.5 Phenolic acids and total antioxidant capacity

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

2.6 Starch properties
Starch susceptibility to $\alpha$-amylase (or damaged starch) was assessed on the uncooked pasta according to the standard method AACC 76-31.01 (AACCI, 2001).

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

The amount of resistant starch was measured in corn flour, uncooked and cooked pasta according to the standard method AACC 32-40.01 (AACCI, 2001).

2.7 Cooking quality

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

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

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

2.8 Statistics

Pasta color was determined on ten pieces of pasta. Starch susceptibility and pasting properties were measured in duplicate. All the chemical analyses were performed in triplicate. Four independent
Cooking trials were carried out for pasta weight increase, cooking loss and texture analysis. For cooking loss determination, two subsamples from each cooking trial were assessed.

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

3. Results and discussion

3.1 Pasta color

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

3.2 Phenolic acids and antioxidant capacity

HA corn flour showed a significantly higher content of CWBPAs and AC (FRAP assay) than CV, while SPA content did not differ among the raw materials (Figure 1 and 2). The higher concentration of CWBPAs in HA flour referred to a high content in ferulic (+41% than CV), p-coumaric (+33%) and sinapic (+31%) acids, which are the main CWBPAs, and in other minor compounds such as protocatechuic, hydroxybenzoic, caffeic and syringic acids (on average +40% than CV).
As is well known for different food matrices, extrusion cooking could lead to changes in phenolic acid content (Hu, Zhang, Hu, Yu, Zho, & Sao, 2018; Zeng, Liu, Luo, Chen, & Gong, 2016), that are strictly related to extrusion conditions (temperature, pressure, time, moisture) (Brennan, Brennan, Derbyshire, & Tiwari, 2011), as well as to the type of food matrix. In a previous work on the dry-extrusion process (Bresciani et al., 2020), the decrease in SPAs detected in CV and HA corn snacks was -63% and -51%, respectively, while no change occurred for CWBPAs. In the present study, wet-extrusion for pasta-making significantly affected the content of phenolic acids, emphasizing the differences among the HA corn substitution levels. SPA concentration was significantly reduced by the pasta-making process: on average Process B resulted in a greater loss (-69%) than Process A (-59%). The decrease in SPAs was significantly higher in CV (-82%, average of process A and B) than HA (-39%), while, as expected, their blends showed an intermediate behaviour (-77% for 25HA and -62% for 50HA). SPA content increased after cooking, with cooked pasta from Process B resulting in a significantly higher SPA concentration than pasta from Process A (+42%). The concentration of CWBPAs significantly increased with pasta-making for 50HA (Process A, +61%) and HA (Processes A and B, +104% and +52%, respectively), while no differences were observed between flour and uncooked pasta when CV or 25HA were used. Furthermore, cooking significantly reduced CWBPA concentration by 13% for 50HA and HA obtained through Process A, while no significant changes were detected in other raw materials X pasta-making process combinations. These results are in accordance with a previous study on black rice pasta, whose cooking promoted a decrease in total bound phenolics and an increase in total free phenolics (Rocchetti et al., 2017). The effect of cooking on phenolic acids may depend on several factors including the cooking procedure, the degree of heating, the leaching into the cooking water, and the surface area exposed to water and oxygen (Rocchetti et al., 2017).

Differences in AC among the raw materials were highlighted by pasta-making (Figure 2). In both processes, HA uncooked pasta resulted in a significantly higher AC than CV (+63% and 151% for ABTS and FRAP assays, respectively), while 25HA and 50HA showed intermediate values. As far as the ABTS method is concerned, pasta-making resulted in a significant decrease in AC, followed by an
increase in cooked pasta only for HA (Processes A and B) and 25HA (Process A). Similarly, the FRAP assay showed an increase in AC after pasta cooking only for HA (+23%).

