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

Andrea Bresciani[1], Debora Giordano[2], Francesca Vanara[2],

Massimo Blandino[2], Alessandra Marti[1,*]

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

[2] Department of Agricultural, Forest and Food Sciences (DISAFA), Università degli Studi di Torino, Largo Braccini 2, 10095, Grugliasco (TO), Italy

* corresponding author: alessandra.marti@unimi.it

Abbreviations: ABTS, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid); AC, antioxidant capacity; AC-ABTS, antioxidant capacity by means of the ABTS assay; AC-FRAP, antioxidant capacity by means of the 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:

This study focused on the suitability of corn milling fractions (break meal, particle size: 250-500 μm; flour, particle size < 150 μm) from hybrids different in amylose content (conventional: 18% amylose; high-amylose: 42% amylose; waxy: 2% amylose) and their blends, to produce co-extruded snacks. Antioxidant capacity, phenolic acid content, pasting properties and snack size, hardness, porosity, and density were considered. As regards the bioactive compounds, corn flour reported a marked antioxidant capacity compared to break meal. High amylose hybrid maintained the highest antioxidant capacity and phenolic acid content even after extrusion. Waxy and conventional hybrids led to more expanded and softer snacks, than the high amylose hybrid that, thus, has to be preferred for co-extruded snacks production. Blends led to snacks whose features did not follow a linear trend with the amylose content, suggesting the need for further studies to better understand starch interactions among the various hybrids.

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

1. Introduction

The snack food market is expanding rapidly and is predicted to continue growth in the future (Brennan, Derbyshire, Tiwari & Brennan, 2013), particularly within the gluten-free sector. Indeed, this product category completely meets the demand for minimally processed, ready-to-eat foods. Among the different technologies used for the production of snacks, the extrusion-cooking process represents one of the most innovative and interesting production processing (Delgado-Nieblas, Aguilar-Palazuelos, Gallegos-Infante, Rocha-Guzmán, Zazueta-Morales & Caro-Corrales, 2012). Extrusion-cooking combines high temperature and high shear stress conditions. High temperature allows to gelatinize the starch keeping the water in the liquid phase. The dough is then processed in the cylinder and subsequently extruded through the die at atmospheric pressure. The sudden breakdown of pressure causes an immediate expansion of the product due to the rapid evaporation of the water. Using this technology, it is possible to produce food that can be designed for shape, taste, texture, and sensory characteristics, helping the food industry to respond to the growing needs of the “modern” consumer.

Nowadays, the majority of the studies have focused on the direct-expanded extrudates and how either formulation (i.e., enrichment in proteins or fibers) or processing conditions (i.e., hydration level, pressure, temperature, shear) play a role in defining the quality of the final product, as recently reviewed by Brennan et al. (2013). Conversely, to the best of our knowledge, little is known about the effect of raw materials and extrusion conditions on the characteristics of co-extruded snacks. The most common snack produced by co-extrusion is a cereal-based outer tube with a sweet or savory filling inside. Direct-expanded and co-extruded snacks are different in texture which is, actually, dependent on the extent of expansion, that is measured by the increase in diameter after extrusion. 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).

Few studies highlighted the relation between expansion rate and starch properties (i.e. amylose content), by adding commercial high-amylose starch in the formulation of puffed snacks (Zhu,

Shukri, de Mesa-Stonestreet, Alavi, Dogan & Shi, 2010; Tacer-Caba, Nilufer-Erdil, Boyacioglu & Ng, 2014). However, none of the available studies make a clear comparison between 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, that play a key role in providing all the features desired for highly acceptable snack products, such as structure, texture, and mouth feel. For most corn-based extruded snacks, corn meal is used (Riaz, 2006). Corn pearl meal (particle size from 600 to 1000 μm), break meal (mainly from 250 to 500), and flour (85% of particles under 150 μm) are the main products obtained from the dry-milling of corn. They differ in both particle size, in the endosperm area they come from, and thus in their end-uses. Specifically, meal is obtained from the vitreous part of the endosperm, while the softer parts are mainly broken down to flour (Blandino, Alfieri, Giordano, Vanara & Redaelli, 2017; Vanara, Scarpino & Blandino, 2018). As regards food applications, corn meal, that 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). In the context of gluten-free products, using flours instead of the isolated starch as a major ingredient will help deliver gluten-free foods with enhanced nutritional quality (Pellegrini & Agostoni, 2015). In the case of corn, this is particularly true for the contribution of phytochemicals, including bioactive compounds such as phenolic acids and carotenoids mainly responsible for flour antioxidant capacity (Blandino et al., 2017). Despite that, the potential use of both corn break meal and flour for the production of co-extruded snacks has not been addressed so far.

Taking into consideration these findings, the objective of the present study was to assess the role corn milling fractions and amylose:amylopectin ratios on the physico-chemical changes during extrusion-cooking and their impact on the features of co-extruded snacks. In particular, three hybrids different in amylose content (i.e., conventional, waxy, and high amylose) and two corn milling fractions (break meal and flour) were considered.

2. Materials & Methods

2.1 Materials

All raw materials were kindly provided by Molino Peila S.p.A. (Valperga, Italy). Three corn varieties different in amylose content were used: i) a conventional hybrid (Pioneer P1547, amylose = 18%; C), ii) high-amylose hybrid (Planta Amylor, amylose = 42%; HA) and iii) waxy hybrid (Pioneer P1547E, amylose = 2%; W). All hybrids have been cultivated in the 2018 growing season in the same growing area in North West Italy. Pioneer P1547 is actually one of the hybrids more cultivated in Italy within the dry-milling supply chain. For each corn hybrid, two types of products were obtained: a flour from the softer part of the endosperm (85% of particles under 150 μm), and a break meal from the vitreous endosperm (77% of particles between 250-500 μm). HA and W were used alone and in combination (50:50) with C (C–HA and C – W).

