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The effect of the amylose content and milling fractions on the physico-chemical features of co-extruded snacks from corn

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Abbreviations: ABTS, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid); AC, antioxidant capacity; AC-ABTS, antioxidant capacity obtained by means of an ABTS assay; AC-FRAP, antioxidant capacity obtained by means of a FRAP assay; C, conventional hybrid; C-HA, conventional:high amylose hybrid blend (50:50); C-W, conventional:waxy hybrid blend (50:50); CWBPAs, cell wall-bound phenolic acids; FRAP, Ferric reducing antioxidant power; HA, high-amylose hybrid; SPAs, soluble phenolic acids; W, waxy hybrid.

Abstract:

The suitability of corn fractions (break meal: 250-500 μm ; flour: < 150 μm) from hybrids with different amylose contents (conventional: 18%; high-amylose: 42%; waxy: 2%) and their blends, to produce co-extruded snacks was assessed. Corn flour exhibited a higher content in total soluble phenolic acids (+34%) than break meal. The high-amylose hybrid maintained a higher antioxidant capacity and phenolic acid content (+52% for soluble and +54% for cell-wall bound phenolic acids), even after extrusion, than the conventional one. Because of its gelatinization properties (high pasting and peak temperatures; low maximum viscosity), the high amylose hybrid produced co-extruded snacks characterized by low section areas and large inner areas. The blends led to snacks whose features (sections and inner areas, porosity and hardness) did not follow a linear trend with the amylose content, suggesting the need for further studies to better understand the starch interactions that take place among the various hybrids.

Keywords: high amylose corn; waxy corn; dry-extrusion-cooking; gluten-free.

1. Introduction

The snack food market has continued to grow over the years (Brennan, Derbyshire, Tiwari & Brennan, 2013), particularly in the gluten-free sector. Indeed, this product category has been able to completely satisfy the food market requirements for minimally processed, ready-to-eat foods.

Among the different technologies used for the production of snacks, the extrusion-cooking process is one of the most innovative and interesting production processes (Delgado-Nieblas, Aguilar-Palazuelos, Gallegos-Infante, Rocha-Guzmán, Zazueta-Morales & Caro-Corrales, 2012). The extrusion-cooking technology involves high temperature and high shear stress conditions. Such high temperatures allow the starch to be gelatinized and the water in the liquid phase to be maintained. The dough is then processed in a cylinder under pressure and subsequently extruded through a die at atmospheric pressure. The high temperature and shear stress in the cylinder cause gelatinization and degradation of the starch, while the sudden drop in pressure at the end of the extruder causes an immediate expansion of the product, due to a rapid evaporation of the water (Brennan, Derbyshire, Tiwari & Brennan, 2013). Using this technology, it is possible to design the shape, taste, texture and sensory characteristics of food, thereby helping the food industry to respond to the growing needs of “modern” consumers.

Several studies have focused on directly-expanded extrudates and how both the formulation conditions (i.e., enrichment in proteins or fibers) and processing ones (i.e., hydration level, pressure, temperature, shear) play roles in defining the quality of the final product, as recently reviewed by Brennan et al. (2013). However, the effects of the raw materials and extrusion conditions on the characteristics of co-extruded snacks have rarely been reported. The most common snack produced by co-extrusion is a cereal-based outer tube, which may be filled with either a sweet or savory filling. Expanded and co-extruded snacks are different from each other in texture, which depends on the extent of the expansion, which is measured as the increase in diameter after extrusion. A high expansion rate is related to increased porosity of the product and either a large number of gas cells, or a number of large gas cells (Brennan et al., 2013).

During extrusion, starch undergoes to several physical changes, including decrease in gelatinization enthalpy, relative crystallinity and amylopectin molecular size, overall suggesting a loss in molecular order, which affects starch hydrolysis (Zhang et al., 2021).

Few studies have highlighted the negative relationship between the expansion rate and starch properties (i.e. amylose content), whereby commercial high-amylose starch (i.e., HYLON VII starch) is added to the puffed snack formulation (Zhu, Shukri, de Mesa-Stonestreet, Alavi, Dogan & Shi, 2010; Tacer-Caba, Nilufer-Erdil, Boyacioglu & Ng, 2014). However, none of the available studies made a clear comparison of conventional, waxy and high amylose corn for the production of co-extruded snacks.

Extruded snacks can be manufactured using a wide variety of starch and/or grains, including corn, which plays a significant role in providing all the features, such as structure, texture, and mouth feel, that are desirable for highly acceptable snack products. Corn meal is frequently used for corn-based extruded snacks (Riaz, 2006). Corn pearl meal (particle size from 600 to 1000 μm), break meal (mainly from 250 to 500 μm), and flour (85% of particles under 150 μm) are the main products obtained from the dry-milling of corn. As they differ in particle size and in the endosperm area they come from, their final use also differs. Meal is in fact obtained from the vitreous part of the endosperm, while the softer parts are mainly broken down into flour (Blandino, Alfieri, Giordano, Vanara & Redaelli, 2017; Vanara, Scarpino & Blandino, 2018). As far as food applications are concerned, corn meal, which is characterized by a higher particle size than flour, is mainly used for *polenta* (pearl meal) or snacks (break meal), whereas corn flour is used as an ingredient in many gluten-free formulations, including snacks, bread and pasta (Marti & Pagani, 2013). Using flours instead of isolated starch as a major ingredient of gluten-free products helps to produce gluten-free food with enhanced nutritional quality (Pellegrini & Agostoni, 2015). Moreover, corn can provide bioactive compounds, such as phenolic acids and carotenoids, which are the compounds that are mainly responsible for the antioxidant capacity (AC) of the obtained flour (Blandino et al., 2017). The consumption of food with high amounts of antioxidant compounds may help prevent various oxidative stress-associated diseases, such as cancer and cardiovascular diseases. In this context, corn has been reported to have the highest total phenolic content and AC of all grains (Adom & Liu, 2002).

Despite all this, the effect of using corn break meal or flour for the production of co-extruded snacks has not been investigated so far.

