

Influence of Genotype and Processing on the *in Vitro* Rate of Starch Hydrolysis and Resistant Starch Formation in Peas (*Pisum sativum* L.)

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The formation of resistant starch (RS) and the rate of starch hydrolysis were evaluated *in vitro* in a wild type of green-seeded pea genotype RRRbRb BC3 (33-Am) with 32.7% amylose content and in two mutants RRrbrb BC3 (23-Am) and rrRbRb BC3 (65-Am) with amylose contents of 23.3 and 65.1%, respectively. Pea samples were intact or homogenized and subjected either to autoclaving or to boiling at atmospheric pressure. The amount of RS (total starch basis) varied from 6.2 to 12.9% in the 23-Am products and from 31.2 to 33.4% in the 65-Am products. The RS level of the 33-Am product with a regular amylose content was 11.0%. Both the 23-Am and the 65-Am products were abundant sources of dietary fiber (39 and 34%, dry matter basis, respectively) versus 23% in the regular pea product. The amylose/amylopectin ratio was an important determinant of the rate of starch hydrolysis. The hydrolysis indices (HI) and predicted glycemic indices were lowest in the 65-Am peas (HI range = 42–59) as compared to the 23-Am peas (HI range = 53–84). It is concluded that the pea genotypes covered a wide range in starch availability, which is likely to affect nutritional parameters such as glycemic responses and colonic delivery of starch.

Keywords: Peas; *Pisum sativum*; amylose; starch digestibility; resistant starch; dietary fiber; hydrolysis index; glycemic index

INTRODUCTION

Besides being a major plant metabolite, starch is also the dominating dietary carbohydrate (CHO) in the human diet. The possibility of optimizing the nutritional characteristics of starch is therefore a subject of great importance. One such characteristic of significance from a health perspective concerns the digestibility of starch, which appears to be affected by its chemical composition, that is, the amylose/amylopectin ratio. Because the starch composition may be modified by plant breeding, a more conscious choice of genotype gives an opportunity to create foods with improved nutritional properties, so-called designer foods.

In the early 1980s it was revealed that not all the ingested starch is digested and absorbed in the human small intestine (Anderson et al., 1981; Stephen et al., 1983). The term resistant starch (RS) was introduced (Englyst et al., 1982) and taken into consideration from both analytical and nutritional points of view. The presence of RS has been associated with the physical entrapment of starch within whole or partly milled grains or seeds (RS₁), ungelatinized granules of B-type starches (RS₂), and starch retrogradation upon food processing (RS₃) (Englyst et al., 1990).

There are several indications that the metabolites, formed during fermentation of RS and other indigestible CHOs in the large intestine [i.e., short-chain fatty acids (SCFAs)], contribute to the maintenance of colon health and also have beneficial effects on glucose metabolism (Bingham et al., 1990; Muir et al., 1993; Thorburn et al., 1993; Hylla et al., 1998). In particular, fermentation of RS appears to generate comparatively high levels of butyrate, which is the main energy source for the colonocytes (Mortensen and Clausen, 1996; ProFibre, 1998). Thus, foods with higher RS contents could be considered as advantageous in most healthy adults.

Another nutritional parameter concerns the rate of starch digestion and absorption in the small intestine. As a tool for ranking foods with respect to their postprandial blood glucose raising potential, as compared with the reference product (glucose or white bread), the glycemic index (GI) concept was introduced by Jenkins et al. (1981). Today there is a growing body of evidence in support of metabolic advantages of low-GI foods. As a consequence, the recent FAO/WHO consultation on CHOs strongly advocates an increased consumption of such foods (FAO, 1998). Extended GI lists are now available. However, one problem from a nutritional point of view is that many basic staple foods have high GIs. Moreover, the products are usually poorly characterized regarding, for example, thermal history or amylose/amylopectin ratio, which may influence the GI features.

Elevated amylose levels appear to lower the rate of glucose delivery to the blood, thus promoting a lower GI. Long-term consumption of high-amylose starch was

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shown to beneficially influence and reduce glucose and insulin responses and to lower triglyceride concentrations, in comparison with high-amylopectin starch in healthy and hyperinsulinemic individuals (Behall and Howe, 1995). Similar effects were observed after the consumption of a diet characterized by low-GI foods (Jenkins et al., 1985, 1987).

Legumes belong to a group that elicits the lowest blood glucose responses (Jenkins et al., 1981; Foster-Powell and Brand Miller, 1995). In general, legume starches differ from cereal or tuber starches both in their chemical composition (they have a higher amylose content) and in their granular structure (Doublier, 1987; Orford et al., 1987; Eliasson, 1988; Bogracheva et al., 1998), which is connected to a more restricted swelling and solubility capacity, and to a higher tendency toward retrogradation and syneresis.