Co-extruded snacks were produced at industrial level by Fudex Group S.p.A. (Settimo Torinese, Italy). Dry-extrusion was performed using a co-rotating twin-screw extruder (model 2FB90; screw speed: 100 rpm; temperature: 117 °C; pressure: 70 bar). For starch susceptibility to α-amylase and pasting properties and phenolic acid content and antioxidant capacity, snacks were milled into flour (particle size less than 250 microns) using a laboratory mill (IKA Universalmühle M20; IKA Laborteknic, Staufen, Germany), with a water-cooling system to avoid the overheating.

2.2. Methods

2.2.1 Chemical composition

The moisture content, determined in order to express all the results on a dry weight (dw) basis, was obtained by oven-drying at 105 °C for 24 h. Total starch content was measured according to the standard method AACC 76-13.01 (Cereals & Grains, 2011). The total protein content (conversion factor: 5.70) was obtained according to the Kjeldahl method by means of a Kjeltec system I (Foss Tecator AB, Höganäs, Sweden) (Sovrani et al., 2012). The total dietary fiber content was determined by means of the Megazyme total dietary fiber analysis kit. The fat

78 (Soxhlet method) and ash (muffle furnace) contents were determined according to the AOAC
79 (2005) procedures.

80 **2.2.2 Extraction and quantification of the soluble (SPAs) and cell wall-bound phenolic**
81 **acids (CWBPAs)**

82 The extraction of free and conjugated SPAs and CWBPAs was performed according to the
83 procedure proposed by Li, Shewry & Ward (2008) and Nicoletti, Martini, De Rossi, Taddei,
84 D'Egidio & Corradini (2013) with some modifications. 3,5-Dichloro-4-hydroxybenzoic was used as
85 an internal standard to ensure that losses due to the extraction method were accounted for.

86 *Extraction of soluble phenolic acids*

87 An aliquot (125 mg) of each sample was extracted with 1 mL of 80:20 (v/v) ethanol:water solution.
88 The mixtures were vortexed for 30 s, and then sonicated (35 kHz, Sonorex Super RK 156 BH,
89 Bandelin Electronic, Berlin, Germany) for 10 min, maintaining the temperature at 4°C to avoid
90 starch gelatinization. Samples were centrifuged at 10,600 x g for 10 min, and a second extraction
91 was carried out with 80:20 (v/v) ethanol:water solution. The pellet was discarded, while the
92 supernatants were collected and then evaporated to dryness under a nitrogen stream. Samples
93 were hydrolyzed with 2 M NaOH for 2 h under continuous stirring at 4°C. After acidification to pH
94 2 with HCl, soluble phenolic acids were extracted with 500 µL of ethyl acetate. After centrifugation
95 at 10,600 x g for 2 min the upper layer was transferred in a clean microcentrifuge tube. The
96 extraction was repeated twice, and the combined supernatants were evaporated to dryness under
97 a nitrogen stream and then reconstituted in 100 µL of 80:20 (v/v) methanol:water solution.

98 *Extraction of cell wall-bound phenolic acids*

99 Samples (125 mg) were extracted two times with 80:20 (v/v) ethanol:water in order to remove
100 soluble phenolic acids. Mixtures were vortexed before being sonicated for 10 min. Samples were
101 then centrifuged at 10,600 x g for 10 min, and the supernatant was removed and discarded. The

remaining pellet was hydrolyzed 4 h under continuous stirring at 4°C, by adding 2 M NaOH. After acidification to pH 2 with HCl, the bound phenolic acids were extracted with 800 µL of ethyl acetate and then centrifuged at 10,600 x g for 2 min. The extraction was repeated another time. The combined supernatants were evaporated to dryness under a nitrogen stream, and then reconstituted in 200 µL of 80:20 (v/v) methanol:water solution.

Quantification of soluble and cell wall-bound phenolic acids by means of RP-HPLC/DAD

The phenolic extracts were filtered through a 0.2 µm PVDF filter and then analyzed by means of a high performance liquid chromatograph Agilent 1200 Series (Agilent Technologies, Santa Clara, CA, US) coupled to an Agilent 1200 Series diode array detector. Separations were carried out using a 150 x 4.6 mm, 5 µm, Gemini RP-18 column (Phenomenex, Torrance, CA, US); the column temperature was set at 35 °C. The mobile phase consisted of 0.1% acetic acid in water (solvent A) and 0.1% acetic acid in methanol (solvent B). The following operating linear gradient was used: 0-22 min, 9-42% B; 22-27 min, 42-90% B; 27-32 min, 90% B. Finally, the mobile phase was brought to 9% B in 3 min, and this was followed by 16 min of equilibration. The flow rate of the mobile phase was 1 mL/min. Phenolic acids were identified using the retention times and the UV/Vis spectra of their respective standards. Solutions of individual phenolic acid standards were also prepared and diluted to different concentrations to obtain calibration curves for quantification purposes. The quantifications were performed at the maximum absorption wavelength of each phenolic acid.

2.2.3 Determination of antioxidant capacity by means of the FRAP assay

Tre FRAP (Ferric Reducing Antioxidant Power) assay adapted into the QUENCHER method was performed as described by Serpen, Gökmen & Fogliano (2012). Briefly, FRAP reagent was prepared by mixing the aqueous solution of 10 mM TPTZ and 20 mM ferric chloride in 300 mM sodium acetate buffer (pH 3.6) at a ratio of 1:1:10 (v:v:v). Samples (2 mg) were analyzed by adding FRAP working solution (2 mL). The reaction was carried out under stirring at 1,000 rpm

(PCMT Thermoshaker, Grant Instruments, Cambridge, UK). After exactly 120 min from the first introduction of FRAP solution onto solid samples, centrifugation was performed for 1 min at 20,800 x g, and the absorbance was measured at 593 nm. The final results were expressed as mmol Trolox equivalents/kg of sample (dw) through a calibration curve.