The objective of the present study, which takes into consideration the abovementioned findings, has been to assess the role of corn milling fractions and amylose:amylopectin ratios on the physico-chemical

changes that take place during extrusion-cooking and their impact on the features of co-extruded snacks. In particular, three hybrids with different amylose contents (i.e., 2%, 18%, and 42%) and two corn milling fractions (break meal and flour) were considered.

2. Materials & Methods

2.1 Materials

Ten corn flour samples, resulting from the factorial combination of two milling fractions with different particle sizes (flour and break meal) and five levels of amylose content, obtained from the milling of grains of three corn hybrids with different amylose contents and their blends were considered in this study. Three corn cultivars were considered: i) a conventional hybrid (Pioneer P1547, amylose = 18%; C), ii) a high-amylose hybrid (Planta Amylor, amylose = 42%; HA) and iii) a waxy hybrid (Pioneer P1547E, amylose = 2%; W). All the hybrids were cultivated in the 2018 growing season in the same growing area in North West Italy. Pioneer P1547 is one of the most frequently cultivated hybrids in Italy for use within the dry-milling supply chain. HA and W were used alone and in combination (50:50) with C (C–HA and C – W).

Two types of products were obtained for each corn hybrid through a dry-milling process carried out in an industrial mill (Molino Peila S.p.A., Valperga, Italy): a flour from the softer part of the endosperm (85% of particles under 150 µm), and break meal from the vitreous endosperm (77% of particles between 250-500 µm). The process was described in detail by Blandino et al. (2017). Briefly, after a cleaning step involving a dry stoner, an intensive horizontal scourer, a vibrating aspirator and an optical sorter, the corn was sent to undergo a dry-degermination step to separate the germ, bran and endosperm fractions. The endosperm fraction was then progressively refined through a series of passages in a grinding and classification system, which was composed of roller mills, plansichters and flour purifiers, in order to reduce the endosperm to a standard meal size and to obtain corn flour and break meal.

Co-extruded snacks were produced at an industrial level by Fudex Group S.p.A. (Settimo Torinese, Italy). Dry-extrusion was performed using a co-rotating twin-screw extruder (2FB90 model; screw speed: 100 rpm; temperature: 117 °C; pressure: 70 bar). The snacks were milled into flour (particle size less than 250 µm) using a laboratory mill (IKA Universalmühle M20; IKA Labortechnik, Staufen, Germany), with a

water-cooling system to avoid overheating, in order to assess the susceptibility of the starch to α -amylase, the pasting properties, the phenolic acid content and the antioxidant capacity.

2.2. Methods

2.2.1 Chemical composition

The moisture content, determined to express all the results on a dry weight (dw) basis, was obtained by oven-drying at 105 °C for 24 h. The total dietary fiber (AOAC 985.29, enzymatic-gravimetric method), fat (AOAC 2003.05, Soxhlet method) and ash (AOAC 923.03, muffle furnace) contents were determined according to the AOAC (2005) procedures. The total protein content (conversion factor: 6.25) was obtained according to the Kjeldahl method, by means of a Kjeltec system I (Foss Tecator AB, Höganäs, Sweden) (AOAC 992.23). Total starch content was measured according to the AACC 76-13.01 method (Cereals & Grains, 2001).

2.2.2 Extraction and quantification of the soluble (SPAs) and cell wall-bound phenolic acids (CWBPAs)

The extraction and quantification of the SPAs (free and conjugated) and CWBPAs were performed according to the procedure proposed by Li, Shewry and Ward (2008) with some modifications, as reported by Giordano, Reyneri, Locatelli, Coisson and Blandino (2019).

The phenolic extracts were filtered through a 0.2 μ m filter and then analyzed by means of an Agilent 1200 Series (Agilent Technologies, Santa Clara, CA, USA) high-performance liquid chromatograph coupled with an Agilent 1200 series diode array detector. Separations were carried out using a 150 x 4.6 mm, 5 μ m particle size Gemini RP-18 column (Phenomenex, Torrance, CA, U.S.A), as reported by Giordano et al. (2019).

2.2.3 Determination of the antioxidant capacity (AC)

The AC was determined by means of FRAP and the ABTS assays adapted from the QUENCHER method, as described by Serpen, Gökmen and Fogliano (2012). The results were expressed as mmol Trolox equivalents kg⁻¹ of sample (dw) through a calibration curve.

2.2.4 Starch properties

Damaged starch was assessed according to the standard AACC 76-31.01 method (Cereals & Grains, 2001).

The pasting properties were evaluated using a Micro Visco-Amylo-Graph (Brabender GmbH., Duisburg, Germany) according to the Alfieri, Bresciani, Zanoletti, Pagani, Marti and Redaelli (2020) procedure. Twelve grams of flour were dispersed in 100 ml of distilled water, and both the sample and water weight were scaled 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⁻¹. The following parameters were considered: pasting temperature (temperature at which an initial increase in viscosity occurs), maximum viscosity (maximum viscosity reached during the analysis), peak temperature (temperature at the maximum viscosity), breakdown (difference between the maximum viscosity and the viscosity reached at the end of the holding period) and setback (difference between the final viscosity at 30°C and the viscosity reached at the end of the holding period).

All the measurements were carried out on both raw materials and snacks.

2.2.5 Snack characterization

2.2.5.1 Area

The snack area was measured, as reported by Brandolini, Lucisano, Mariotti and Hidalgo (2018). Cylindrical shaped snacks were cut using a blade, and images of the cross sections were acquired at 300 dots per inch with a digital scanner (Epson Perfection 550 Photo, Seiko Epson Corp., Suwa, Japan). Image analyses were performed using Image ProPlus software (v6; Media Cybernetics, Inc., Rockville, US). The images were processed at the gray level (8 bits). The section area, cell wall area, and inner area were considered.

2.2.5.2 Porosity and bulk density

Total porosity and bulk density were assessed with a Pascal Mercury Porosimeter (P240; Thermo Fisher Scientific, Waltham, US), according to Lucisano, Pagani, Mariotti and Locatelli (2008). Samples were subjected to an increasing pressure of up to 200 MPa, and pores with a radius of 3.7×10^{-3} to 7.5 μm were measured.

