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Processing of Novel Elevated Amylose Wheats: Functional Properties and Starch Digestibility of Extruded Products

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Different types of novel wheat lines with different starch contents and amylose/amylopectin ratios, relating to defined alterations in the number and activity of starch synthase IIa genes, were processed by pilot-plant extrusion. Two types of products were produced: pure wholemeal products and breakfast cereals made from wholemeal/maize blends. Lower apparent shear viscosity was obtained in the extruder with lower starch content and higher amylose/amylopectin ratio flours (SSIIa-deficient line). The bulk density of the products decreased with increasing extrusion temperature and was always higher for the triple-null line. The bulk density was not completely explained by the melt shear viscosity, suggesting the importance of the fillers (fibers, brans) in the process of expansion and structure acquisition. The different mechanical properties were explained by the density and by the material constituting the cell walls. Enzyme-resistant starch (RS) content and hydrolysis index (HI) were not correlated to the extrusion temperature, but RS was higher in pure wholemeal products and in the SSIIa-deficient line. These results are discussed in terms of starch molecular architecture and product microstructure.

KEYWORDS: Breakfast cereals; wheat; maize; extrusion; resistant starch; hydrolysis index; crispness; microstructure

INTRODUCTION

Processing of novel wheat varieties is of interest for the creation of novel extruded products such as breakfast cereals or snacks, with decreased starch digestibility including increased resistant starch content and reduced glycaemic index.

Resistant starch (RS) is defined as the sum of starch and products of starch degradation not absorbed in the small intestine of healthy individuals. RS is by definition not absorbed in the small intestine (*1*); thus, it does not contribute to postprandial hyperglycemia (*2*). RS might have physiological properties similar to those of dietary fibers and have significant positive effects on intestinal function (*3*). RS is present in a wide variety of carbohydrate-based foods, and numerous studies indicate that its presence in food might be beneficial for health (*4*). Four classes of RS are commonly identified (*5*, *6*); these are the physically inaccessible starch (RS1) (found in uncooked legumes, lentils); the resistant starch granules (RS2, including raw potato and banana starch containing B-type starches, known to be very resistant to enzymic hydrolysis); the retrograded starch (RS3), present in most starchy foods that have been cooked, cooled, and stored; and chemically modified starch (RS4), including starch ethers and esters.

The glycemic index (GI) is a comparative measure of the capacity of a food's available carbohydrate to raise blood glucose level. The glycemic response curve for a particular food is compared to that of a reference food containing an identical quantity of available carbohydrate. Resistant starch is a quantitative measure of starch that reaches the colon undigested: these values are not necessarily correlated. However, the substitution of a digestible starch by resistant starch generally leads to a decrease of the glycemic response.

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Extrusion–cooking is a process that involves many changes at the crystal and molecular length scale of starch structures. Consequently, in most cases, depending on the processing conditions, extrusion will destroy RS1 and RS2 and may form RS3. The transformation starch undergoes under thermomechanical processing in terms of structural modifications such as melting of the crystallites, granule fragmentation, and depolymerization has been well described (*7–9*). Many studies have related starch transformation to extrusion conditions such as screw geometry, barrel temperature, or water content of the product (*10*). The extent of starch transformation, controlled by adjusting the extrusion conditions, also depends on the starch origin and other components present in flour, such as proteins or fibers, and added ingredients such as sugars.

Functional properties of the end-products are affected by the state of starch in the products; the molecular weight of macromolecules depends on the raw material and on the extrusion conditions, and the amylose/amylopectin ratio will affect the flow behavior of the molten system through the extruder and consequently affect the expansion of the product (*11*). The mechanical/textural properties of the products are affected by the expanded structure and density (*12*) and by the intrinsic material properties, in relation to the molecular weight of the starch macromolecules and their organization. The presence of V-type crystals normally assigned to amylose–lipid complexes and of B-type crystals from the retrogradation of starch when stored above the glass transition temperature also modify the mechanical properties of starchy materials (*10, 13*). The final molecular order of starch in the product will affect its ability to be digested by enzymes, which result in a change in the starch digestibility (*14*).

Alteration of the activity of starch biosynthetic enzymes provides a powerful method of generating novel ingredients. The relationships between specific genetic changes and starch structure and properties have been extensively reviewed and a number of specific alterations in wheat starch properties reported (*15–17*). In this study, lines of wheat with combinations of mutations in the starch synthase IIa gene have been first studied at laboratory scale to understand the impact of the genetic changes on the processability of the novel flours and on the mechanical behavior of the processed products (*18*). The hexaploid nature of wheat allows the isolation of eight specific genotypes based on combinations of mutations in the SSIIa genes from each of the three wheat genomes. The generation of these lines and the structure of their starch have been extensively analyzed previously (*19*).

The aims of this study were to conduct a pilot-plant extrusion to validate the results obtained at the laboratory scale on selected novel wheat flour and to produce model products to conduct relevant in vitro nutritional and mechanical behavior analysis. The objective of this study was to evaluate the behavior of novel wheat varieties when submitted to extrusion–cooking and to evaluate the effects of processing conditions and wheat flour composition on the properties of the end-products, in terms of structural changes, mechanical properties, and nutritional quality focusing on starch digestibility.

MATERIALS AND METHODS

Materials. Novel wholemeal wheat flours were developed by CSIRO, Plant Industry (Canberra, Australia). These novel wheat varieties represent different gene mutations (single, double, or triple null) of starch synthesis enzymes (SSIIa) as described by Konik-Rose et al. (*19*). Composition of the wholemeal flours is shown in **Table 1**. Maize polenta was purchased from Allied Mills, Australia.

Table 1. Composition of the Flours from the Wheat Lines with Different Null Alleles for SSIIa

	no. of gene mutations			
	wild type	single null (aBD)	double nulls (aBd)	triple nulls (abd)
flour name	A9	A38	B44	B63
starch content[a] (%)	62.2 (2.0)	64.6 (1.6)	58.5 (1.2)	52.0 (2.6)
amylose content (%)	21.5	22.9	20.7	22.8
amylose content (starch basis) (%)	34.5	35.4	35.4	43.9
amylopectin content (%)	40.1	41.7	37.8	29.2
amylose/amylopectin ratio	0.53	0.55	0.55	0.78
proteins (%)	11.1	11.3	14.0	14.3

[a] Standard deviation is given in parentheses.

