

## Effect of Process Parameters on Slowly Digestible and Resistant Starch Content in Extrudates

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### Abstract

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A laboratory single-screw Kompaktextruder KE 19/25 was used at speeds 120–140 rpm of the screw with either a 2 : 1 or 3 : 1 compression ratio; the head had a 3 or 4 mm die. The temperature of the head was set at 131–144°C. The applied raw materials were maize grits, and mixtures with wheat starch, pea flour or chemically modified starch. Input mixtures differed in water addition (50 or 100 g water/kg). The highest amount of resistant starch (3.1% per total starch) in extrudate was found for mixture of pea flour and maize grits (with addition of 100 g water/kg of dry mixture), the output(extrudate)/input(mixture) ratio of the resistant starch was 45.8%. The highest SDS content was obtained for the maize grits and addition of 100 g water/kg using a die with a 3 mm diameter and a screw with a 3 : 1 compression ratio; the temperature of the head was approximately 140°C. Transportation rates: screw 140 rpm, dosing 15 rpm.

**Keywords:** extrusion cooking; legumes; starch digestibility; distarch phosphate

Starch is the main component of digestible carbohydrates consumed in the human diet and can generally be classified as rapidly digestible (RDS), slowly digestible (SDS) and resistant starch (RS) according to its susceptibility to pancreatic amylase (*in vitro*). RDS is degraded to glucose within 20 min after enzymatic activity. SDS is changed into glucose while being digested in the small intestine for up to 120 min (ENGLYST *et al.* 2007). RS passes into the colon, where it is metabolised into secondary products by the colonic microflora (WRONKOWSKA & SORAL-ŚMIETANA 2012) and behaves in a way similar to dietary fibre (KUČEROVÁ *et al.* 2013). Interior starch crystallites with shorter double helices in cereal starches with the typical A-type X-ray pattern are more susceptible to pancreatic  $\alpha$ -amylase than tuber starches, banana starch and high-amylose cereal starches (PLANCHOT *et al.* 1997).

Starch granules can irreversibly absorb water and swell when their aqueous dispersion is heated. Dur-

ing this process (gelatinisation), the starch granule undergoes three main changes: granule swelling, crystal or double helix decomposition, and amylose leaching (SINGH *et al.* 2003). This increases randomness and decreases crystallinity in the granule. The molecules in the starch granule vibrate vigorously, breaking intermolecular bonds and thus allowing increased interaction with water molecules. The flow behaviour of the slurry changes significantly as the suspension becomes a mixture of swollen granules, partially disintegrated granules, and molecularly dispersed granules (mostly amylose).

After cooling, starch molecules gradually aggregate into a gel. The association among the chains of amylose-amylose, amylose-amylopectin, and amylopectin-amylopectin combines with the embedded water. High-amylose starches require more energy to break bonds in order to gelatinise into starch molecules, leading to a rigid and stiff gel. The term retrogradation

denotes changes during the aging of starch solutions, pastes, or gels. The main feature is crystallisation due to the formation of hydrogen bonds between hydroxyl groups of neighbouring starch molecules; amylose forms a double helical crystalline structure.

In some biscuits having very low moisture levels during the treatment, the extent of gelatinisation is reduced, and partially intact granules and gelatinised starch co-exist. The preservation of partially intact granules results in higher SDS content compared to other baked products. This can be reached using prior extrusion of wheat bran with low water content as well (REYES-PEREZ *et al.* 2013).

SDS formed in this way results in the slow increase of both postprandial blood glucose levels and sustained blood glucose levels over time, compared to the RDS blood glucose curve with its fast growth, high peak and rapid decline. In addition, metabolic responses correspond to postprandial glycaemia. SDS therefore has implications for physical performance, obesity and diabetes management.