2.2.4 Determination of antioxidant capacity by means of the ABTS assay

The ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) assay adapted into QUENCHER method was performed as described by Serpen et al. (2012). Briefly $ABTS^{\cdot+}$ working solution was obtained by diluting the aqueous stock solution (7 mM ABTS, 2.45 mM potassium persulfate) with water until the absorbance at 734 nm was 1.5. Samples (1.4 mg) were analyzed by adding 700 µl of ethanol and 700 µl of the $ABTS^{\cdot+}$ working solution (final solvent ratio - water:ethanol 50:50). The reaction was carried out in the dark under stirring at 20°C and 1000 rpm for 30 min. The samples were promptly centrifuged for 1 minute at 20,800 x g, and the absorbance of the supernatant was measured at 734 nm. A control solution, without the ground sample, was tested under the same conditions, in order to calculate the $ABTS^{\cdot+}$ inhibition percentage of the samples. The final results were expressed as mmol Trolox equivalents/kg of sample (dw) through a calibration curve.

2.2.5 Starch properties

Starch susceptibility to α -amylase was carried according to the standard method AACC 76-31.01 (Cereals & Grains, 2011).

Pasting properties were evaluated using a Micro Visco-Amylo-Graph (Brabender GmbH., Duisburg, Germany) according to the procedure of Marengo et al. (2017). 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. The following parameters were considered: beginning of gelatinization

(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 raw materials and snacks.

2.2.6 Snack characterization

Area

Cylindrical shape snacks were cut using a blade and images of 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). Images were processed into gray level (8 bits). Section area, cell wall area, and inner area were considered.

Porosity and bulk density

Total porosity and bulk density were assessed with a Pascal Mercury Porosimeter (P240; Thermo Fisher Scientific, Waltham, US). Samples were subjected to an increasing pressure of up to 200 MPa that makes it possible to measure pores with a radius from 3.7×10^{-3} to 7.5 µm.

Texture analysis

Mechanical properties of the snacks were determined by 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 1 mm/s to 5 mm of 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 for hardness.

2.2.7 Statistics

Three individual extractions were carried out for each sample for both SAPs and CWBPAs. The SPAs and CWBPAs content, AC-FRAP, AC-ABTS, and starch susceptibility were measured in triplicate, whereas pasting properties, porosity and bulk density in duplicate. Image and texture analysis were carried out on ten and thirty pieces, respectively. One-way analysis of variance was performed by 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 & Discussion

3.1 Raw materials chemical composition

Corn fractions resulted in different composition according to the particle size: corn flour resulted in a higher content of starch (76%), fat (2%), and ash (1%) compared to break meal (starch 72%, fat 1.5%, ash 0.6%). On the other hand, the percentage of protein (7.0% vs 4.6%) and fiber (4.4% vs 2.6%) was higher in break meal than corn flour.

3.2 Phenolic acids and antioxidant capacity of raw materials and snacks

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 concentration of phenolic acids. The corn flour showed on average a higher concentration of both SPAs and CWBPAs in comparison with the break meal (+34% and +4%, respectively). Comparing the hybrids, the highest concentration of CWBPAs was observed in HA (686 and 629 mg/kg dw, for break meal and flour, respectively), while no significant difference was observed between the C and W hybrids. Less differences were observed comparing hybrids for their SPA content.

The antioxidant capacity, measured by means of FRAP and ABTS assays, was higher in the HA hybrid than the other hybrids (+32% and +20%, respectively). Results are in accordance with the ones of Li, Wei, White & Beta (2007), who stated that among corn genotypes, the high-amylose

one had the best antioxidant capacity and the highest concentration of ferulic acid. Similarly, the HA hybrid analyzed in the present study showed the highest concentration of CWB ferulic acid (+49%) if compared with the C and W hybrids (data not shown).

Several changes occur during extrusion as far as both phenolic acids and AC are concerned (Figure 1). Specifically, AC increased significantly during processing, resulting - as far as the HA hybrid is concerned - about 2 times higher in snacks than the related raw material. The increase in the AC seems not to be related to the changes in SPAs and CWBPAs occurred during extrusion. Indeed, even if phenolic acids are the main antioxidant compounds in cereals, and several studies showed a correlation between their concentration and the AC (Beta, Nam, Dexter & Sapirstein, 2005; Li et al., 2007), both SPAs and CWBPAs decreased during extrusion. The only exception is the cell wall-bound sinapic acid, whose concentration increased after extrusion. Interestingly, as far as the HA hybrid is concerned, the concentration of soluble sinapic acid during processing decreased of 32 mg/kg, whereas in the bound form the concentration increased of 25 mg/kg.