Differences in starch characteristics arise from several reasons. In addition to the type of organ, developmental stage of plants, and environmental conditions, the pathway of starch synthesis is of major importance. It is controlled by enzymes comprised in the whole process (Stark et al., 1992; Smith et al., 1995). To be more precise, the amount of starch and its chemical and granular structure are greatly determined by genes encoding the activity of key enzymes at each step in the starch synthesis (Hedley et al., 1998). Therefore, mutations of appropriate genes could divert the biochemistry of starch synthesis in plants, which then results in mutants with starch properties different from those of the ancestor.

In the case of peas, the mutations found at the *r* and *rb* loci provide pea lines (rrRbRb and RRrbrb) with changes in the structure and the physicochemical properties of starch granules as compared to the wild type (RRRbRb) (Bogracheva et al., 1995; Lloyd et al., 1996). A mutation at the *r* locus more significantly affects the starch synthesis pathway and results in a high proportion of amylose and also in changes in the amylopectin structure, whereas a mutation at the *rb* locus increases the proportion of amylopectin in pea starch (Hedley et al., 1998).

In the GI tables (Foster-Powell and Brand Miller, 1995), data for green peas are scarce. In most cases, processing conditions are not described, and the origin of the pea material is unknown. As discussed above, variation in the amylose content of peas could probably affect the GI and RS features. In the present study, pea genotypes with known and different amylose/amylopectin ratios were subjected to autoclaving or boiling at atmospheric pressure (to resemble either industrial canning or domestic processing) and tested either intact or following homogenization. In vitro enzymatic procedures were then used to estimate the GI and RS features of the processed pea genotypes.

MATERIALS AND METHODS

Samples. Three different green-seeded pea genotypes (*Pisum sativum* L.) were obtained from Dr. Cliff L. Hedley, Department of Applied Genetics, John Innes Centre, Norwich, U.K. The set of lines was nearly isogenic, except for the genes at the *r* and *rb* loci. The genetic background of the lines and their development were published previously (Hedley et al., 1994). One wild-type pea genotype (RRRbRb BC3) with medium content of amylose (32.7%; 33-Am) and two mutants (rrRbRb BC3 and RRrbrb BC3) with high (65.1%; 65-Am) and low (23.3%; 23-Am) contents of amylose (Bogracheva et al., 1995), respectively, were studied. The mature dry seeds of pea

genotypes 65-Am and 23-Am were available whole (intact) or milled, whereas the 33-Am peas were available only as milled material.

Sample Processing. Prior to analyses the milled samples were remilled in a Cyclotec mill (Tecator, Sweden) to a particle size <0.8 mm.

Autoclaved Pea Purées. An accurate amount of the particular milled sample was weighed in a 100-mL glass bottle. Distilled water was added to reach the final concentration of 7% starch (w/w). For the 65-Am and 23-Am peas, a higher concentration was prepared as well (10% w/w, starch basis). To avoid sedimentation during autoclaving, the pea sample was allowed to swell during mixing in a boiling water bath. The samples were sealed, autoclaved for 1 h at 1.35 bar, and subsequently stored at room temperature for 3 days. The purées based on 7% starch concentration were like the commercial purées as opposed to the much thicker and viscous purées with a higher starch concentration. Thus, for studying the rate of starch hydrolysis only the purées with 7% starch concentration were chosen.

Autoclaved Whole Seeds. In the case of 65-Am and 23-Am peas, 150 dried seeds were placed in a 100-mL bottle, which was then filled with salt solutions (22 g/L of NaCl and 5 g/L of CaCl₂). The salts were added to attenuate the disruption of seeds and the leaking of starch during thermal treatment. The seeds were soaked overnight and autoclaved for 1 h at 1.35 bar in the same water. Prior to analysis, samples were stored for 3 days at room temperature.

Boiled Whole Seeds. The seeds were soaked in distilled water overnight (without addition of salts), boiled in an excess of water for 1 h in a saucepan, and then cooled for 2 h before analysis. The starch content of the seeds was corrected for the amount of starch that was leaked out into the water.

Boiling the whole seeds simulated a domestic way of processing. Usually such products are not stored, and thus the cooling/storage period was shorter than for the autoclaved material.