2.2.5.3 Texture analysis

The mechanical properties of the snacks were determined by means of a three-point bend method, using a TA – XT plus texture analyzer (Stable Micro Systems Ltd., Godalming, UK) equipped with a 10 kg (100 N) load cell. Samples were compressed with the HDP/3PB probe at a crosshead speed of 1 mm s⁻¹ to 5 mm of the original diameter of the extrudate. The compression generated a curve with the force over distance. The highest value of force was taken as a measurement of the hardness.

2.2.6 Statistics

Three individual extractions were carried out for each sample for both the SPAs and CWBPAs. The SPA and CWBPA contents, AC-FRAP, AC-ABTS and starch susceptibility to α -amylase were measured in triplicate, whereas the pasting properties, porosity and bulk density were measured in duplicate. Image and texture analyses were carried out on ten and thirty pieces, respectively. One-way analysis of variance was performed with the SPSS for Windows statistical package Version 24 (SPSS Inc., Chicago, Illinois, US). Significant differences ($p < 0.05$) among the samples were determined using the REGW-F test.

3. Results and discussion

3.1 Chemical composition of the raw materials

The chemical composition of the raw materials is shown in Supplementary Table 1. The milling fractions resulted in different compositions, according to the particle size: the corn flour had higher fat content than the break meal; on the other hand, the percentage of protein and fiber was higher in the break meal than in the corn flour. The HA hybrid had the highest fiber, protein and fat contents. These results agreed with those reported by Alfieri et al. (2020), who found -by screening a set of 23 Italian inbred lines of corn - that high amylose lines had the highest protein and lipid contents. Starch content was similar in corn flour and break meal, with W showing the lowest values in both milling fractions.

3.2 Phenolic acids and the AC of the raw materials and snacks

The SPAs, CWBPAs and AC detected in the corn fractions and snacks are reported in Table 1. As regards the raw materials, both the milling particle size and the type of hybrid affected the phenolic acid concentration. The corn flour showed a higher concentration of SPAs (+34%) than the average break meal concentration. The high SPA concentration in the corn flour could be related to the high germ content in this milling fraction (Blandino et al., 2017). Indeed, germ has a greater phenolic acid content than the endosperm (Ndolo & Beta, 2014).

Nevertheless, in order to obtain healthier foods, the opportuneness of using these finer milling fractions needs to be carefully considered, in particular for those genotypes that suffer more from environmental stress, such as HA, taking in consideration their higher risk of fumonisin and other mycotoxin contaminations (Vanara et al., 2018).

As far as the hybrids are concerned, the highest concentration of CWBPAs was observed in HA (686 and 629 mg kg⁻¹ dw, for break meal and flour, respectively), while no significant difference was observed between the C and W hybrids. No significant difference was observed in the SPA content among the hybrids for the corn flours. A significantly higher concentration of SPAs was reported in the HA break meal than that of the C and W hybrids (+42% and +17%, respectively). Phenolic acids are the main antioxidant compounds in cereals, and several studies have shown a direct and positive correlation between their concentration and AC (Beta, Nam, Dexter & Sapirstein, 2005; Li, Wei, White & Beta, 2007). The AC resulted higher in HA than in the C and W hybrids, regardless of the milling fraction. Nevertheless, as far as break meal is concerned, more consistent differences were observed between hybrids; indeed, the AC of the HA hybrid was +48% (FRAP assay) and +34% (ABTS assay) higher than the C and W hybrids. These findings are in agreement with those of Li et al. (2007), who stated that among corn genotypes, HA had the best AC and the highest ferulic acid concentration. This phenolic acid was always significantly higher in HA (mean content of 2917 mg kg⁻¹) than in the C and W hybrids, with a ferulic acid content that ranged between 1552 and 2135 mg kg⁻¹. Similarly, the HA hybrid showed a higher concentration of CWB ferulic acid (+49%) than the C and W hybrids (data not shown). Several changes occurred in both the phenolic acids and AC during extrusion (Figure 1). In fact, AC increased significantly during processing: the snacks made from HA hybrid flour and break meal had an AC, as measured by the FRAP assay, that was 1.8 times higher than the raw material. As observed in previous

studies (Yilmaz & Toledo, 2005; Yu & Nanguet, 2013), the increase in the AC during extrusion could be due to the Maillard reaction products that formed during the processing at high temperatures. Several studies have shown that the extrusion process can affect the composition of both free and conjugated phenolic acids in cereals to a great extent, depending on the type of grain (Zeng, Liu, Luo, Chen & Gong, 2016; Ruiz-Armenta, Zazueta-Morales, Delgado-Nieblas, Carrillo-López, Aguilar-Palazuelos & Camacho-Hernández, 2019). In the present study, the SPAs showed the maximum decrease after extrusion. The snacks obtained from the HA hybrid showed a decrease of -51-59% of SPAs, compared to the raw material. Similarly, Altan, McCarthy and Maskan (2009) observed that the extrusion cooking of barley significantly reduced the total phenolics by -46-60%. Extrusion cooking could lead to a decrease in the free phenolic acids, because of the decomposition caused by high temperatures. Moreover, the extractability of free phenolic acids after extrusion could decrease because of an increased polymerization that can occur during extrusion. At the same time, CWBPAs could be released from the cell wall during extrusion, and their extractability could increase because of changes in the organizational structure of the extruded cereals (Zeng et al., 2016). Nevertheless, in the present study, no significant changes were observed in the CWBPAs after extrusion. Interestingly, as far as the HA hybrid is concerned, the concentration of soluble sinapic acid during processing decreased by 32 mg kg⁻¹, whereas the concentration increased by 25 mg kg⁻¹ in the conjugated form. As hypothesized by Hu, Zhang, Hu, Yu, Zho and Sao (2018), during extrusion, some SPAs could transform into insoluble-bound forms, which are not linked to the cell walls, but are combined with a complex formed of lipids, protein, starch and high molecular weight compounds.