As observed in previous studies (Ylmaz & Toledo, 2005; Yu, Nanguet & Beta, 2013), the increase in the AC during extrusion could be due to the products of the Maillard reaction formed during the processing at high temperatures. As far as phenolic acids are concerned, several studies showed that the extrusion process greatly affect the composition of both free and bound phenolic acids in cereals, and that changes could differ depending on the cereal processed because of great difference of cereal matrix (Zeng, Liu, Luo, Chen & Gong, 2016; Ruiz-Armenta, Zazueta-Morales, Delgado-Nieblas, Carrillo-López, Aguilar-Palazuelos & Camacho-Hernández, 2019). In the present study, SPAs suffered the greatest decrease after extrusion. Snacks obtained from the HA hybrid showed a decrease by 51-59% of SPAs compared to the raw material. Similarly, Altan, McCarthy & Maskan (2009) observed that extrusion cooking of barley significantly reduced total phenolics by 46-60%. Extrusion cooking could lead to a decrease of free phenolic acids because of decomposition caused by high temperatures. Moreover, the extractability of free phenolic acids

after extrusion could decrease because of the increased polymerization which could 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 extruded cereals. Nevertheless, even if the concentration of CWBPAs slightly decreased during extrusion, differences observed in the present study were not significant.

3.3 Starch properties

3.3.1 Starch susceptibility to α -amylase hydrolysis

Starch susceptibility to 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 which are physically broken during the milling or grinding process to make flour. Indeed, damaged granules are more susceptible to enzymatic hydrolysis having a high contact surface. Taking into consideration that, as expected, corn break meal samples presented less amount of damaged starch (Table 2) and higher particle size (data not shown), coming from the vitreous endosperm of the kernel. On the other hand, the flour samples are obtained from the softer part of the endosperm (Blandino et al., 2017).

As regards the hybrids different in amylose content, the highest damaged starch in snacks from W flour has been associated with the low hydrogen bonding due to the low amylose content that may decrease the resistance to crushing and thus increase the starch damage (Bettge, Giroux & Morris, 2000). On the other hand, the more compact structure in HA might account for the low values in damaged starch found in both 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 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, suggesting starch destructuring. Specifically, snacks from HA exhibited the lowest starch susceptibility when either

flour or break meal were used for their production, suggesting that the more compact structure due to the high amylose content mitigated the effect of processing.

3.3.2 Pasting properties

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

As regards the amylose content, both W flour and break meal showed a lower pasting temperature, peak viscosity, and retrogradation tendency (i.e., low final viscosity and setback values) compared to C, in agreement with literature (Caramanico et al., 2018; Liu, Yuan, Wang, Reimer, Isaak & Ai, 2019). On the other hand, both HA flour and break meal did not show 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. Previous studies showed that heating HA starch at temperatures above 120 °C completely gelatinized starch (Liu et al., 2019). As expected, blending either W or HA with C resulted in an intermediate behaviour. Regardless of the type of milling fractions and corn hybrid, snacks did not show a pasting profile (data not shown), as a result of starch degradation occurring during the extrusion process and confirming the starch susceptibility data in Table 2. Dry-extrusion leads to a high gelatinization degree of the starch causing the loss of its gelatinisation and retrogradation properties. This behavior was 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 snacks in terms of total and section area, bulk density, porosity, and hardness are summarized in Table 4. The overall quality of snacks greatly depends on the type of product, i.e. direct-expanded *vs* co-extruded snack. The former is characterized by low bulk density and high expansion rate; the latter by a compact structure, in which voids are undesired. In this study we focused on co-extruded snacks that typically have an extrusion-cooked outer shell that will be filled with a savory or sweet filling. Since the filling needs to be contained inside the snack, a compact structure is desirable. In this kind of products, structure compactness might be assessed by low volume and consequently high bulk density and porosity. Last but not least, various sensory attributes, including crispy texture, contribute to the definition of product quality. Specifically, usually, the higher the expansion rate, 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 the final product density (Robin, Schuchmann & Palzer, 2012).

The section area could be considered an index of the degree of expansion of the product. Indeed, the higher the area, the higher is the expansion rate, considering that the die of the extruder did not change during the extrusion trials.

The section area of snacks made from corn flours is generally higher than the area of corn meal snacks (Table 4), suggesting high expansion degree and confirming previous findings about the relation between increased particle size and decreased expansion of extrudates (Garber, Hsieh & Huff, 1997; Shevkani, Kaur, Singh, Singh & Singh, 2014). The lower expansion of snacks from powders of larger particles could be due to incomplete starch gelatinization, as shown by the pasting profiles in Figure 2. The differences in section area among the snacks made from either corn flour or break meal were similar when HA was used, suggesting that the type of hybrid prevails on particle size.

299 Regardless of the particle size, snacks from C and HA showed the highest and lowest section
300 and cell wall area (which is the difference between section and inner area), respectively. Previous
301 studies emphasized the role of HA on the expansion rate of corn starch snacks (Mercier & Feillet,
302 1975; Zhu et al., 2010). Specifically, HA starch required higher extrusion temperature to reach an
303 expansion degree comparable to starches with less amylose content (Mercier & Feillet, 1975).
304 The amount of amylose in the products and both section and cell wall area did not follow a linear
305 trend (data not shown), suggesting that it is not only the amylose content, but likely also the starch
306 structure to determine the characteristics of the final product.
307 Snacks from HA showed the highest values of the inner area (Table 4), suggesting the potential
308 application of this raw material to produce co-extruded snacks to be filled. While using corn flour,
309 the snack with the highest inner area was obtained when HA was blended with C (Table 4).
310 The highest values of both bulk density and porosity were obtained when corn break meal was
311 used, in agreement with the lowest area (Table 4). Other authors found a similar relation between
312 bulk density and expansion rate (Zhu et al., 2010; Tacer-Caba et al., 2014). As regards the hybrid
313 type, using HA resulting in a snack with high bulk density and porosity. The higher particle size of
314 break meal - together with its gelatinization difficulty - masks the effect of HA on these indices.
315 As regards hardness, snacks from corn break meal were generally firmer than those made from
316 flour, because of their reduced section area (or diametric expansion) and increased bulk density
317 (Table 4).
318 The effect of the type of hybrid and amylose content is evident just in the case of corn flour.
319 Specifically, using HA flour determined the production of a firmer snack, suggesting that amylose
320 played a significant role in determining the mechanical strength of the products, as already shown
321 in soy protein - high amylose starch extrudates (Zhu et al., 2010). Several factors might account
322 for the mechanical properties of snacks, including volume, average cell size and cell size
323 distribution, thus porosity (Zhu et al., 2010). This result could be justified by the high bulk density