Resistant starch is classified into several categories. Type RS1 is physically inaccessible starch – such as that in legumes – embedded in cell wall material or protein matrix. RS2 is native granular starch, such as in raw potato, banana, and high-amylose maize starch. RS3 is retrograded amylose; common methods of cooking cannot dissociate the amylose crystallites. Thus, the crystalline amylose remains highly resistant to enzyme hydrolysis. RS4 is chemically modified starch. RS5 is a lipid complex of starch (JANE *et al.* 2010).

One interesting characteristic of RS is its fermentation into short chain fatty acids (SCFAs) produced by the colonic microflora, considered to be the main nutrient of the colonocyte. Created butyrate inhibits proliferation and induces apoptosis of colorectal cancer cells (CRC) (FUNG *et al.* 2012). There is an inverse correlation between resistant starch intake and the risk of CRC (TOPPING & CLINGTON 2001). It is probable that gases are absorbed from the colon at a sufficient rate to avoid gas accumulation in the lumen, which is responsible for the symptoms of flatulence. In contrast to traditional fibre sources such as cellulose, RS decreases postprandial glucose and insulin responses and decreases the glycaemic index of food; thus, the metabolism of SCFAs may contribute to improvements in glycaemic control and lipid metabolism in diabetic subjects (MUIR *et al.* 1994).

Extrusion cooking may be defined as a continuous process in which raw materials are forced through an orifice and transformed into cooked and formed products. Due to thermal treatment, high pressure and

shear forces, starch granules disrupt and merge into dispersion (gelatinisation). The process is connected with molecular fragmentation of starch polymers. There is a loss of organisation of starch granules starting in the amorphous regions. As shearing proceeds, breakup between the crystalline lamellae occurs; the amorphous lamellae act as sites of weakness for fracture. This type of fracture also implies that the amylopectin molecules themselves are broken, facilitating crystallite breakdown. That extrusion leads to molecular weight degradation (higher digestibility), with greater degradation occurring at high temperatures, high screw speeds and lower water contents (DONALD 2004). On the other hand, fragmentation of starch polymers could ameliorate starch retrogradation (BRENT 1999).

MAHASUKHONTHACHAT *et al.* (2010), WOLF (2010) and other authors reported a decrease in RS content (or increased digestibility) after extrusion processing. Further changes during extrusion are protein denaturation and the formation of complexes between the lipids and starch and/or proteins.

The formation of new amyloso-lipid complexes during extrusion (BHATNAGAR & HANNA 1994) or formation of double-helices of amylose by retrogradation when extrudates are stored is connected with higher resistant starch content (RS) in extrudates and therefore RS can be influenced by higher amylose content such as in pea starch. Similarly, ROHLFING *et al.* (2010) found that the high-amylose tortillas had high amounts of resistant starch or CHIU *et al.* (1994) prepared a patented technology using high-amylose starch with pullulanase to produce resistant starch.

The integrity, and thus digestibility, of starch granules depends on such conditions as water content, temperature, cooking time, pressure, and pH (SINGH *et al.* 2003). E.g. low initial water content in raw material for snack food predetermines that this process will result in products having a high content of slowly digestible starch. The extent of cooking reactions also depends on the construction of the apparatus employed (FRAME 1995; BRENT 1999).

Besides the nutritional values of the extrudates, important parameters are sensorial and mechanical ones, e.g. colour, flavour, shear stress at break, water solubility index (ZENG *et al.* 2011), rehydration and expansion ratio.

The aim of the paper was to seek suitable conditions for the extrusion process to save resistant starch in the maize grits and/or to obtain high levels of slowly digestible starch, and to observe changes when pea flour, wheat starch or chemically modified starch was added.

## MATERIAL AND METHODS

**Material.** Fine maize grits were provided by the KONKORDIA Ltd. maize mill, Mrzkovice, Czech Republic. Pea flour (starch content 30.4% dry matter – DM) was milled from pea seeds (*Pisum sativum* L. var. *medullare*, cv. Radovan; supplied by SEMO Ltd., Smržice, Czech Republic). High-grade wheat A-starch (Amylon Ltd., Havlíčkův Brod, Czech Republic) and Moramyl ZBH distarch phosphate (P 0.98 g/kg DM; Krnov Starch Company, Ltd., Krnov, Czech Republic) were applied.