3.3 Starch properties

3.3.1 Starch susceptibility to α -amylase hydrolysis

The susceptibility of starch to the α -amylase hydrolysis of corn and the related snacks is shown in Table 2. In the raw materials, this index is related to the amount of damaged starch, i.e. the starch granules that are physically broken during the milling or grinding process to make flour. Indeed, damaged granules are more susceptible to enzymatic hydrolysis as they have a high contact surface. As expected, the corn break meal samples presented a smaller amount of damaged starch (Table 2) and a higher particle size

(data not shown), which come from the vitreous endosperm of the kernel. The flour samples were instead obtained from the softer part of the endosperm (Blandino et al., 2017).

As regards the hybrids with different amylose contents, the high amount of damaged starch in the W flour was associated with low hydrogen bonding, due to the low amylose content, which may have decreased the resistance to crushing and thus increased starch damage (Bettge, Giroux & Morris, 2000). On the other hand, the more compact structure of HA might account for the low damaged starch values found in both the corn flour and break meal from HA.

In the case of processed foods, the starch susceptibility index provides information about the effects of processing on the starch structure (Marti, Seetharaman & Pagani, 2010). As expected, the combination of both thermal and mechanical stresses applied during the dry-extrusion process resulted in a significant increase in starch susceptibility to α -amylase hydrolysis, thus suggesting starch destructuring. The snacks from HA exhibited the lowest starch susceptibility when either flour or break meal was used for their production; this suggests that the more compact structure, due to the high amylose content, mitigated the effect of processing.

3.3.2 Pasting properties

The pasting profiles of the raw materials are reported in Figure 2 and the relative indices are summarized in Table 3. The corn break meal samples showed low viscosity values, compared to corn flours, likely due to differences in the particle size. In addition, the starch in the break meal samples reached a plateau rather than a peak of viscosity, thereby suggesting a low hydration and gelatinization capacity. Similar differences were observed between flour and semolina samples (Mariotti, Zardi, Lucisano & Pagani, 2005).

As regards the amylose content, the W flour and break meal both showed a lower pasting temperature, peak viscosity and retrogradation tendency (i.e., low final viscosity and setback values) than C, in agreement with the literature (Caramanico, Marti, Vaccino, Bottega, Cappa, Lucisano, & Pagani, 2018; Liu, Yuan, Wang, Reimer, Isaak & Ai, 2019). On the other hand, neither the HA flour nor the break meal showed viscosity, even at 95 °C, which could be ascribed to their high gelatinization temperatures (Liu et al., 2019). Therefore, HA did not show re-association to provide a high final viscosity. A similar behavior was found by Alfieri et al. (2020). Thus, despite the slight differences in chemical composition

(Supplementary Table 1), it was the amylose content that accounted for the pasting behavior shown in Figure 2. Previous studies showed that heating HA starch at temperatures above 120 °C completely gelatinized the starch (Liu et al., 2019). As expected, blending either W or HA with C resulted in an intermediate viscosity.

Regardless of the type of milling fractions and corn hybrid, the snacks did not show any pasting profile (data not shown) as a result of the starch degradation that occurred during the extrusion process, thus confirming the starch susceptibility data shown in Table 2. Dry-extrusion led to a high gelatinization degree of the starch and caused the loss of its gelatinization and retrogradation properties. This behavior is common in extruded products obtained from various raw materials (Gomez & Aguilera, 1983; Ozcan & Jackson, 2005; Tacer-Caba et al., 2014).

3.4 Snack features

The features of the snacks, in terms of total and section area, bulk density, porosity and hardness, are summarized in Table 4, whereas the images of the snacks are reported in supplementary Figure 1. The overall quality of snacks depends to a great extent on the type of product, i.e. expanded vs co-extruded snack. The former is characterized by a low bulk density and high expansion rate; the latter by a compact structure, in which voids are undesirable. This study has focused on co-extruded snacks that generally have an extrusion-cooked outer shell that is later filled with either a savory or sweet filling. Since the filling needs to be contained within the snack, a compact structure is desirable. In this kind of product, structure compactness might result by measured as low volume and consequently high bulk density and porosity. Last but not least, various sensory attributes, including a crispy texture, contribute to the definition of product quality. In fact, in general, the higher the expansion rate is, the lower the bulk density and the hardness (Tacer-Caba et al., 2014). Moreover, hardness is related to some other parameters, such as porosity, cell size and cell wall thickness, and to the final product density (Robin, Schuchmann & Palzer, 2012).

The section area may be considered as an index of the degree of expansion of the product. Indeed, the higher the area is, the higher the expansion rate, although it should be considered that the die of the extruder was not changed during the extrusion trials. The section area of the snacks made from corn

flours was generally larger than the area of the corn meal snacks (Table 4), thus suggesting a high expansion degree and confirming previous findings about the relationship between the increased particle size and decreased expansion of extrudates (Garber, Hsieh & Huff, 1997; Shevkani, Kaur, Singh, Singh & Singh, 2014). The lower expansion of the snacks from the powders of larger particles could be due to incomplete starch gelatinization, as shown by the pasting profiles in Figure 2. In addition, the snacks from the break meal corn generally showed the highest bulk density, porosity and hardness. Such differences might be due to both the particle size and the chemical composition. Indeed, the break meal contained more proteins and fiber than the corn flour (Supplementary Table 1), and thus a lower starch content. The differences in section area and porosity among the snacks made from either corn flour or break meal were similar when HA was used, thus suggesting that the effect of the type of hybrid prevailed over the effect of the particle size.

Regardless of the particle size, the snacks from C and HA showed the highest and lowest sections and cell wall areas (the difference between the section and inner area; data not shown), respectively. Previous studies emphasized the role of HA on the expansion rate of corn starch snacks (Mercier & Feillet, 1975; Zhu et al., 2010). It was in fact shown that HA starch required a higher extrusion temperature to reach a comparable expansion degree than starches with a lower amylose content (Mercier & Feillet, 1975).

Neither the amount of amylose in the products nor the section and cell wall areas followed a linear trend (data not shown), which suggests that it is not only the amylose content, but probably also the starch structure, and its interaction with proteins, that determines the characteristics of the final product. However, this aspect needs to be further investigated.

The snacks from HA showed the highest inner area value (Table 4), which would suggest that this raw material could be used to produce co-extruded snacks for a later filling. When using corn flour, the snack with the largest inner area was obtained when HA was blended with C (Table 4).