of this sample compared to the others. Confirming the above, this sample showed the smallest section area, which indicated a more compact structure which better resisted compression load. A different behaviour was observed about the samples obtained from corn break meal; in this case, the hardest samples were the one obtained from C-W blend. However, it is important to note that C, W and HA were not significantly different in hardness. This could be due to the coarse particle size that limits starch gelatinisation even in C and W, that are usually able to gelatinize, in agreement with both pasting profiles (Figure 2).

As for samples from blends, in both corn flour and break meal, hardness values are different from what we might expect analysing the raw materials. These behaviours are probably due to a particular rearrangement of starch during the dry-extrusion process, that is worthy of further investigation.

4. Conclusions

This study provided an insight into the interactions between amylose content and type of milling fraction in defining the characteristics of corn snacks.

As regards bioactive compounds, HA confirmed the highest antioxidant capacity and phenolic acid content, maintaining superior content of these phytochemicals in the related snacks. Moreover, for each hybrid the corn flour fraction reported a marked antioxidant capacity compared to break meal. Furthermore, in order to obtain healthier foods, the opportunity to use these finer milling fractions, need to be carefully considered, in particular for genotypes more subject to environmental stress such as high amylose corn, taking in consideration their higher risk for fumonisin and other mycotoxin contamination (Vanara et al., 2018).

W and C corn (with 2 and 18% amylose content, respectively) led to more expanded and softer snacks. In the case of co-extruded snack, the best results in terms of texture, porosity, bulk density, and section area expansion were obtained using flour from HA (40% amylose). The high compact structure of this kind of snack is related to the peculiar starch properties of HA, which

results in the lowest gelatinization degree. Starch gelatinization properties were also restricted when corn break meal (with higher particle size) was used instead of corn flour. In this case, the milling fraction seems to mask the effect of amylose content. Blending both HA and W hybrids to the C one led to snacks whose features did not follow a linear trend with the amylose content, suggesting that beside the latter, also the starch structure/properties are strategic in defining the quality of the final product. Taking into consideration these findings, the particular rearrangement of starch when different hybrids are blended during the dry extrusion process is worthy of further studies.

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

Acknowledgements: This work was supported by Regione Piemonte (POR FESR 2014-2020), as a part of the EXFREE Project. The authors thank Mr. Davide Carrara (DeFENS, Università degli Studi di Milano), Dr. Alessandro Peila (Molino Peila S.p.A.), Dr. Umberto Lenzi (Fudex Group S.p.A.) for technical support.

References

AACC (2011). Approved Methods of Analysis, 11th Ed. Cereals & Grains Association, St. Paul, MN, US.

Altan, A., McCarthy, K. L., & Maskan, M. (2009). Effect of extrusion process on antioxidant activity, total phenolics and β-glucan content of extrudates developed from barley-fruit and vegetable by-products. *International Journal of Food Science & Technology, 44(6)*, 1263-1271.

AOAC (2005). Official Methods of Analysis of AOAC International, 18th Ed., Horwitz W., ed. AOAC International, Gaithersburg, MD, US.

Beta, T., Nam, S., Dexter, J. E., & Sapirstein, H. D. (2005). Phenolic content and antioxidant activity of pearled wheat and roller-milled fractions. *Cereal Chemistry, 82(4)*, 390-393.

Bettge, A. D., Giroux, M. J., & Morris, C. F. (2000). Susceptibility of waxy starch granules to mechanical damage. *Cereal Chemistry, 77(6)*, 750-753.

Blandino, M., Alfieri, M., Giordano, D., Vanara, F., & Redaelli, R. (2017). Distribution of bioactive compounds in maize fractions obtained in two different types of large scale milling processes. *Journal of Cereal Science, 77*, 251-258.

Brennan, M. A., Derbyshire, E., Tiwari, B. K., & Brennan, C. S. (2013). Ready-to-eat snack products: the role of extrusion technology in developing consumer acceptable and nutritious snacks. *International Journal of Food Science & Technology, 48(5)*, 893-902.

Caramanico, R., Marti, A., Vaccino, P., Bottega, G., Cappa, C., Lucisano, M., & Pagani, M. A. (2018). Rheological properties and baking performance of new waxy lines: Strengths and weaknesses. *LWT, 88*, 159-164.

Delgado-Nieblas, C., Aguilar-Palazuelos, E., Gallegos-Infante, A., Rocha-Guzmán, N., Zazueta-Morales, J., & Caro-Corrales, J. (2012). Characterization and optimization of extrusion cooking for the manufacture of third-generation snacks with winter squash (Cucurbita moschata D.) flour. *Cereal Chemistry, 89(1)*, 65-72.

Garber, B.-W., Hsieh, F., & Huff, H.-E. (1997). Influence of particle size on the twin-screw extrusion of corn meal. *Cereal Chemistry, 74(5)*, 656–661.

Gomez, M. H., & Aguilera, J. M. (1983). Changes in the starch fraction during extrusion-cooking of corn. *Journal of Food Science, 48(2)*, 378-381.

Li, W., Wei, C., White, P. J., & Beta, T. (2007). High-amylose corn exhibits better antioxidant activity than typical and waxy genotypes. *Journal of Agricultural and Food Chemistry, 55(2)*, 291-298.