Pancreatic  $\alpha$ -amylase (3 Ceralpha U/mg), amyloglucosidase (3260 U/ml on soluble starch), invertase (300 U/mg), and a glucose oxidase/oxidase colorimetric assay kit (GOPOD) were purchased from Megazyme International Ireland Ltd. (Bray, Ireland)

**Extrusion process and storage of extrudates.** At first the maize grits were mixed with pea flour, native wheat or modified starch (0, 100, 200 or 500 g/kg mixtures) in a dry state. Subsequently, 50 or 100 g water/kg dry mixtures were added over a 5-min period. The resistant starch content of input samples ranged from 3.18–12.93% DM. The highest value was found for the input mixture of maize grits and distarch phosphate.

Extrusion of the wetted premix (40 experiments) was performed using a laboratory single-screw Kompaktextruder KE 19/25 (Brabender, Duisburg, Germany). The extruder consisted of a screw (2 : 1 or 3 : 1 compression ratio) of 19 mm in diameter and 475 mm in length, with areas of controlled heating in the barrel and in the head with a die (diameter 3 or 4 mm). The extruder was used at various speeds of both the main and dosing screws. The prepared extrudates were stored at laboratory temperature for 90 days. We came from our preliminary trials with extrudates from Croatia maize hybrids (unpublished results) in which we did not reveal any changes in RS content between 5 and 90 days storage.

**Analytical methods.** Total starch content was quantified enzymatically by a Megazyme total starch assay

kit (AACC 76-13). The SDS fractions were measured according to the enzymatic procedure of ENGLYST *et al.* (1992) as modified by OVANDO-MARTÍNEZ *et al.* (2011). Determination of the resistant starch was carried out using the Megazyme Resistant starch assay kit commercial procedure (2005).

Rheological properties of the modified starch were measured by a Viscograph<sup>®</sup> E (Brabender, Duisburg, Germany).

Diameter measurement of the product was done using a digital calliper at 10 points of the extrudate and averaged. The expansion ratio of the puffed product was calculated according to FRAME (1995): ER = diameter of product/diameter of die opening.

## RESULTS AND DISCUSSION

This section provides only part of the results obtained in experiments (12 out of 40 performed), hence just those which show the selected influences on product parameters.

Table 1 illustrates the influence of revolutions of the main and dosing screws on the expansion ratio, SDS and RS content. The better value of expansion ratio was reached for the revolution of the dosing screw at 15 rpm and for that of the main screw at 120 rpm. RS content was the same, but increased SDS content was observed at higher revolutions.

The water content played an important role for the expansion ratio. Compare Line 2 (in Table 1) with Line 1 (in Table 2): the lower the water content, the higher the expansion ratio. On the other hand, higher water content caused increased RS content. The reason can be found rather in higher amylopectin defragmentation for lower water content (DONALD 2004) than in retrogradation or in new formed amylose-lipid complexes during extrusion.

Tables 2–4 describe the results where various starchy materials were added to the maize grits.

Table 2 shows that 200 g wheat starch/kg mixture slightly increased the expansion ratio (ER), but on the

Table 1. Influence of revolutions of the main screw and dosing screw on technological and nutritional parameters (sample of maize grits with added 50 g water/kg dry mixture – water content of the premix 13.7%, die diameter 4 mm, screw compression ratio 2 : 1)

Experiment No.	$t_1$	$t_2$	$t_3$	$t_4$	Screw (rpm)	Dosing (rpm)	Pressure (bar)	Expansion ratio (1)	SDS content (% of total starch)	RS content
1	43	90	124	141	140	20	166	2.09	49.2	0.19
2	43	80	122	141	120	15	164	2.69	63.0	0.19

SDS – slowly digestible starch; RS – resistant starch;  $t_1$ – $t_4$  – the temperatures of extruder zones