Table 2. Temperature Profile of the Extruder for Two Breakfast Cereals with Flour from Wheat Lines with Different Null Alleles for SSIIa

	zone 1 (°C)	zone 2 (°C)	zone 3 (°C)	zone 4 (°C)	zone 5 (°C)	melt temperature (°C)
pure wholemeal product	30	60	90	108 (±2)	108 (±2)	110
	30	60	90	120 (±2)	120 (±2)	120
	30	60	90	140 (±5)	150 (±5)	140
breakfast cereals	30	60	90	100 (±2)	100 (±2)	110
	30	60	90	115 (±2)	115 (±2)	120
	30	60	90	145 (±2)	150 (±2)	140

Pilot-Plant Scale Extrusion. Extruded products were produced by twin-screw extrusion (APV Baker MPF40). Two type of products were extruded: products composed of pure wholemeal wheat flour, named as pure wholemeal products, and products composed of wholemeal flour/maize with a 60:40 ratio, named as breakfast cereals. Initial moisture content of the flour was between 10 and 13%. As a result, the barrel moisture content was adjusted to 23% with a feed moisture between 10 and 13%. The screw configuration consisted of 5D feed screw − 5 × 30° forward paddles − 3D feed screw − 2 × 60° forward paddles − 3 × 60° reverse paddles − 1D feed screw − 3 × 60° forward paddles − 4 × 60° reverse paddles − 1.5D lead screw. The barrel length was 14.75*D*, where *D* corresponds to the screw diameter (40 mm). The die geometry was 3.5 mm in diameter and 2 mm in length. The temperature profiles used to obtain a temperature of the molten product measured at the die of 110, 120, and 140 °C are shown in **Table 2**.

Differential Scanning Calorimetry (DSC): Residual Gelatinization Enthalpy. To check the extent of starch transformation after processing by extrusion, samples were ground to a powder. Eight milligrams of powder was placed in the DSC pan (Pyris Diamond DSC, Perkin Elmer) and excess water added (40 μL). The scan was carried out immediately to avoid retrogradation. The run was carried out from 20 to 180 °C at 10 °C/min to observe the presence of any enthalpy around 60 °C corresponding to gelatinization.

Mechanical Testing and Bulk Density. Mechanical testing was performed with a TAXT2 Texture Analyzer (Texture Technologies) on extrudates (moisture content = 2.9 ± 0.6%) in the compression mode with a 5 kg cell load. A pin indentation probe was used for the test. The test speed was set to 2 mm/s and completed to 50% of the distance of sample thickness. The average breaking force and distance were determined at the first maximum peak force on the force versus distance curve for 10 replicates of each product. Standard deviations were between 10 and 12% for the breaking force and distance.

Bulk density of the extrudates was determined by weighing a specific volume (500 mL) of the products. Standard deviation of the bulk density was 5%.

In Vitro Enzyme Resistant Starch Content and Hydrolysis Index Determination. In vitro procedures developed by CSIRO were used to predict the hydrolysis index (HI) and resistant starch content of the

Table 3. Extrusion Variables and Temperatures for Two Breakfast Cereals with Flours from the Wheat Lines with Different Null Alleles for SSIIa

		product 1: pure wholemeal breakfast cereals		product 2: breakfast cereals [wheat/maize blend (60:40)]	
sample composition	melt temperature (°C)	pressure (Pa)	SME (kg/kJ)	pressure (Pa)	SME (kg/kJ)
wheat flour A9	110	698	378	650	357
	120	596	340	582	349
	140	464	273	475	291
wheat flour A38	110	757	361	696	345
	120	706	354	650	327
	140	546	306	515	276
wheat flour B44	110	747	384	669	344
	120	683	363	626	318
	140	584	315	520	281
wheat flour B63	110	601	281	549	263
	120	579	294	527	253
	140	395	204	439	237

studied products. The methods simulate the process of food digestion that typically occurs in the upper human gastrointestinal tract. Briefly, duplicate samples along with appropriate standards were chopped using a simple domestic food processor and then mixed with artificial saliva. The bolus was subjected to a series of incubations, at physiological pH and temperature, which essentially mimic gastric and pancreatic digestion. Protein, fat, sucrose, and starch were hydrolyzed using various enzymes of bacterial, fungal, and mammalian origin. After 300 min, the glucose concentration of the digesta was quantitated, using standard spectrophotometric procedures and the HI of the test food calculated as the quantity of glucose released by digestion as a proportion of available carbohydrate content of the sample. For resistant starch, samples were incubated for a further 11 h, and starch remaining at the end of this process was isolated, dispersed, and hydrolyzed to completion using high-purity α-amylase and amyloglucosidase. The resulting glucose was quantitated by spectrophotometry and the (resistant) starch yield calculated.

Microscopy. To observe the expanded structure of the extruded products, 10 and 20 μm layers of each cereal product were cut using a cryotome, and specimens were placed on slides and observed by light microscopy.

To observe the protein structure the specimens were stained with Acid Fuschin (1%) according to the procedure described by Chanvrier et al. (*20*) used for extruded corn products. The samples were observed on a Leica DMLB microscope by fluorescence microscopy (light source OSRAM HBO Mercury Short Arc lamp) using an excitation band pass filter (515–560 nm).

X-ray Diffraction (XRD). XRD was carried out on a Panalytical X'Pert Pro diffractometer. The instrument was equipped with a Cu long fine focus tube, a programmable incident beam divergence slit, a programmable diffracted beam scatter slit, and an X'celerator high-speed detector. The samples were examined over the angular range of 2–40° with a step size of 0.0332° and a count time of 800 s per point. Crystallinity determination was carried out using X'Pert software. This program automatically determines the amorphous hump of the diffraction pattern, and the crystallinity can be easily calculated from the intensity ratio of the diffraction peaks (I_{net}) and of the sum of all intensity measured (I_{total}):

$$\text{crystallinity (\%)} = 100 \times (I_{net}/I_{total})$$

RESULTS

Behavior of the Novel Wheat Flours with Gene Mutations for SSIIa under Extrusion. *Extrusion Parameters and Variables.* The extrusion variables of pressure and specific mechanical energy (SME) as a function of extrusion temperature are presented in **Table 3**. For every product, that is, wholemeal breakfast cereals and wheat/maize breakfast cereals, the pressure and SME decreased with increasing temperature. This is related to the flow rate, which increases due to a decrease in friction of the product in the barrel with increasing temperature (*21*). Regardless of the wheat line used, the applied SME was generally lower for wheat/maize blends than for wholemeal products. It may be possible that maize polenta provides a lubricating effect due to the different viscosity of maize starch and maize protein compared to wheat flour. The applied SME was also significantly lower for B63 (triple-null line) than for A9, A38, and B44, for both types of formulation. This might be due to the increased protein level in B63, which can act as a better lubricant, particularly if it is present as a more extensive phase.