The highest values of both the bulk density and porosity were obtained when corn break meal was used, which is in agreement with the lowest area (Table 4). Other authors found a similar relationship between the bulk density and expansion rate (Zhu et al., 2010; Tacer-Caba et al., 2014). As regards the hybrid

type, using HA resulting in a snack with high bulk density and porosity. The larger particle size of break meal - together with its difficulty to gelatinize - masked the effect of HA on these indices.

As regards hardness, the snacks from corn break meal were generally firmer than those made from flour, because of their reduced section area (or diametric expansion) and increased bulk density (Table 4).

The effect of the type of hybrid and amylose content is only evident in the case of corn flour. Using HA flour in fact determined the production of a firmer snack, which suggests that amylose played a significant role in determining the mechanical strength of the products, as already shown for soy protein - high amylose starch extrudates (Zhu et al., 2010). In addition, the role of fiber and proteins (which were higher in HA than C and W, as shown in Supplementary Table 1) in limiting starch gelatinization should not be disregarded. Several factors could account for the mechanical properties of snacks, including volume, average cell size and cell size distribution, and thus porosity (Zhu et al., 2010). This result could be justified by considering the high bulk density of this sample, compared to the others. This sample in fact showed the smallest section area, which indicated a more compact structure with better resistance to compression loads.

A different behavior was observed for the samples obtained from the corn break meal; in this case, the hardest samples were the ones obtained from the C-W blend. However, it is important to note that the hardness of C, W and HA was not significantly different. This could be due not only to the higher fiber and protein contents (Supplementary Table 1), but also to the coarse particle size, which limited starch gelatinization, even in C and W, which in general were able to gelatinize, as shown by the pasting profiles (Figure 2).

As for the samples from the blends, the hardness values of both the corn flour and break meal were different from what could be expected from an analysis of the raw materials. These behaviors were probably due to a particular rearrangement of starch during the dry-extrusion process, which is worthy of further investigation. Understanding the relation between extrusion conditions and the supramolecular structure of starch is of interest especially in relation to product digestibility. Indeed, in a previous work, Li et al. (2021) found a strong association between the supramolecular structure of jackfruit seed starch and its digestibility. Specifically, extrusion promoted the breakage of α -1,4-glycosidic bonds in highly branched amylopectin, thus weakening the van der Waals forces between the chains and generating

many more amylopectin fragments with branched chains, likely improving starch digestibility (Li et al. 2021).

4. Conclusions

This study provided insight into the interactions that take place between the amylose content and type of milling fraction in determining the characteristics of corn snacks. Although the market interest in this type of snacks, to the best of our knowledge, the structural features of co-extruded snacks have been poorly investigated. The outcomes of this study might help industries to reformulate products with enhanced nutritional and physical features by selecting suitable corn hybrids and milling fractions.

As regards the bioactive compounds, HA showed the highest AC and phenolic acid contents, and the greater contents of these bioactive compounds were also confirmed in the corresponding snacks. Moreover, the corn flour fraction of each hybrid showed a higher SPA content than the break meal.

The W and C corns (with 2 and 18% amylose contents, respectively) led to more expanded and softer snacks. The best results for the co-extruded snacks, in terms of texture, porosity, bulk density and section area expansion, were obtained using HA flour (40% amylose). The high compact structure of this kind of snack could be related to the particular starch properties of HA, which resulted in the lowest gelatinization degree. The gelatinization properties of starch were also restricted when corn break meal (with a higher particle size) was used instead of corn flour. Blending both HA and W hybrids with the C one led to snacks whose features (i.e., section and inner area, porosity and hardness) did not follow a linear trend with the amylose content, thus suggesting that not only the amylose content, but also the structure/properties of the starch and its interactions with proteins could be important in defining the quality of the final product. Taking into consideration all these findings, the particular rearrangement of starch, when different hybrids are blended together during the dry extrusion process, is worthy of further study.

Declaration of Competing Interest. The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

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Figure captions

Figure 1. Figure 1. Antioxidant capacity (FRAP and ABTS assay, panel A), total, sinapic and ferulic soluble phenolic acids (SPAs; panel B) and total, sinapic and ferulic cell wall-bound phenolic acids (CWBPA; panel C) detected in the high-amylose hybrid raw materials (gray bars) and snacks (white bars).

Data are expressed on a dw basis. The ANOVA significance level between the raw material and snack is reported for each compound and milling fraction: ns = p -value >0.05 ; * = p -value <0.05 ; ** = p -value <0.01 ; *** = p -value <0.001 . The reported values are based on 3 replications.

Figure 2. Pasting profiles of the break meal (panel A) and flours (panel B) for the conventional hybrid (gray), waxy hybrid (yellow), high-amylose hybrid (orange), conventional-high amylose blend (green) and conventional-waxy blend (blue).

Supplementary Materials

Table S1. Chemical composition of the raw material (g 100g⁻¹).

Figure S1. Images of the snack section.

Table 1. Soluble (free and conjugated forms) phenolic acids (SPAs), cell wall -bound phenolic acids (CWBPAs), and antioxidant capacity (AC) detected in the corn raw material and derived snacks.