3.3 Starch properties

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

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

3.3.2 Pasting properties

The effects of the pasta-making process on starch pasting properties is shown in Figure 3. CV flour showed a pasting profile typical for corn flour, with a pasting temperature around 67 °C, a high tendency to gelatinization (peak viscosity: 560 BU) and retrogradation (final viscosity: 925 BU; setback: 620 BU). On the other hand, HA flour did not present a gelatinization profile, and consequently no retrogradation tendency. Differences in pasting profiles between the two flours might be related to the amylose content and, thus, to starch structure. Indeed, in HA flour, amylose is
packed in a more compact structure in the starch granules, limiting their gelatinization (Liu, Yuan, Wang, Reimer, Isaak, & Ai, 2019). Consequently, replacing CV flour with 25% and 50% of HA flour led to a gradual increase in pasting temperature (72 and 76 °C for 25HA and 50HA blends, respectively), decrease in both peak (350 and 190 BU for 25HA and 50HA blends, respectively) and final (665 and 300 BU for 25HA and 50HA blends, respectively) viscosity values. As the HA flour level increased, the breakdown value decreased (CV: 248 BU; 25HA: 130 BU; 50HA: 45 BU), suggesting that the starch granules are more stable with regards to both thermal and shear actions. Once again, this behavior is related to the low gelatinization properties of HA flour, which resisted the gelatinization phenomenon maintaining its structure even at high temperatures, so that the breakdown value was not detectable. Regardless of the type of process, all pasta samples showed lower gelatinization properties than the related flours (Figure 3). Specifically, the pasting temperature and the maximum viscosity decreased, showing a plateau during the holding period at 95 °C and suggesting the formation of a new macromolecular organization during the pasta-making process. Indeed, the new structure showed reduced viscosity values, probably due to either a relevant compactness or the contribution of those starch granules that did not undergo gelatinization and reorganization during the process. Similar results were found when either rice (Marti, Caramanico, Bottega, & Pagani, 2013) or durum wheat semolina (Marti, Seetharaman, & Pagani, 2013) were processed into pasta. As regards the type of process, pasta samples from either process A or B showed similar profiles of gelatinization and retrogradation indicating that the process has a similar effect in modifying the starch pasting properties. It should also be considered that the use of a high-temperature drying cycle might have lowered the effect of the type of process on starch pasting properties. Despite that, some differences between process A and B were observed. Specifically, all pasta samples made using process A showed a higher viscosity at the beginning of the test (30 °C). This could be due to the presence of gelatinized starch granules that are able to absorb water even at low temperatures. In addition, pasta from process A required higher temperature for gelatinization to begin (+ 15.3 °C for the pasta from CV) and for reaching maximum viscosity (+ 2.5 °C for the pasta from CV), which is the peak of gelatinization, suggesting that a part of the starch was organized in a more compact structure. These results agreed with the data on starch susceptibility in Table 1. Finally, pasta from process A
showed higher final viscosity (+ 60 UB for the pasta from CV) indicating that the gelatinized starch granules were more prone to retrograde during the cooling step. Previous studies compared the processing conditions used in our study for Process A to a conventional extrusion, using parboiled rice as raw material (Marti et al., 2010; Marti et al., 2011). Applying a multidisciplinary approach, the authors stated that extrusion-cooking (i.e. Process A) was able to create a structure with an external region characterized by an amorphous structure and a core characterized by a crystalline structure. On the other hand, data about Process B might suggest the formation of a more homogeneous structure, thanks to the flour steaming treatment on a conveyor belt that could promote homogenous gelatinization, in agreement with data in Table 1.

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

3.3.3 Resistant starch

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

In the case of the CV sample, neither the pasta-making or the cooking process affect the RS content, which remained very low (< 1%) for all processes. Conversely, pasta-making significantly decreased the RS content in HA-enriched pasta, resulting on average in a drop of -41% and -48% for process A and B, respectively, due to starch gelatinization during processing. Similar results were obtained by
Zhang et al. (2016), while assessing the effect of dry-extrusion on RS. Specifically, dry-extrusion in a co-rotating twin extruder significantly reduces RS by 60% for high amylose samples. The cooking process did not affect the RS content for any HA substitution levels or pasta-making processes. Interestingly, even after cooking, 50HA and HA pasta samples exhibited the highest RS content (> 6 g/100g). In addition, as expected, RS values for HA-enriched pasta were higher compared to those measured in commercial pasta from conventional corn (i.e., about 3 g/100g as reported by Marti, Abbasi Parizad, Marengo, Erba, Pagani, & Casiraghi, 2017).