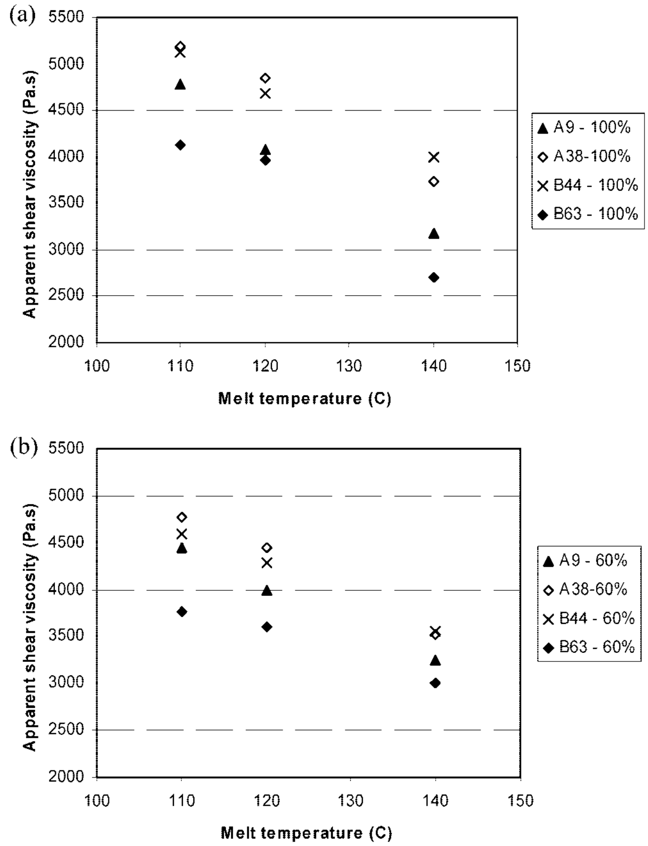

Figure 1. Apparent shear viscosity versus melt temperature for the two types of extruded products: (**a**) 100% wholemeal products; (**b**) 60:40 wholemeal/maize blends.

From the extrusion data, the apparent shear viscosity was determined from the pressure and flow rate data (**Figure 1**). For both types of products (wholemeal breakfast cereals, **Figure 1a**, and wheat/maize breakfast cereals, **Figure 1b**), the apparent viscosity versus extrusion temperature was highest for A38 and lowest for B63. A9 and B44 represented intermediate apparent shear viscosity values.

Product Bulk Density. *Product Bulk Density versus Product Type.* The bulk density of each product was determined to evaluate the expansion of the product at the end of the die as a function of the extrusion temperature (**Figure 2**). The bulk density was lower in the wheat/maize products (between 275 and 325 kg/m^3 for A9-60%, A38-60%, and B44-60% when extruded at 110 °C) than in the pure wholemeal products (between 325 and 350 kg/m^3 for A9-100%, A38-100%, and B44-100%). This trend was also observed at 120 and 140 °C. All wheat/maize products exhibited a lower density than the

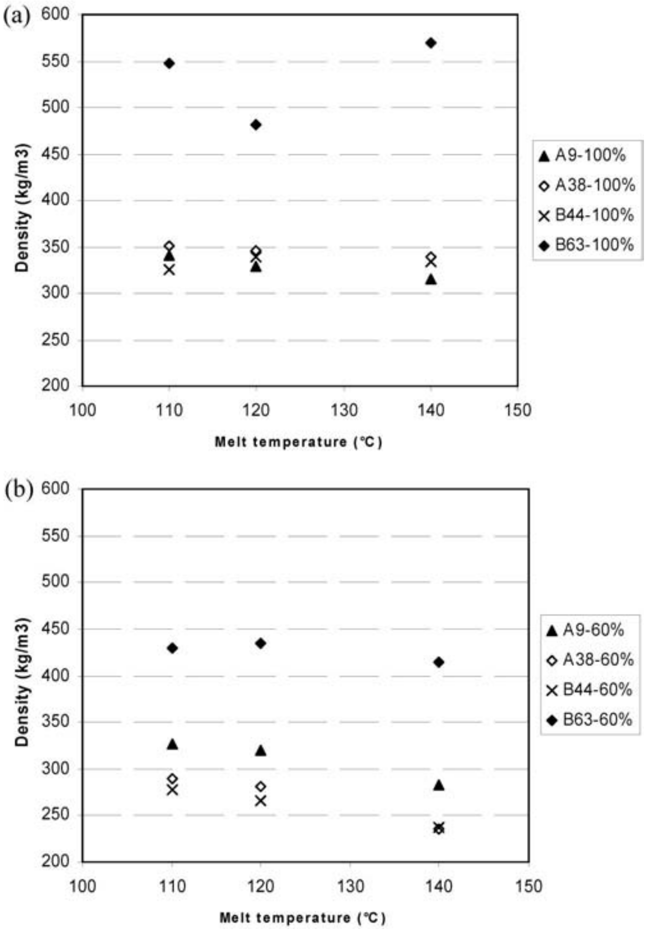

Figure 2. Bulk density of the novel wheat products versus melt temperature (°C): (**a**) 100% wholemeal products; (**b**) 60:40 wholemeal/ maize blends (standard deviation of 0.02% for density measurements).

wholemeal products. This might be explained by the different ability of maize to expand under certain extrusion conditions (*22*). In some studies (*22*), the authors observed a better sectional expansion of the corn extrudates than the wheat extrudates at a screw speed of 200 rpm, but no explanation was given.

Product Bulk Density versus Temperature. For all flour lines except B63 (triple-null line), the density tended to decrease with increasing temperature regardless of the type of product (**Figure 2a**,**b**), and the decrease was greater for wheat/maize products than for pure wholemeal products. For example, the density of A9-100% decreased from 340 kg/m^3 at 110 °C to 315 kg/m^3 at 140 °C, whereas for A9-60% the density decreased from 330 kg/m^3 at 110 °C to 280 kg/m^3 at 140 °C. The decrease in density can be attributed to the lower shear viscosity of the melt when processed at higher temperatures (**Figure 1**) and the concurrent increase in steam pressure generating the expansion. However, this was not true for B63, which, although also showing a decrease in shear viscosity with increasing temperature, did not exhibit a decrease in density with increased temperature (**Figure 2a**,**b**).