Product	Milling fraction	Corn hybrids	SPAs ¹ mg kg ⁻¹	CWBPAs ¹ mg kg ⁻¹	AC-FRAP mmol TE kg ⁻¹ ₁	AC-ABTS mmol TE kg ⁻¹
Raw materials	break meal (250-500 µm)	C	87.3±5.1 ^d	385.2±4.6 ^c	3.6±0.0 ^f	7.0±0.1 ^e
		C-HA	104.7±10.6 ^{cd}	561.1±26.4 ^b	4.4±0.2 ^{def}	8.2±0.2 ^{cd}
		HA	124.4±8.2 ^b	685.7±78.2 ^a	5.5±0.5 ^{ab}	9.6±0.5 ^a
		C-W	94.2±2.2 ^{cd}	347.9±23.3 ^c	3.6±0.1 ^f	7.8±0.1 ^{de}
		W	106.3±6.7 ^c	398.6±41.8 ^c	3.9±0.3 ^{ef}	7.4±0.5 ^{de}
	flour (< 150 µm)	C	131.3±3.8 ^{ab}	448.3±34.4 ^c	5.2±0.1 ^{abcd}	8.6±0.5 ^{bcd}
		C-HA	140.7±0.7 ^{ab}	538.6±53.2 ^b	5.3±0.6 ^{abc}	8.9±0.4 ^{abc}
		HA	145.7±10.1 ^a	629.3±17.8 ^{ab}	6.2±0.7 ^a	9.6±0.4 ^{ab}
		C-W	130.2±4.4 ^{ab}	442.4±26.0 ^c	4.5±0.1 ^{cde}	7.9±0.2 ^{de}
		W	143.7±9.0 ^a	426.7±19.6 ^c	4.8±0.1 ^{bcde}	8.0±0.5 ^{cde}
Snacks	break meal (250-500 µm)	C	32.8±3.0 ^c	380.6±21.9 ^e	7.0±0.2 ^{cde}	8.5±0.2 ^d
		C-HA	32.8±0.6 ^c	508.7±62.5 ^{cd}	5.9±0.8 ^e	10.1±0.3 ^{bc}
		HA	51.4±9.9 ^b	666.6±41.2 ^a	9.9±1.0 ^b	10.9±0.6 ^b
		C-W	30.8±5.7 ^c	422.5±41.4 ^{de}	6.2±0.2 ^{de}	8.7±0.3 ^d
		W	34.7±0.7 ^c	453.2±0.7 ^{cde}	6.2±0.2 ^e	8.4±0.1 ^d
	flour (< 150 µm)	C	48.3±2.3 ^b	466.3±40.2 ^{cde}	8.6±0.2 ^{cd}	10.1±0.3 ^{bc}
		C-HA	35.0±0.7 ^c	553.2±45.2 ^{bc}	7.6±0.9 ^{cde}	9.8±0.4 ^c
		HA	71.4±0.7 ^a	619.6±41.2 ^{ab}	11.4±1.0 ^a	12.3±0.5 ^a
		C-W	26.5±0.9 ^c	489.5±18.4 ^{cd}	6.8±0.4 ^{de}	8.1±0.3 ^d
		W	35.3±0.5 ^c	493.2±36.8 ^{cd}	7.8±0.5 ^{cd}	8.6±0.3 ^d

Data are expressed on a dw basis. Within each product (raw materials or snacks), means ± standard deviation followed by different letters in the same column are significantly different, according to the REGW-Q test (p<0.001). Reported values are based on 3 replications. ¹ sum of the SPAs and CWBPAs determined by means of the RP-HPLC/DAD

C, conventional hybrid; C-HA, conventional:high amylose hybrid blend (50:50); C-W, conventional:waxy hybrid blend (50:50); HA, high-amylose hybrid; W, waxy hybrid; AC-ABTS, antioxidant capacity by means of the ABTS assay; AC-FRAP, antioxidant capacity by means of the FRAP assay.

Table 2. Starch susceptibility to α -amylase hydrolysis measured as damaged starch (g 100g⁻¹ dw).

	Raw materials		Snacks	
	Break meal (250-500 μ m)	Flour (<150 μ m)	Break meal (250-500 μ m)	Flour (<150 μ m)
C	3.6 \pm 0.1 ^d	4.7 \pm 0.2 ^c	61.3 \pm 1.7 ^{AB}	59.5 \pm 1.7 ^B
C- HA	3.0 \pm 0.2 ^e	5.7 \pm 0.2 ^b	51.9 \pm 2.7 ^{DE}	52.5 \pm 3.3 ^D
HA	2.5 \pm 0.2 ^f	5.6 \pm 0.1 ^b	48.1 \pm 1.0 ^E	48.4 \pm 2.1 ^D
C-W	3.4 \pm 0.2 ^{de}	5.8 \pm 0.1 ^b	56.3 \pm 3.6 ^c	58.2 \pm 1.8 ^B
W	3.4 \pm 0.2 ^{de}	6.8 \pm 0.3 ^a	59.6 \pm 2.2 ^B	66.5 \pm 3.0 ^A

Within each product (raw materials or snacks), means \pm standard deviation followed by different letters are significantly different, according to the REGW-Q test ($p < 0.001$). Lowercase letters refer to break meal and flour as raw materials ; uppercase letters refer to snacks from break meal and flour.

Reported values are based on two replications.

C, conventional hybrid; C-HA, conventional:high amylose hybrid blend (50:50); C-W, conventional:waxy hybrid blend (50:50); HA, high-amylose hybrid; W, waxy hybrid.

Table 3. Pasting properties of break meal and flour fractions.

Parameters	Milling fraction	C	C-HA	HA	C-W	W
Pasting temperature (°C)	Break meal (250-500 µm)	77.1±1.8 ^c	89.5±0.7 ^a	-	72.2±1.3 ^d	70.1±0.2 ^{de}
	Flour (<150 µm)	68.2 ± 1.2 ^e	76.9±1.5 ^c	86.2±1.2 ^b	66.8±0.1 ^{ef}	64.1±1.4 ^f
Peak temperature (°C)	Break meal (250-500 µm)	94.9±0.1 ^a	95.1±0.1 ^a	-	92.0±0.1 ^{ab}	89.2±3.4 ^b
	Flour (<150 µm)	88.9±0.1 ^b	93.9±1.9 ^a	95.1±0.1 ^a	90.5±0.1 ^b	73.1±2.1 ^c
Maximum viscosity (UB)	Break meal (250-500 µm)	347.5±16.2 ^b	178.5±0.7 ^c	-	380.5±0.7 ^b	300.0±24.0 ^b
	Flour (<150 µm)	556.0±2.8 ^a	201.5±16.3 ^c	58.1±1.4 ^d	358.3±53.7 ^b	347.2±11.3 ^b
Breakdown (UB)	Break meal (250-500 µm)	37.0±5.6 ^{de}	13.5±6.3 ^{ef}	-	55.0±2.8 ^d	44.0±5.6 ^{de}
	Flour (<150 µm)	247.5±4.9 ^a	44.5±11.9 ^{de}	-	96.5±21.9 ^c	154.5±18.1 ^b
Final viscosity (UB)	Break meal (250-500 µm)	952.5±33.2 ^a	376.5±4.9 ^{de}	-	781.0±5.6 ^b	559.5±19.0 ^c
	Flour (<150 µm)	922.0±2.8 ^a	327.5±39.3 ^e	47.5±0.7 ^f	617.0±69.3 ^c	461.5±27.6 ^d
Setback (UB)	Break meal (250-500 µm)	637.0±48.0 ^a	220.0±11.3 ^{de}	-	479.0±15.5 ^b	294.5±9.1 ^d
	Flour (<150 µm)	625.5±4.9 ^a	174.5±12.2 ^e	-	370.0±42.4 ^c	265.5±21.9 ^d

Means ± standard deviation followed by different letters in the same row are significantly different, according to the REGW-Q test (p<0.001). Reported values are based on two replications.