Li, L., Shewry, P. R., & Ward, J. L. (2008). Phenolic acids in wheat varieties in the HEALTHGRAIN diversity screen. *Journal of Agricultural and Food Chemistry, 56(21)*, 9732-9739.

Liu, S., Yuan, T. Z., Wang, X., Reimer, M., Isaak, C., & Ai, Y. (2019). Behaviors of starches evaluated at high heating temperatures using a new model of Rapid Visco Analyzer–RVA 4800. *Food hydrocolloids, 94*, 217-228.

Marengo, M., Barbiroli, A., Bonomi, F., Casiraghi, M. C., Marti, A., Pagani, M. A., Manful, J., Graham-Acquaah, S., Ragg, E., Fessas, D., Hogenboom, J. A., & Iametti, S. (2017). Macromolecular traits in the african rice Oryza glaberrima and in Glaberrima/Sativa crosses, and their relevance to processing. *Journal of Food Science, 82(10)*, 2298–2305.

Mariotti, M., Zardi, M., Lucisano, M., & Pagani, M. A. (2005). Influence of the heating rate on the pasting properties of various flours. *Starch-Stärke, 57(11)*, 564-572.

Marti, A., Seetharaman, K., & Pagani, M. A. (2010). Rice-based pasta: A comparison between conventional pasta-making and extrusion-cooking. *Journal of Cereal Science, 52(3)*, 404-409.

Marti, A., & Pagani, M. A. (2013). What can play the role of gluten in gluten free pasta?. *Trends in Food Science & Technology, 31(1)*, 63-71.

Mercier, C., & Feillet, P. (1975). Modification of carbohydrate component by extrusion cooking of cereal product. *Cereal Chemistry, 52(1)*, 283-297.

Nicoletti I., Martini D., De Rossi A., Taddei F., D'Egidio M.G., Corradini D. (2013). Identification and quantification of soluble free, soluble conjugated, and insoluble bound phenolic acids in

durum wheat (Triticum turgidum L. var. durum) and derived products by RP-HPLC on a semimicro separation scale. J*ournal of Agricultural and Food Chemistry, 61(48)*, 11800-11807.

Ozcan, S., & Jackson, D. S. (2005). Functionality behavior of raw and extruded corn starch mixtures. *Cereal Chemistry, 82(2)*, 223-227.

Pellegrini, N., & Agostoni, C. (2015). Nutritional aspects of gluten-free products. *Journal of the Science of Food and Agriculture, 95(12)*, 2380-2385.

Riaz, M, N. (2006). Extruded snacks. In: Handbook of Food Science, Technology, and Engineering, Vol 4. Y. H. Hui, Frank Sherkat (Eds.). Taylor & Francis, Boca Raton, US, pp. 168-1 - 168-8.

Robin, F., Schuchmann, H. P., & Palzer, S. (2012). Dietary fiber in extruded cereals: limitations and opportunities. *Trends in Food Science & Technology, 28(1)*, 23-32.

Ruiz-Armenta, X. A., Zazueta-Morales, J. D. J., Delgado-Nieblas, C. I., Carrillo-López, A., Aguilar-Palazuelos, E., & Camacho-Hernández, I. L. (2019). Effect of the extrusion process and expansion by microwave heating on physicochemical, phytochemical, and antioxidant properties during the production of indirectly expanded snack foods. *Journal of Food Processing and Preservation, 43(12)*, e14261.

Serpen, A., Gökmen, V., & Fogliano V. (2012). Solvent effects on total antioxidant capacity of foods measured by direct QUENCHER procedure. *Journal of Food Composition and Analysis, 26 (1-2)*, 52-57.

Shevkani, K., Kaur, A., Singh, G., Singh, B., & Singh, N. (2014). Composition, rheological and extrusion behaviour of fractions produced by three successive reduction dry milling of corn. *Food and Bioprocess Technology, 7(5)*, 1414-1423.

437 Sovrani, V., Blandino, M., Scarpino, V., Reyneri, A., Coïsson, J. D., Travaglia, F., Locatelli, M.,
438 Bordiga, M., Montella, R., & Arlorio M. (2012). Bioactive compound content, antioxidant activity,
439 deoxynivalenol and heavy metal contamination of pearled wheat fractions. *Food Chemistry,*
440 *135(1)*, 39–46.

441 Tacer-Caba, Z., Nilufer-Erdil, D., Boyacioglu, M. H., & Ng, P. K. (2014). Evaluating the effects of
442 amylose and Concord grape extract powder substitution on physicochemical properties of wheat
443 flour extrudates produced at different temperatures. *Food Chemistry, 157*, 476-484.

444 Vanara, F., Scarpino, V., & Blandino, M. (2018). Fumonisin distribution in maize dry-milling
445 products and by-products: impact of two industrial degermination systems. *Toxins, 10(9)*, 357.

446 Ylmaz, Y., & Toledo, R. (2005). Antioxidant activity of water-soluble Maillard reaction products.
447 *Food Chemistry, 93(2)*, 273-278.

448 Yu, L., & Nanguet, A. L. (2013). Comparison of antioxidant properties of refined and whole wheat
449 flour and bread. *Antioxidants, 2(4)*, 370-383.

450 Zeng, Z., Liu, C., Luo, S., Chen, J., & Gong, E. (2016). The profile and bioaccessibility of phenolic
451 compounds in cereals influenced by improved extrusion cooking treatment. *PloS one, 11(8)*,
452 e0161086.