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

3.4 Cooking behaviour

Adding HA hybrid to CV flour led to a decrease in cooking time (Table 2). In the case of HA and 50HA pasta samples, the indicated cooking time did not represent the optimal cooking time but the time within which the pasta maintained its structure before breaking up in the cooking water. Indeed, samples produced with more than 25% of HA flour tended to break easily during cooking indicating a less compact structure, unable to withstand cooking stresses. As regards water absorption, the value decreased as the percentage of HA flour increased, due to the packed structure of HA starch that limited gelatinization during the pasta-making process and therefore pasta water absorption during cooking. Comparing the processes, process B produced a pasta with higher water absorption. This
result agrees with the data related to starch susceptibility to $\alpha$-amylase hydrolysis (Table 1). Indeed, the higher starch susceptibility to hydrolysis of pasta from Process B might suggest the presence of external layers able to absorb more water during cooking. The cooking loss represents - together with pasta firmness - one of the main criteria for defining pasta quality. Regardless of the type of process, pasta from CV flour showed values for both cooking loss and firmness similar to those measured for a commercial corn pasta (data not shown). As the level of HA flour increased, an increase in cooking loss and a decrease in firmness were observed, suggesting the presence of a less compact structure and reaching unacceptable values in the case of HA pasta. In the case of Process A, the increase in cooking loss seemed to be proportional with the level of HA flour. The effect of the process was not evident in pasta from CV or 25HA; conversely, lower cooking losses were found when process B was applied to high HA blends. The particular starch organization coming from the pasting profile would account for the differences in cooking loss. Moreover, the lower cooking loss in pasta from process B might account for the higher SPA content found in the related cooked samples, compared to cooked pasta from process A (Fig. 1). On the other hand, the Kramer-shear cell test was not able to highlight the effect of the process, except for 50HA pasta where applying process B decreased the firmness of the cooked product. However, such data should be confirmed by future studies focused on sensory analysis.

4. Conclusion

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

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**References**


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


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 α-amylase of pasta samples (g/100 g uncooked pasta)

<table>
<thead>
<tr>
<th>Pasta</th>
<th>Process A</th>
<th>Process B</th>
</tr>
</thead>
<tbody>
<tr>
<td>CV</td>
<td>14.33 ± 0.67 b</td>
<td>16.42 ± 0.06 a</td>
</tr>
<tr>
<td>25HA</td>
<td>12.99 ± 0.18 bc</td>
<td>14.50 ± 0.54 b</td>
</tr>
<tr>
<td>50HA</td>
<td>11.79 ± 0.54 c</td>
<td>13.48 ± 0.24 bc</td>
</tr>
<tr>
<td>HA</td>
<td>8.86 ± 0.96 d</td>
<td>12.27 ± 0.25 c</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Pasta</th>
<th>Optimal cooking time (min)</th>
<th>Weight increase (g/100 g pasta)</th>
<th>Cooking loss (g/100 g pasta)</th>
<th>Firmness (N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CV</td>
<td>13</td>
<td>14</td>
<td>89.8 ± 2.8 ab</td>
<td>91.6 ± 1.9 a</td>
</tr>
<tr>
<td>25HA</td>
<td>12</td>
<td>12</td>
<td>80.7 ± 1.2 c</td>
<td>88.2 ± 0.3 b</td>
</tr>
<tr>
<td>50HA</td>
<td>13</td>
<td>13</td>
<td>81.3 ± 1.2 c</td>
<td>89.4 ± 0.6 ab</td>
</tr>
<tr>
<td>HA</td>
<td>9</td>
<td>11</td>
<td>17.1 ± 0.9 e</td>
<td>65.4 ± 3.6 d</td>
</tr>
</tbody>
</table>

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 (CWBPAAs) 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.