Product Bulk Density versus Wheat Lines. A9, A38, and B44 flours produced similar density values when processed as pure wholemeal products (300–350 kg/m^3), and their behaviors were different from that of the flour from the triple-null alleles for SSIIa B63. B63 showed a significantly higher density (>400 kg/m^3, which corresponded to a denser breakfast cereal). These trends were mirrored for wheat/maize products in which A9, A38, and B44 had density values of 250–300 kg/m^3 and B63

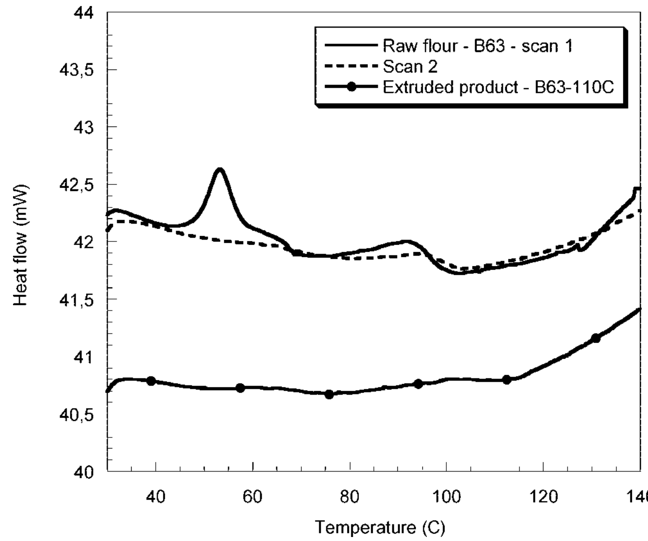

Figure 3. Typical DSC thermograms in excess water of native B63 (and rescan) and extruded B63 (110 °C).

showed the highest density value of >400 kg/m^3. The incorporation of maize tended to modify the trends compared to wholemeal products; its addition improved the expansion of A9, B44, A38, and B63, by helping to lower the density. This has been observed in earlier work in which maize grit extrudates were observed to show higher section expansion index (SEI) than wheat extrudates (*23*). The better ability of maize extrudates to expand could be explained by having a different degree of starch transformation under the same processing conditions and by the different characteristics of maize proteins and level compared to wheat proteins. Consequently, the viscoelastic properties of the wheat/maize blends are different and can affect the rate and extent of cereal product expansion.

Changes of Starch Structure during Processing. *Changes Observed by DSC.* No residual gelatinization enthalpy was observed by DSC, regardless of the processing temperature, suggesting that all products were completely gelatinized after processing. **Figure 3** presents a typical DSC thermogram of native B63 (triple null) compared to B63 in excess water and after processing at 110 °C. Although the melting temperatures of the flour were >110 °C (between 120 and 130 °C), at 23% moisture content, starch seemed completely transformed when processed at 110 °C. In principle, different starch transformation degrees were expected when the processing temperatures were varied, but all of the samples were gelatinized at least within the detection limits of DSC. This can be explained by the specific mechanical energy applied, which was higher at 110 °C than at 140 °C. The granular structure of starch should completely disappear when the SME is >400 J/g (*24*) or 500–600 J/ g (*25*). In the current study, SMEs were between 200 and 400 J/g, and therefore when processing was performed at a temperature lower than the melting temperature, complete starch transformation was enhanced via mechanical shear. However, there was still some crystallinity left as detected by XRD (see below), which was not measurable by DSC, although no Maltese cross typical of native starch granules within the samples was observed by polarized microscopy. Although B63 was subjected to a lower SME (**Table 3**) than the other flours, the product also showed no residual gelatinization enthalpy, probably because of its lower melting temperature, as previously observed (*18*). Additionally, no sign of starch retrogradation was observed in the DSC thermograms (**Figure 3**), indicating that the products were completely amorphous. This was expected

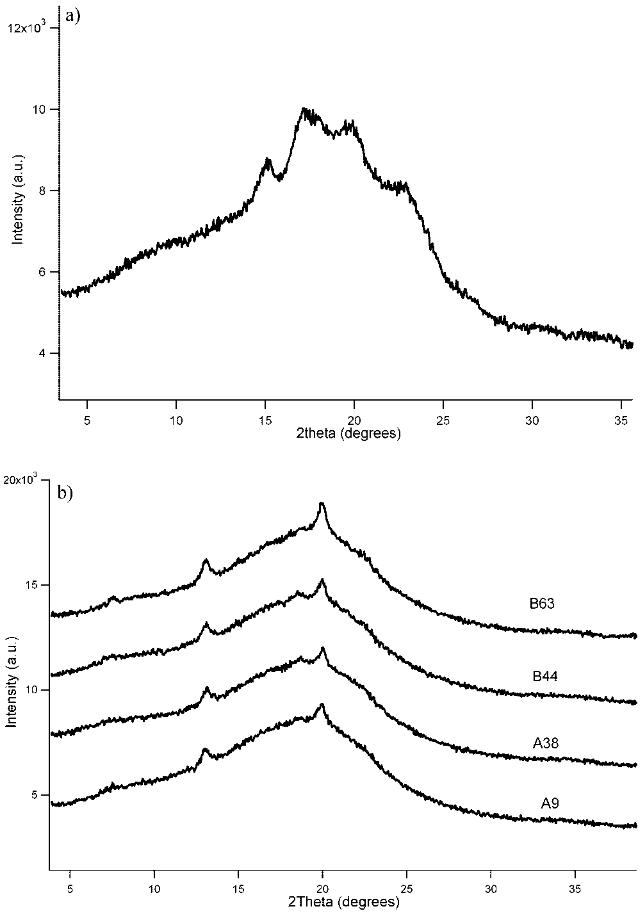

Figure 4. X-ray diffractograms of the wholemeal products: novel wheat varieties (B63, B44, and A38) and wild-type wheat.

because the samples were dried immediately following extrusion to a final moisture content of 3.0 ± 0.7%, conditions that do not generally allow for any molecular rearrangement.

Changes Observed by XRD. Changes in the structure of the wholemeal products at the molecular level were investigated using XRD. Cereal starches, such as wheat, are known to have A-type crystallinity (*26*), with typical reflections at $2\theta \sim 15.1°$, 17.2°, 18.1°, and 23.2°. However, as can be observed in **Figure 4a** for the unprocessed B63 flour, a C-type crystallinity (a mixture of A- and B-type crystals) is observed, as the reflection around $\sim 23°\ 2\theta$ hides the contribution of two other reflections at $\sim 22.4°$ and $24.1°\ 2\theta$, which are characteristic of B-type crystal morphologies. This structural transformation of starch with increasing amylose content has been described before for maize starches (*27*).