453 Zhu, L. J., Shukri, R., de Mesa-Stonestreet, N. J., Alavi, S., Dogan, H., & Shi, Y. C. (2010).
454 Mechanical and microstructural properties of soy protein–high amylose corn starch extrudates in
455 relation to physiochemical changes of starch during extrusion. *Journal of Food Engineering,*
456 *100(2)*, 232-238.

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 (CWBPAs; panel C) detected in the high-amylose hybrid raw materials (grey bars) and snacks (white bars).

Data are expressed on a dw basis. Level of significance of ANOVA: ns=p-value >0.05; *=p-value <0.05; **=p-value <0.01; ***=p-value<0.001. Reported values are based on 3 replications.

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

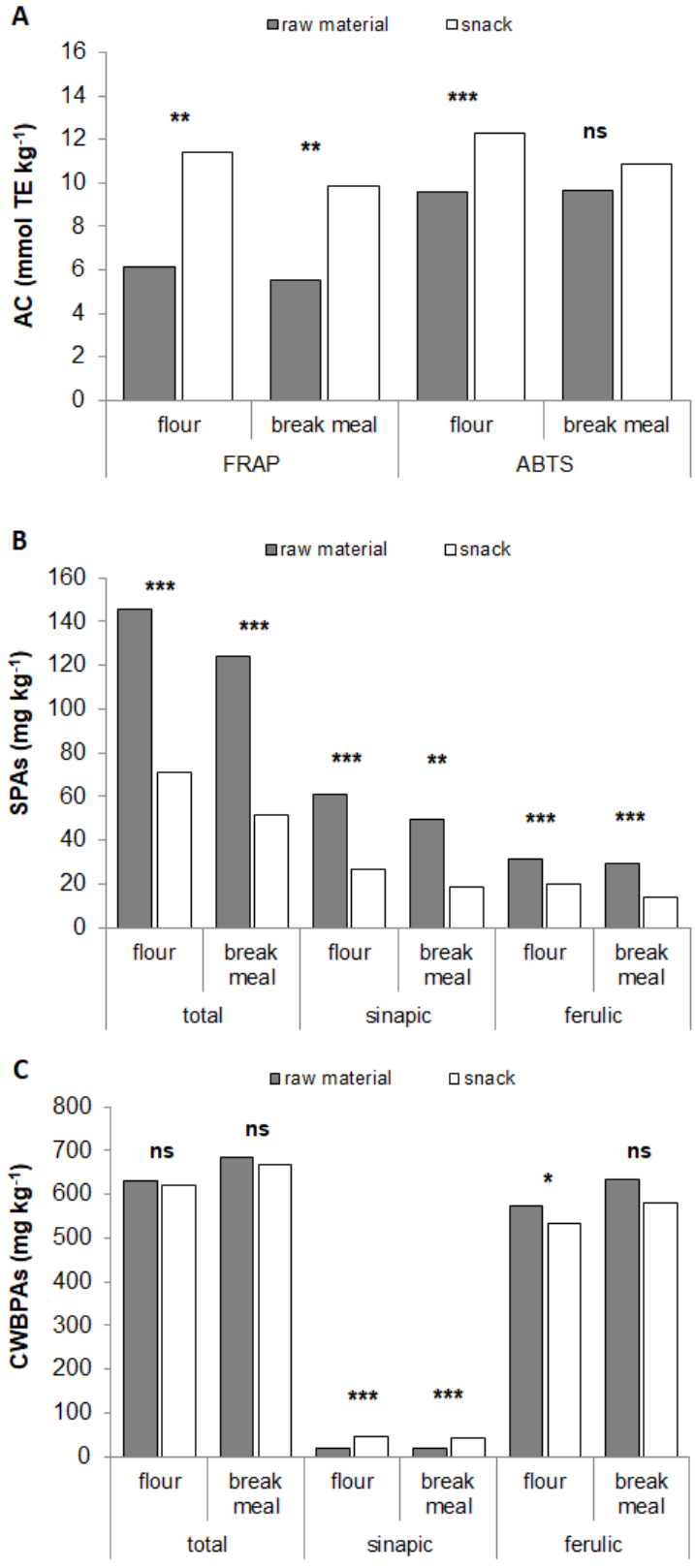
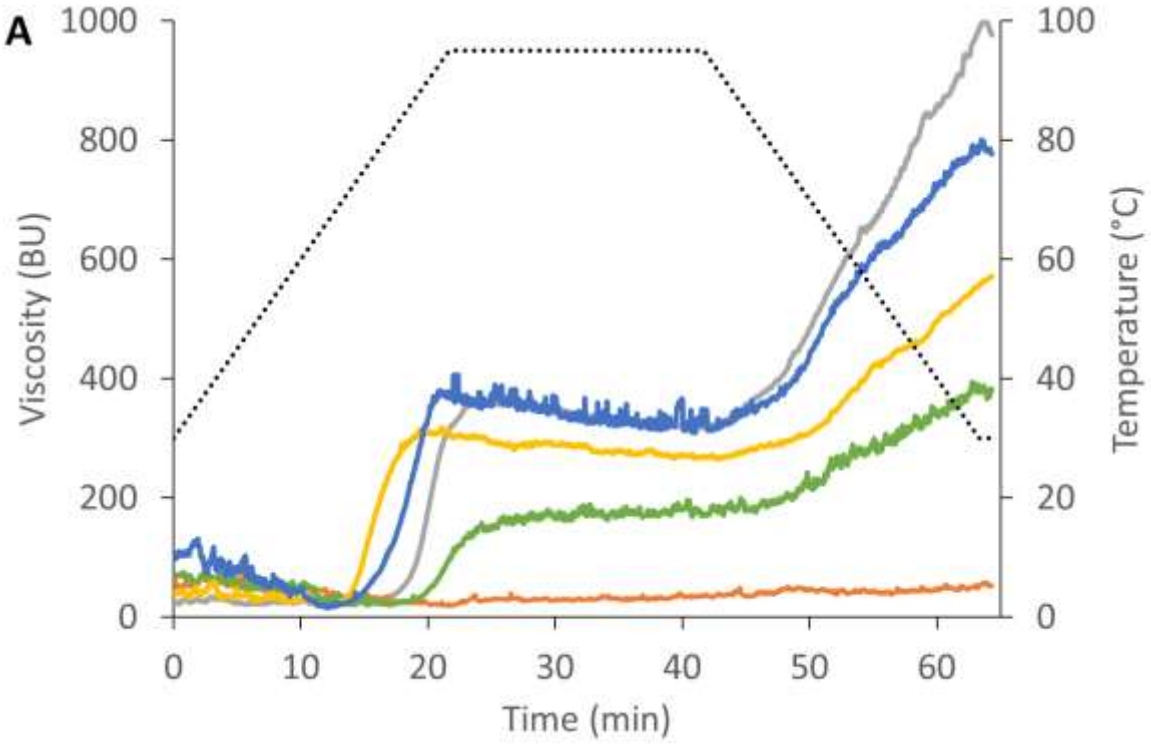