Pasting temperature, temperature at which an initial increase in viscosity occurs; maximum viscosity, maximum viscosity reached during the analysis; peak temperature, temperature at the maximum viscosity; breakdown, difference between the maximum viscosity and the viscosity reached at the end of the holding period; setback, difference between the final viscosity at 30°C and the viscosity reached at the end of the holding period.

C, conventional hybrid; C-HA, conventional:high amylose hybrid blend (50:50); C-W, conventional:waxy hybrid blend (50:50); HA, high-amylose hybrid; W, waxy hybrid

Table 4. Features of snacks made from either break meal and flour fractions.

Parameters	Milling fraction	C	C-HA	HA	C-W	W
Section area (mm ²)	Break meal (250-500 µm)	356.3 ± 19.7 ^a	294.0 ± 24.8 ^c	235.2 ± 17.7 ^d	300.0 ± 18.2 ^{bc}	282.5 ± 34.3 ^c
	Flour (<150 µm)	376.2 ± 21.7 ^a	294.5 ± 21.4 ^c	232.7 ± 15.6 ^d	353.1 ± 14.7 ^a	322.1 ± 16.2 ^b
Inner area (mm ²)	Break meal (250-500 µm)	21.0 ± 7.4 ^{bcd}	43.5 ± 5.7 ^a	42.5 ± 6.0 ^a	27.3 ± 4.2 ^{bc}	23.5 ± 3.8 ^{bcd}
	Flour (<150 µm)	23.5 ± 5.9 ^{bcd}	39.6 ± 5.6 ^a	27.4 ± 2.9 ^b	17.6 ± 7.4 ^d	19.5 ± 4.3 ^{de}
Bulk density (gcm ⁻³)	Break meal (250-500 µm)	1.60	1.65	2.20	1.67	1.60
	Flour (<150 µm)	1.16	1.30	1.38	1.51	1.40
Porosity (%)	Break meal (250-500 µm)	51.8	45.4	67.1	64.0	60.9
	Flour (<150 µm)	30.7	56.8	67.3	50.4	39.5
Hardness (N)	Break meal (250-500 µm)	29.4 ± 1.2 ^a	21.3 ± 1.1 ^{bc}	28.2 ± 1.2 ^a	12.9 ± 1.4 ^d	33.1 ± 2.5 ^a
	Flour (<150 µm)	19.2 ± 0.5 ^c	20.5 ± 0.5 ^c	27.9 ± 0.5 ^a	11.8 ± 0.5 ^d	23.3 ± 0.5 ^b

Means ± standard deviation followed by different letters in the same row are significantly different, according to the REGW-Q test (p<0.001). Reported values are based on thirty replications for harness and on ten replications for section area and inner area.

C, conventional hybrid; C-HA, conventional:high amylose hybrid blend (50:50); C-W, conventional:waxy hybrid blend (50:50); HA, high-amylose hybrid; W, waxy hybrid.

21 Table S1. Chemical composition of the raw material (g 100g⁻¹).

Milling fraction	Corn hybrids	Moisture	Starch	Protein	Fat	Total dietary fiber	Ash
Break meal (250-500 µm)	C	14.0	83.3	7.0	1.5	4.4	0.6
	HA	13.2	82.1	8.3	2.0	5.9	0.7
	W	12.4	77.5	6.3	1.2	2.5	0.5
Flour (< 150 µm)	C	14.0	81.6	4.6	2.0	2.6	1.0
	HA	12.2	81.6	6.6	2.2	4.9	0.7
	W	12.1	77.6	5.5	1.5	2.5	0.6