After extrusion, most of the original reflections are almost completely erased (see **Figure 4b**), but in contrast to the DSC results, some degree of crystallinity can be detected by XRD. This crystallinity, however, is mostly due to a V-type structure formed during processing, which has been previously attributed to the formation of amylose–lipid complexes (*28*). Although the crystallinity left after processing is around 3% for each wholemeal product, in the case of the B63 flour, the contribution of the reflections from the V-type crystal, that is, from the amylose–lipid complex, is higher than in the other three flours (2.7% for B63 versus 1.9% for A9, A38, and B44).

Expanded Cereal Extrudate Structure and Microstructure. *Expanded Structure of the Extrudates.* Expanded structures of the extrudates were observed by light microscopy and are shown in **Figure 5** for the different products. At the same magnification, B63-100% exhibited a different expanded structure compared to other wholemeal products. The structure appeared to be more compact with closely packed structures within the starchy matrix. The average cell size ranged from 0.2 to 0.4 mm, whereas the much more expanded product A9 (**Figure 5**) had cell sizes ranging from 1 to 2 mm. The smaller bubble size of B63 products was reflected in the higher density measured for the range of B63 products (**Figure 2**). For all of the higher expanded products (A9, A38, and B44), most of the cells contained particles, possibly plant cell walls or fibers, at the periphery (see arrow in **Figure 5** for A38-140-100%). These particles assumed to be nonsoluble fibers were resistant to the thermomechanical treatment applied by extrusion–cooking, similar results to those shown in the study of Guy (*29*). Unexpanded small cells looking like the nuclei of cell were also observed next to these particles, and these observations suggest that the nucleation of bubbles occurred at the interface between particles and the starch matrix. As previously described (*30*) in the case of corn flour extrudates, there is a weak adhesion between the starch matrix and the particles present in the flour that might favor the nucleation of bubbles at the interface. Similar nuclei were also observed in specimen samples of B63 wholemeal products; however, no large cells were observed. The B63 specimen had a higher number of particles (**Figure 5**), which might be related to a relatively higher amount of fibers and a corresponding decrease in starch content. If the particles are the origin of nucleation, the higher number of particles might also explain the higher number of nuclei in B63 products. However, although B63 samples contained a higher number of cells, the cells were smaller, mostly broken, and not uniformly surrounded by the starch matrix. **Figure 5** also shows that in the more highly expanded wheat/maize products (B63-140-60%) with bigger cells, particles could be contained within a cell wall and were surrounded by starch matrix. No empty space that could be attributed to nuclei between the particle and the matrix was visible.

Microstructure of the Extrudates. To evaluate the distribution of other major components within the wheat flour, such as proteins within the cell walls, fluorescence microscopy was also used (**Figure 6**) to characterize the extruded products. The micrographs show that for all of the systems protein particles were dispersed in the continuous starch phase, as observed for laboratory-scale processed products (*18*). The particle sizes ranged between 50 and 100 μm for the extrudate products. There were no clear structural differences between the products processed at 110 °C and those processed at 140 °C. In all cases the high temperatures applied during extrusion–cooking tend to lead to protein aggregates, whereas the high mechanical shear tends to disrupt or disperse them. In the extrudates, protein particles appeared to be smaller than the cell wall thickness and also much smaller than the fibers shown in **Figure 6** for A38-140-100%. Protein dispersed in products made from the different flours, A9-110-60% and B63-140-60%, appeared to show a similar pattern and were of similar particle size (50–100 μm), and none of the products showed a continuous phase of proteins.

Thus, the difference in behavior of the different flours may relate more to the protein content of the flours than to the protein particle size and distribution as the latter two look quite similar from one product to another. The number of large particles such as fibers may also play an important role.

Mechanical Properties. Figure 7 shows typical force versus displacement plots for the extrudates made from wholemeal wheat flour and processed at 140 °C. For all of the samples tested the mechanical testing showed mostly a brittle fracture

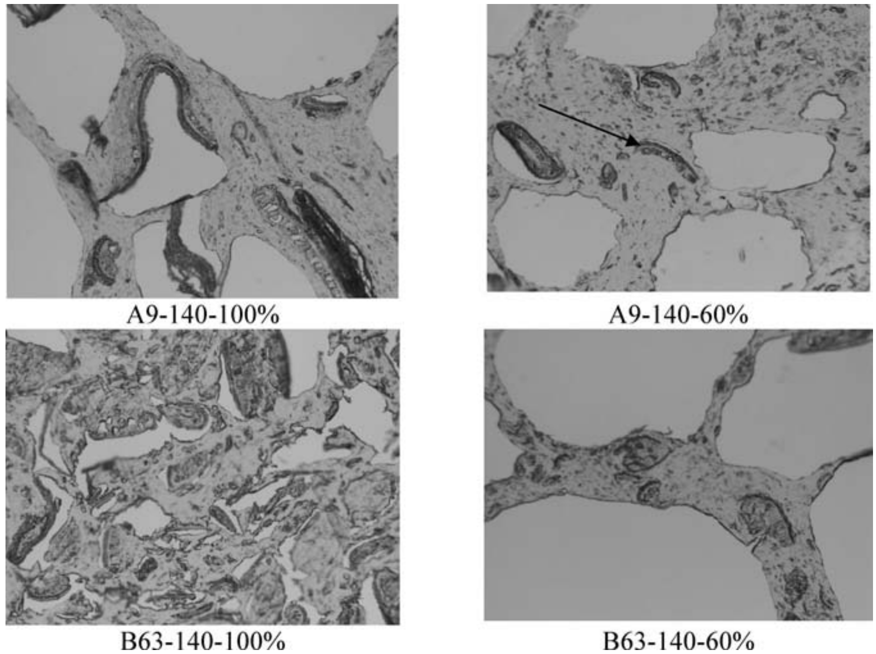

Figure 5. Light microscopy observations of extruded cereals products (section of 2.80 × 2.15 mm^2).