Table 2. Influence of native wheat starch addition on technological and nutritional parameters (die diameter 4 mm, screw compression ratio 2:1, screw 120 rpm, with addition of 100 g water/kg dry mixture)

Sample No.	input composition	$t_1$	$t_2$	$t_3$	$t_4$	Pressure (bar)	Dosing (rpm)	Expansion ratio (1)	SDS content (% of total starch)	RS content
		(°C)								
3	K + 10	53	87	117	138	42	15	1.62	54.2	0.35
4	80K + 20A + 10	45	90	110	132	53	20	1.81	63.4	0.29
5	50K + 50A + 10	46	92	109	134	48	20	1.47	60.2	0.31

Samples (labeling): K + 10 – maize grits + 10% water addition (1000 g + 100 g); 80K + 20A + 10 – mixture of 80% maize grits and 20% wheat A-starch + 10% water addition (800 g + 200 g + 100 g); 50K + 50A + 10 – mixture of 50% maize grits and 50% wheat A-starch + 10% water addition (500 g + 500 g + 100 g); SDS – slowly digestible starch; RS – resistant starch;  $t_1$ – $t_4$  – the temperatures of extruder zones

other hand, 500 g caused the fall of ER. RS content was nearly the same.

100 g distarch phosphate/kg the mixture, with 50 g added water/kg dry mixture caused the pressure fall and a slight increase of ER (Table 3). The other advantage was an increase of RS content in the extrudate, although this was not verified for higher water addition (Table 3). This confirmed assumptions (ŠÁRKA *et al.* 2013) that it is more effective to apply cross-linked starch with a higher degree of substitution to increase the RS amount. Otherwise, the gelatinisation destroys the starch granules (Figure 1) and increases starch digestibility. Additionally, a slight SDS content increase was observed for 100 g distarch phosphate/kg mixture.

The trials with the addition of pea flour (Table 4) were done using a die with a 3 mm diameter, which enabled the increase of ER and SDS content. The

addition of high-amylose pea flour resulted in the worsening (decrease) of ER. The value of 1.71 resulted from high protein content and is comparable with the data of Yu *et al.* (2013). However, the conclusions of Yu *et al.* (2013) concerning the optimal higher

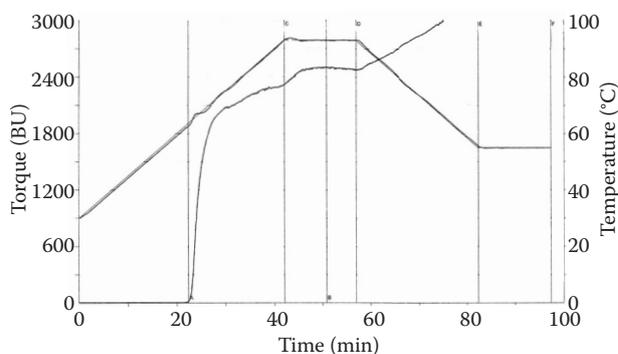


Figure 1. Result from the Brabender Viscograph E of Moramyl ZBH modified starch

Table 3. Influence of cross-linked starch addition on technological and nutritional parameters (die diameter 4 mm, screw compression ratio 2:1, screw 120 rpm, with addition of 50 and 100 g water/kg dry mixture)

Sample No.	input composition	$t_1$	$t_2$	$t_3$	$t_4$	Pressure (bar)	Dosing (rpm)	Expansion ratio (1)	SDS content (% of total starch)	RS content
		(°C)								
<b>Addition 50 g water/kg dry mixture</b>										
2	K + 5	43	80	122	141	164	15	2.69	63.0	0.19
6	90K + 10MZBH + 5	43	89	121	140	138	15	2.98	66.8	0.36
7	80K + 20MZBH + 5	43	90	120	140	130	15	3.00	62.8	1.13
<b>Addition 100 g water/kg dry mixture</b>										
3	K + 10	53	87	117	138	42	15	1.62	54.2	0.35
8	80K + 20MZBH + 10	45	88	109	131	66	20	1.98	60.5	0.10
9	50K + 50MZBH + 10	45	90	110	131	57	20	1.94	59.8	0.16