22 C, conventional hybrid; HA, high-amylose hybrid; W, waxy hybrid.

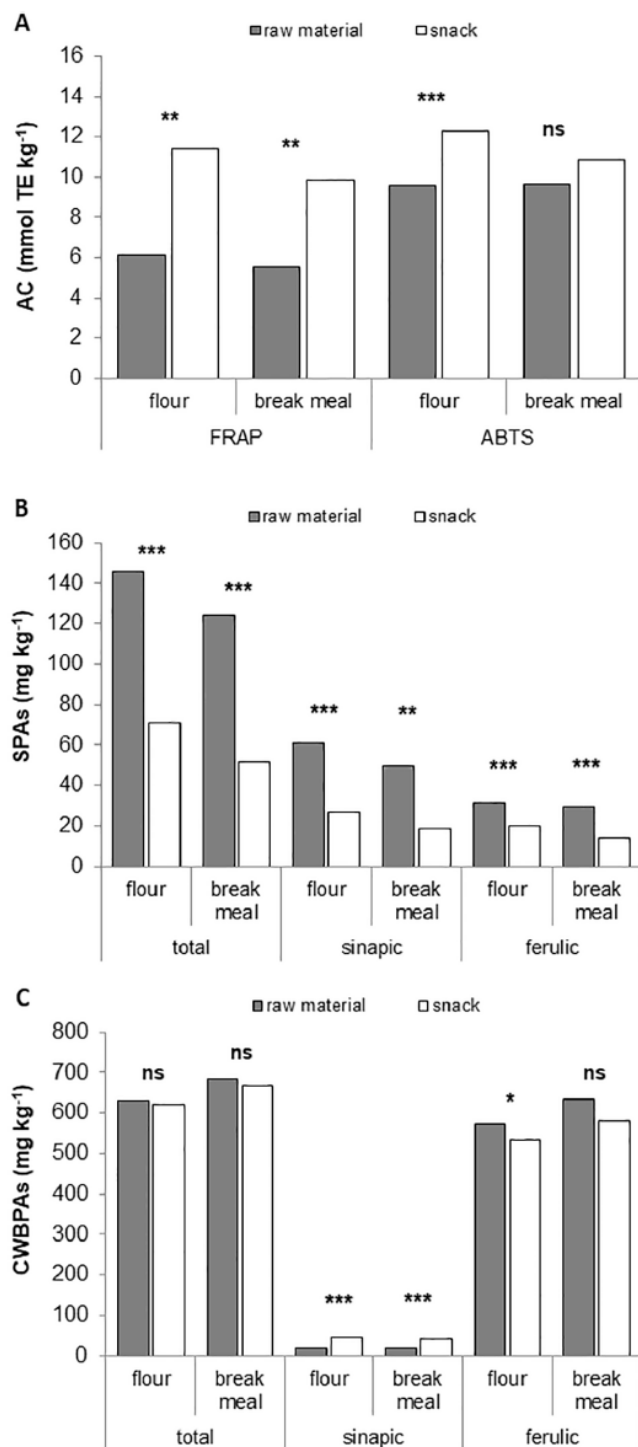


Fig. 1. Antioxidant capacity (FRAP and ABTS assay, panel A), total, sinapic and ferulic soluble phenolic acids (SPAs; panel B) and total, sinapic and ferulic cell wall-bound phenolic acids (CWBPAs; panel C) detected in the high-amylose hybrid raw materials (gray bars) and snacks (white bars). Data are expressed on a dw basis. The ANOVA significance level between the raw material and snack is reported for each compound and milling fraction: ns = p -value > 0.05; * = p -value < 0.05; ** = p -value < 0.01; *** = p -value < 0.001. The reported values are based on 3 replications.

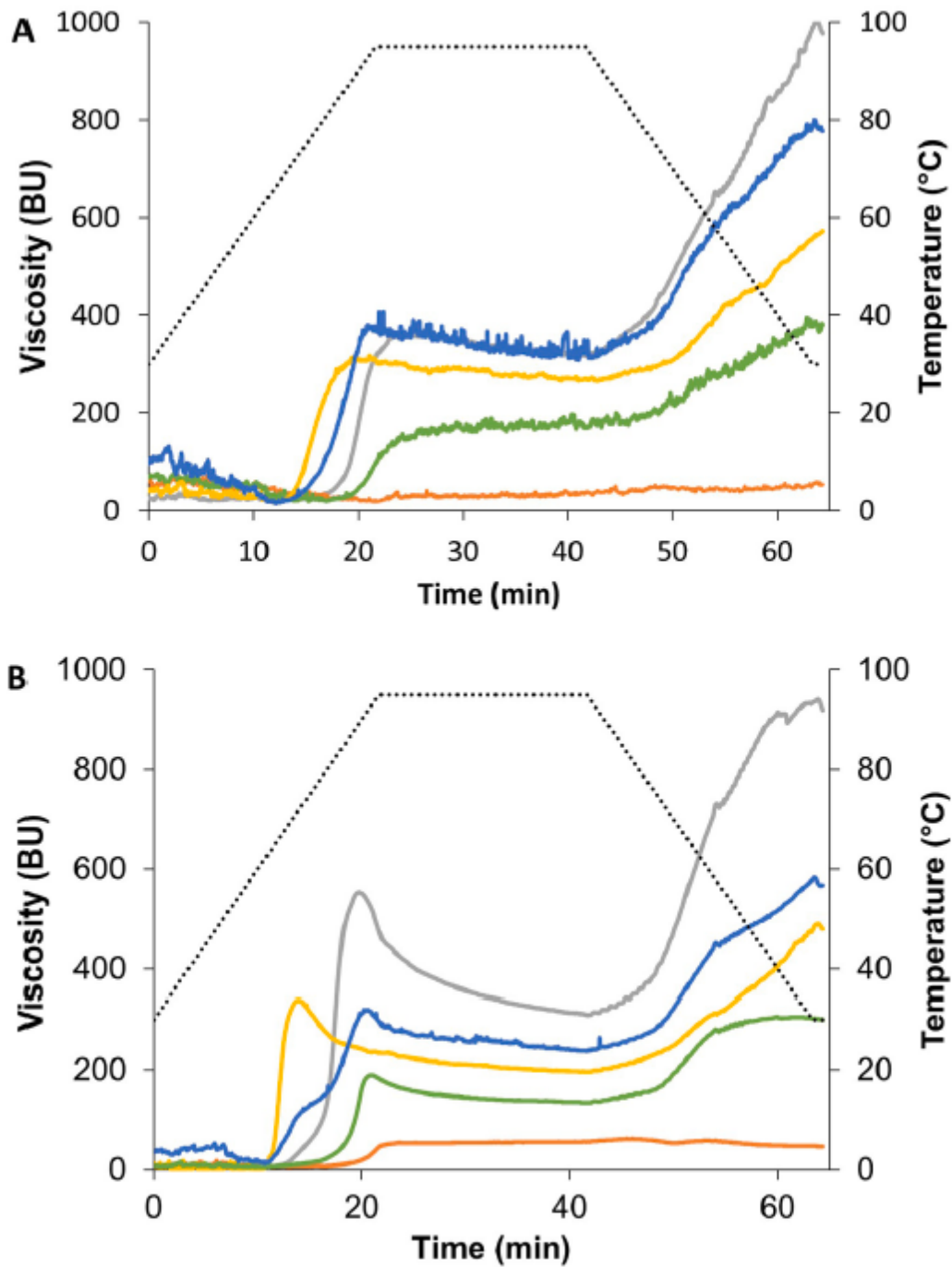


Fig. 2. Pasting profiles of the break meal (panel A) and flours (panel B) for the conventional hybrid (gray), waxy hybrid (yellow), high-amylose hybrid (orange), conventional-high amylose blend (green) and conventional-waxy blend (blue).