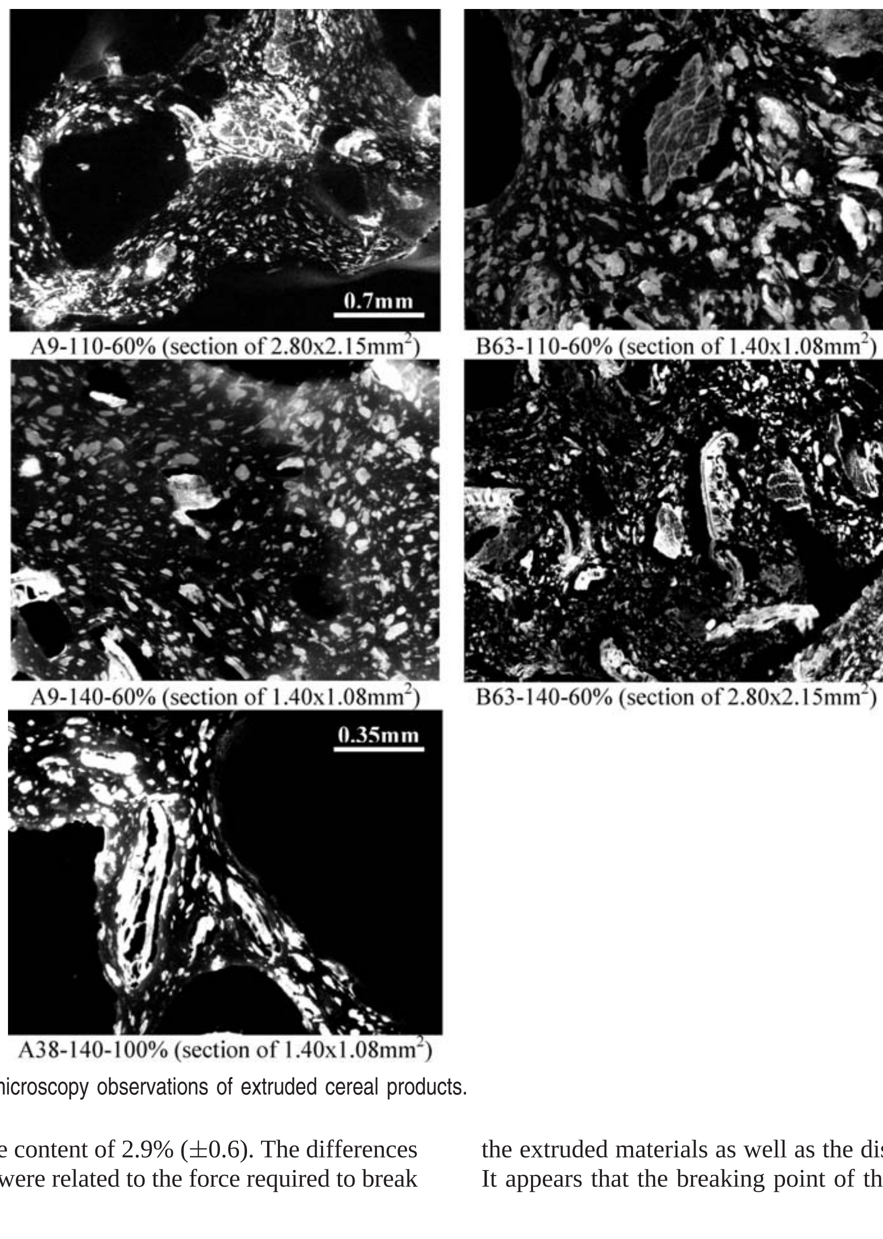

Figure 6. Fluoresence microscopy observations of extruded cereal products.

behavior, at a moisture content of 2.9% (±0.6). The differences between the products were related to the force required to break the extruded materials as well as the displacement at breaking. It appears that the breaking point of the wild type and single-

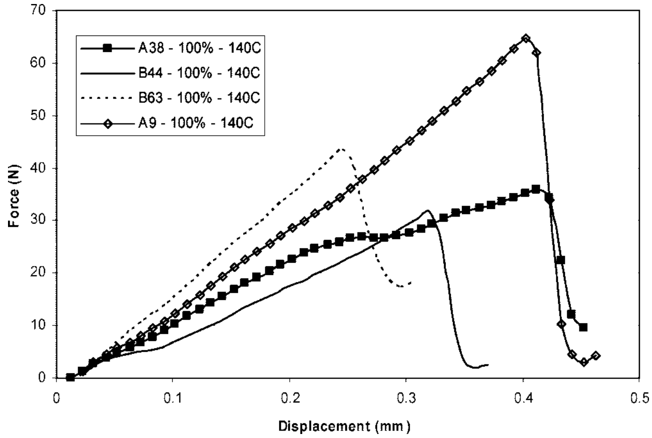

Figure 7. Force versus displacement plots for the extrudates made from wholemeal wheat flour and processed at 100 °C.

null line (A9 and A38) took place at a larger displacement than for the double- and triple-null lines (B44 and B63).

The mechanical properties of the pure wholemeal products were thus compared by the breaking force and the breaking distance plotted against the density of the products (**Figure 8**). Although B63-100% products had a higher density, the average breaking force was of the same order of magnitude as those for A9-100% and B44-100% (between 55 and 70 N). The breaking distance of B63-100% was lower than that of the other products. In general, higher density products would be expected to have a higher modulus and breaking stress, according to the Gibson–Ashby relationship (*31*). The lower breaking distance of B63 extrudates can be explained by the B63 materials being more brittle due to possibly having more protein and less starch, which may weaken the mechanical properties of the extrudate matrix and/or the cell wall materials. The increased brittleness and weakness of corn extrudates has been related to the change of microstructure with increased protein content (*20*). The mechanical properties of the wheat/maize breakfast cereals exhibited different trends compared to those of pure wholemeal products for the same moisture content of 2.9% (±0.6) (**Figure 9**). The breaking force tended to increase with density (**Figure 9a**); at low density (200–250 kg/m^3), the forces at breaking were between 45 and 55 N, whereas at high densities (300–450 kg/m^3), the forces at breaking were between 55 and 65 N. Thus, it is likely that the breaking force required to cause failure in the product is due to the density of the matrix phase and the microstructure where cell size distribution and orientation are also known to influence the mechanical properties (*32*).

The distance at breaking was smaller for B63-60% compared to other products; that is, they were more brittle. This might be due to the high amount of particles (brans, insoluble fibres) in B63 products, which favors the initiation of product fracture. As observed by light microscopy, the cohesion between the starch matrix and the particles appeared to be weak (Figure **5**).

Starch Digestibility in the Products. Resistant starch content and glycemic index of the novel wheat breakfast cereal products are presented in **Table 4**. For the same product composition, there are no significant differences or trends in the starch digestibility with extrusion temperatures. This can be explained by the DSC results, which showed no residual gelatinization enthalpy for any of the products and no endotherm related to the melting of retrograded starch. It might be assumed, therefore, that the starch structure was not significantly different with extrusion temperature and that the extrudates may exhibit similar behavior toward in vitro enzyme digestion.