Samples (labeling): K + 5 – maize grits + 5% water addition (1000 g + 50 g); 90K + 10MZBH + 5 – mixture of 90% maize grits and 10% starch Moramyl ZBH + 5% water addition (800 g + 100 g + 50 g); 80K + 20MZBH + 5 – mixture of 80% maize grits and 20% starch Moramyl ZBH + 5% water addition (800 g + 200 g + 50 g); K + 10 – maize grits + 10% water addition (1000 g + 100 g); 80K + 20MZBH + 10 – mixture of 80% maize grits and 20% starch Moramyl ZBH + 10% water addition (800 g + 200 g + 100 g); 50K + 50MZBH + 10 – mixture of 50% maize grits and 50% starch Moramyl ZBH + 10% water addition (800 g + 200 g + 100 g); SDS – slowly digestible starch; RS – resistant starch;  $t_1$ – $t_4$  – the temperatures of extruder zones

Table 4. Influence of pea flour addition on technological and nutritional parameters (die diameter 3 mm, screw compression ratio 3 : 1, screw 140 rpm, dosing 15 rpm)

Sample No.	input composition	$t_1$	$t_2$	$t_3$	$t_4$	Pressure (bar)	Expansion ratio (1)	SDS content (% of total starch)	RS content
		(°C)							
10	K + 10	43	93	125	144	100	3.66	71.6	0.33
11	80K + 20pea + 10	44	90	121	141	80	1.71	62.7	3.13
12	80K + 20pea + 5	44	90	121	140	150	2.92	59.4	1.01

Samples (labeling): K + 10 – maize grits + 10% water addition (1000 g + 100 g); 80K + 20 pea + 10 – mixture of 80% maize grits and 20% pea flour + 10% water addition (800 g + 200 g + 100 g); 80K + 20 pea + 5 – mixture of 80% maize grits and 20% pea flour + 5% water addition (800 g + 200 g + 100 g); SDS – slowly digestible starch; RS – resistant starch;  $t_1$ – $t_4$  – the temperatures of extruder zones

humidity were not confirmed – on the contrary, it was still advantageous to work with a low water addition to achieve a higher ER. RS content in the extrudates increased in agreement with TOVAR and MELITO (1996) due to high amylose content and its follow-up retrogradation. The amount of SDS was reduced. The other explanation of the high SDS and RS content, originating from the result of competition between protein and starch for water (GUARDEÑO *et al.* 2013) with a large amount of ungelatinised starch, seems improbable.

## CONCLUSIONS

The best sensorial and nutritional results (acceptable RS and high SDS content, and high ER) of extrudates were found using a die with a 3 mm diameter and a screw with a 3 : 1 compression ratio. The temperature of the head was approximately 140°C. Transportation rates: screw 140 rpm, dosing 15 rpm.

The highest ERs were found for mixtures with addition of 50 g water/kg dry mixtures. Besides, the ratio of 100–200 g distarch phosphate/kg the premix increased the values of ER. On the other hand, the addition of pea flour or of native wheat starch decreased ER, probably as a result of competition of proteins and starch for water during extrusion. Our preliminary results (ŠÁRKA *et al.* 2014) showed that ER is negatively correlated with the stress at break of extrudates, which is very important for our sensorial evaluation.

High RS content was found for the addition of 100 and 200 g water/kg dry mixture due to high amylose content and its follow-up retrogradation. We can expect similar or even better potential results for high amylose wheat starch. Higher results of RS would be reached using cross-linked starch with a higher degree of substitution as well.

During the extrusion process the level of RDS slowly increased and that of RS slowly decreased in all samples. The highest SDS content was obtained for the mixture of maize grits and water (100 g water/kg dry mixture) by the technological parameters described above. Additionally, a slight SDS content increase was observed for the ratio of 100–200 g distarch phosphate/kg premix.

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