Figure 1. Antioxidant capacity (FRAP and ABTS assay, panel A), total, sinapic and ferulic soluble phenolic acids (SPAs; panel B) and total, sinapic and ferulic cell wall-bound phenolic acids

(CWBPAs; panel C) detected in the high-amylose hybrid raw materials (grey bars) and snacks (white bars).

Data are expressed on a dw basis. Level of significance of ANOVA: ns=p-value >0.05; *=p-value <0.05; **=p-value <0.01; ***=p-value<0.001. Reported values are based on 3 replications.

475
476
477



478
479
480

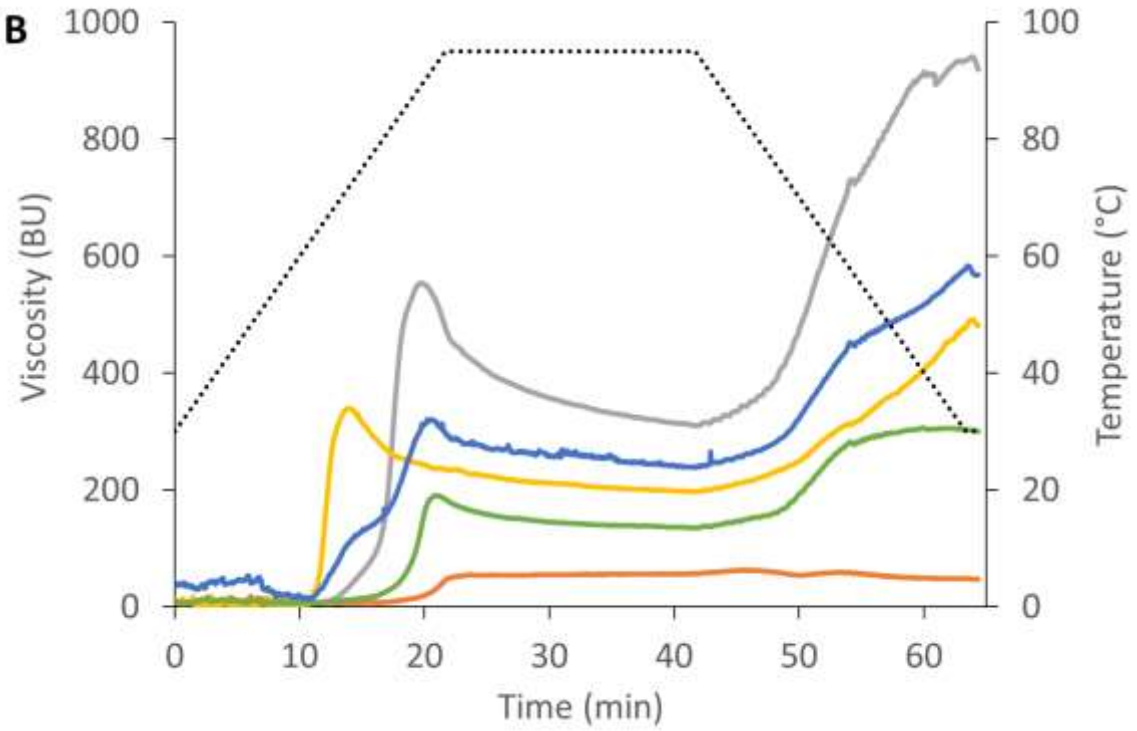


Figure 2. Pasting profiles of break meal (panel A) and flours (panel B) from conventional hybrid (grey), waxy hybrid (yellow), high-amylose hybrid (orange), conventional-high amylose blend (green) and conventional-way blend (blue).

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	CWBPAs[1] mg/kg	AC-FRAP mmol TE/kg	AC-ABTS mmol TE/kg
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^{e}	8.2±0.2^{cd}
		HA	124.4±8.2^{b}	685.7±78.2^{a}	5.5±0.5^{def}	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^{bcd}	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±62.5c^{d}	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±0.2c^{d}	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 (g/100g dw).

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

Within each product (raw materials or snacks), means ± standard deviation followed by different letters are significantly different, according to the REGW-Q test ($p<0.001$). Reported values are based on two replications. Lowercase letters refer to raw materials; uppercase letters refer to snacks.

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}
Maximum viscosity (°C)	Break meal (250-500 μm)	94.9±0.01^{a}	95.1±0.1^{a}	-	92.0±0.01^{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±2.83^{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 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 ± 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 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.