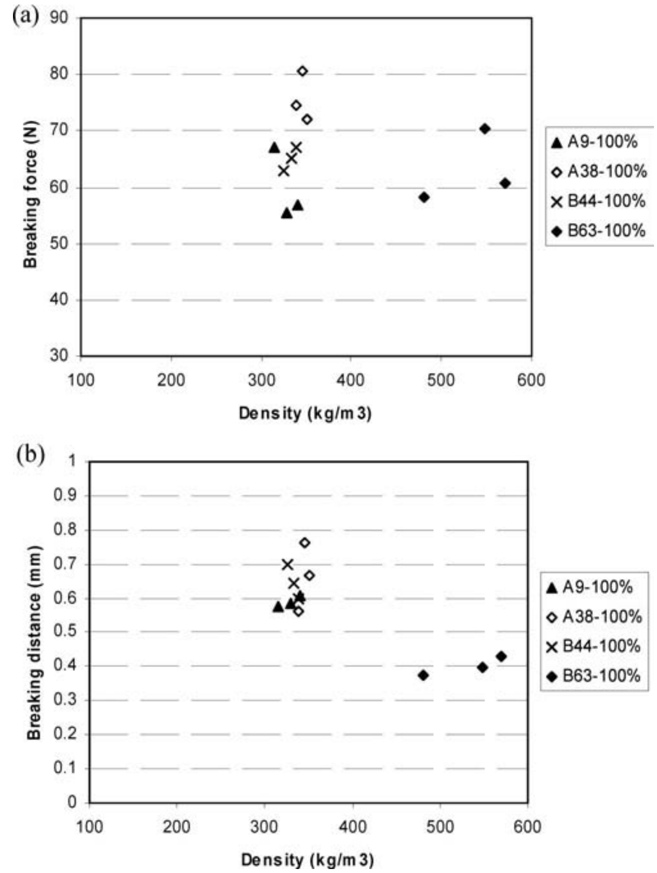

Figure 8. Breaking force (**a**) and breaking distance (**b**) versus density for 100% wholemeal products (standard deviations of 5 and 10% for breaking force and distance, respectively).

Differences in starch digestibility made with the wild wheat line (A9) were observed between the pure wholemeal products and the breakfast cereals. Wholemeal products had higher RS content (1.5 g/100 g of pure wholemeal products compared to 0.8 g/100 g of breakfast cereals for A9) and lower HI values compared with the mixed-starch breakfast cereals (respectively, 80 compared to 90 for A9). The presence of maize can provide a less resistant structure to enzyme digestion than that offered by the novel wheat. The presence of maize within the blend can also change the product microstructure and enhance the accessibility of the enzymes to starch.

Comparing the RS content and HI values between wheat variety lines showed that the triple-suppression gene flours exhibited a much different response compared to that of the other flours (i.e., wild type and single and double mutations). B63 wheat line products showed significantly higher RS contents and lower HI values than the other lines (**Table 4**). RS content was about 4% and HI value 75 for the wholemeal product.

DISCUSSION

All extruded products displayed different densities and different degrees of expanded structures, as observed by microscopy (**Figure 5**). To understand expansion behavior, the melt viscosity was determined and the apparent shear viscosity was determined from the extrusion data. **Figure 10** is a plot of bulk density versus apparent shear viscosity and shows for wholemeal products made from wild type and single- and double-null alleles for SSIIa (A9, A38, and B44) that the density increased with the apparent shear viscosity (**Figure 10a**) and therefore suggests that density is influenced by shear viscosity.

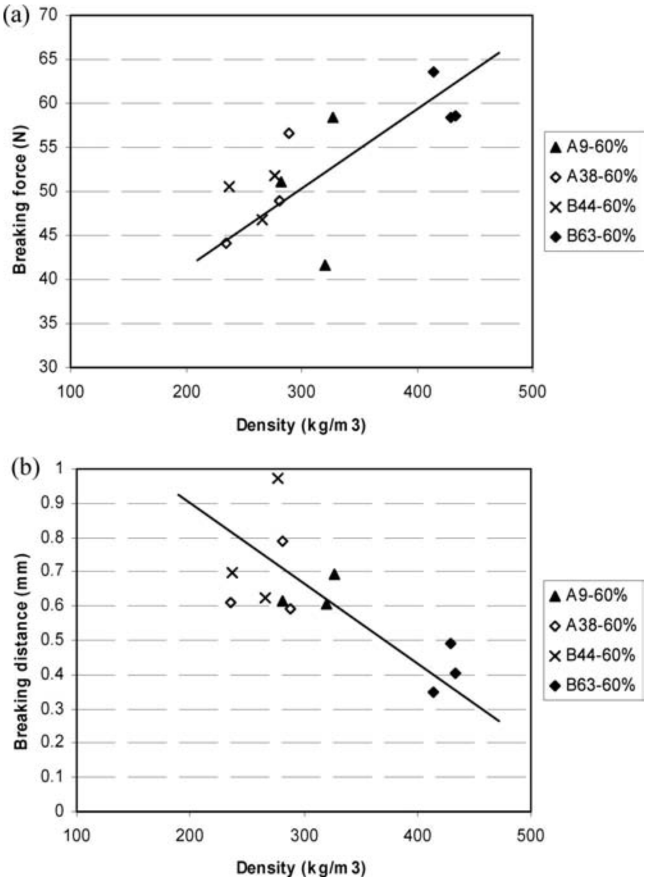

Figure 9. Breaking force (**a**) and breaking distance (**b**) versus density for 60:40 wholemeal/maize blends (standard deviations of 5 and 10% for breaking force and distance, respectively).

Table 4. Resistant Starch (RS) Content and Hydrolysis Index (HI) Values Measured in Vitro for Two Extruded Breakfast Cereals with Flours from the Wheat Lines with Different Null Alleles for SSIIa

sample composition	melt temperature (°C)	product 1: wholemeal breakfast cereals RS (g/100 g)	product 1: HI	product 2: breakfast cereals [wheat/maize blend (60:40)] RS (g/100 g)	product 2: HI
wheat flour A9	110	1.3	82	0.8	91
	120	1.2	79	0.7	87
	140	1.5	79	0.8	90
wheat flour A38	110	1.0	80	0.2	93
	120	0.8	86	0.4	90
	140	1.1	82	0.4	86
wheat flour B44	110	0.7	81	0.3	91
	120	0.9	84	0.5	90
	140	1.0	79	0.5	93
wheat flour B63	110	4.3	72	3.0	86
	120	3.1	76	3.0	86
	140	4.0	79	2.9	89

However, B63 did not behave in the same way because although its shear viscosity was similar to or lower than that of the other flour lines, the density of the B63 products was significantly higher. This suggested that shear viscosity is not necessarily the only determinant of density. This was also confirmed by the behavior of the breakfast cereals (wheat/maize blends), for which the density could not be completely explained by the shear viscosity trend (**Figure 10b**). During the extrusion process,

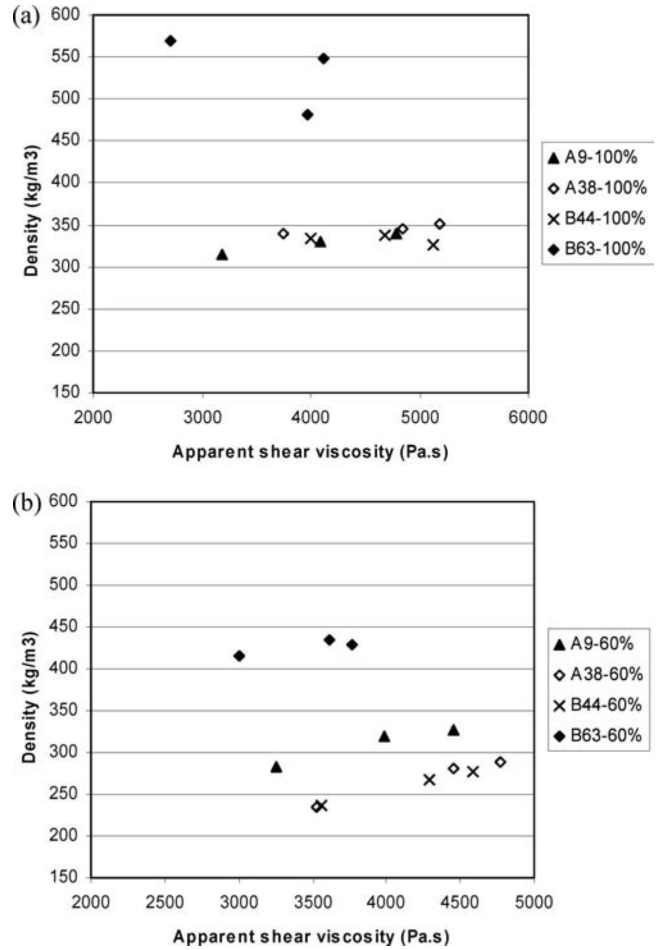

Figure 10. Density versus apparent shear viscosity (Pa·s): (**a**) 100% wholemeal products; (**b**) 60:40 wholemeal/maize blends.

the expansion of the products is influenced by the melt viscosity and by its multiphase nature, as observed in extruded composite materials (*33*). Dispersed particles can cause early breakage of the cell walls during product expansion, which involves a release of water vapor and a decreased driving force for expansion. Shrinkage can also be the cause of increased density in a product and is related to the glass transition temperature (T_g) of the material, which determines the temperature of fixation of the structure (*34*), that is, vitrification. Modulus or breaking stress of solid foams is known to depend not only on the density and cell wall properties (*31*) but also on the cell size and wall size distributions. It is likely for the novel wheat products that the breaking stress is, due to the combined effects of density, related to their expansion behavior and the cell wall properties affected by the amylose content, as indicated in the study of Lourdin et al. (*35*). They reported a tensile stress and elongation increase at breaking with increased amylose content for cast starch films. The increased brittleness of B63 shown by a lower deformation at breaking may be explained by its microstructure: a greater extension of the protein phase may increase the number of stress concentration or breaking points within the product, by causing weaker adhesion between starch and gluten, which has been similarly observed for corn extrudates (*30*).

Higher RS contents and lower HI values were obtained for products made from the flour (B63) containing the triple-null alleles for SSIIa. This variety has higher amylose content; up to 44% of the starch content (*19*) and flour from SSIIa–triple-null lines has previously been shown to increase the RS content of breads (*36*). The highest RS value obtained with this flour was 4.3%. For normal wheat, Murray et al. (*37*) observed that at high temperature (143 °C) a RS content of 0.6 was obtained. Moreover, RS contents of high-amylose rice flour extrudates were shown to be between 0.7 and 3.1, depending on the extrusion conditions (*38*). The triple gene mutated flour, thus, provides the best option for increasing RS content compared with wild type and single- and double-null alleles for SSIIa.

For wheat with different null alleles for SSIIa, no relationship was observed between the starch digestibility and the processing temperatures. In these samples, DSC thermograms showed no residual enthalpy, suggesting that starch was completely transformed, and no melting endotherm of retrograded starch was observed as the samples were dried immediately after extrusion. However, XRD determined the presence of V-type crystals after extrusion processing. This type of crystallinity, as previously stated, arises from amylose–lipid complexes that were not detected by DSC. The amount of V-type crystals was higher for B63 than for the other three wholemeal products. Because B63 has a higher amylose/amylopectin ratio (**Table 1**), the increased amount of amylose–lipid crystals could have contributed to the higher RS content obtained in the wholemeal breakfast cereals with the triple-null alleles for SSIIa. It is thought that these amylose–lipid complexes are able to resist digestion, which would imply that the B63 flour could provide higher amounts of fermentable fats for the colonic flora, with its associated benefits from a nutritional viewpoint. The higher RS content obtained for the breakfast cereals made from triple-null alleles for SSIIa lines is consistent with the higher RS observed in bread (*39, 40*).

Moreover, B63 presented a much more compact structure compared to the well-expanded wild-type products (A9). Differences in accessibility of the digestive enzyme toward starch due to its compact structure may also be an explanation for the higher RS content in the wholemeal breakfast cereals with the triple-null alleles for SSIIa. This means that the hierarchical structures of starch-based products at different length scales (molecular structure, granule architecture and product microstructure) could be combined to control the starch sensitivity to enzyme digestion.

In conclusion, the behavior of novel wheat varieties with different null alleles for SSIIa subjected to a thermomechanical treatment such as extrusion was studied. Different behaviors were observed for the wild-type flour and for the triple gene mutated flour with the triple-suppression flour having the lowest apparent shear viscosity values. Bulk densities also varied considerably: the triple-null line product was not as well expanded as the wild-type product. Under all conditions studied, extrusion leaves no residual ungelatinized starch in the novel wheat products and leads to different expanded structures. The higher RS content in the triple-null extruded products can be explained by the presence of V-type structure and also by its microstructure, which may limit the accessibility by digestive enzyme. These results are encouraging and lend strength to the approach to build interesting microstructure through advanced design in raw wheat grain ingredients and process control to target enhanced starch digestibility characteristics.

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