Preparation of White Wheat Reference Bread. Bread from white wheat flour (Kungsörnen AB, Järna, Sweden) was made in a baking machine (El-Gennel HB-021E, Korea) as described previously (Liljeberg and Björck, 1994). The cooled bread was sliced, the crust was removed, and the slices were frozen. Before analysis, the slices were thawed at room temperature. White wheat bread was used as a reference product to evaluate both the in vitro rate of starch hydrolysis and the RS formation in the pea products.

Determination of Total Starch. In general, the procedure followed the method described by Tovar et al. (1990); however, before the alkali treatment, the samples were soaked in a phosphate buffer (0.1 M; pH 6.0). Total starch was determined enzymatically following solubilization in alkali (4 M KOH) and incubation with a thermostable α -amylase (Termamyl 300L DX; Novo Nordisk A/S, Copenhagen) and amyloglucosidase (EC 3.2.1.3, 3500 U/25 mL; Boehringer Mannheim, No. 1202 367). The content of glucose was assayed with the glucose oxidase-peroxidase reagent (GLOX), and the starch content was calculated using the conversion factor 0.9.

In Vitro Rate of Starch Hydrolysis. The original dialysis procedure based on chewing (Granfeldt et al., 1992) was followed with some modifications regarding the enzyme system. Thus, instead of α -amylase, a mixture of α -amylase and amyloglucosidase was used. Thereafter, a lower molecular weight cutoff (MWCO) was introduced, and as a measure of the rate of starch hydrolysis the released glucose was assayed. A detailed description of the procedure is as follows: Prior to the experiment, six healthy subjects brushed their teeth and then chewed the sample (corresponding to 500 mg of total starch) 15 times (15 s). The content was expectorated into the beaker. The subjects rinsed their mouths with 5 mL of water, which was expectorated into the same beaker with the chewed sample. In the case of autoclaved purées, glass beads were used and the collected saliva was transferred to the samples. To the chewed sample was added also 5 mL of 0.1 M succinate buffer (pH 4.7). The content was stirred and the pH adjusted to 1.5 with 2 M HCl. Prior to incubation at 37 °C for 30 min,

Table 1. Amylose, Total Starch, and DF Contents in Different Nonprocessed Pea Lines

sample	amylose ^a (g/100 g of starch)	total starch (g/100 g of dm)	DF (g/100 g of dm)		
			total	insoluble	soluble
23-Am	23.3	26.8	39.2	31.3	7.9
33-Am	32.7	46.2	22.6	16.3	6.3
65-Am	65.1	28.8	33.6	26.6	7.0

^a Bogracheva et al. (1995).

1 mL of pepsin (EC 3.4.23.1, 2000 FIP-U/g; Merck, Darmstadt, Germany) solution (50 mg/mL) was added to each sample. Thereafter, the mixtures were adjusted with 1 M NaOH to pH 4.7 and transferred into dialysis tubings (Spectra Por No. 3, width = 45 mm, MWCO = 3500) with 400 μ L of amyloglucosidase (EC 3.2.1.3, 3500 U/25 mL; Boehringer Mannheim, No. 1202 367), 125 μ L of pancreatine (8 \times U.S.P., 400 mg/10 mL; Sigma, No. P 7545), and 125 μ L of mineral mix (0.3 mmol/mL CaCl₂ + 0.06 mmol/mL MgCl₂). The tubings were placed into 1000-mL beakers containing 800 mL of 0.05 M succinate buffer (pH 4.7; 37 °C) and stirred during the incubation (3 h, 37 °C). Every 30 min, aliquots of 200 μ L were withdrawn in duplicates and added to the tubes containing 1.8 mL water. Furthermore, 4 mL of GLOX reagent was added to the samples, which were incubated for 1 h before the absorbance was read at 450 nm. A standard curve was prepared using glucose. The amount of glucose released was multiplied by 0.9 and expressed as the percentage of total starch in the chewed sample. The hydrolysis index (HI) was calculated as follows: for each product, the area under the hydrolysis curve was expressed as a percentage of the corresponding area obtained after hydrolysis of white wheat bread chewed by the same person. To predict the glycemic index (GI) from the HI, the equation introduced by Granfeldt (1994) was used: $GI = 0.862 \times (HI) + 8.198$.

Parallel Determination of Potentially Available Starch and RS. A detailed description of the method was recently published by Åkerberg et al. (1998). This *in vitro* procedure mimics physiological conditions and includes chewing before digestion with proteolytic and amylolytic enzymes. In summary, the samples (corresponding to 500 mg of total starch) were treated similarly as described above in the study of the rate of starch hydrolysis. However, the samples were incubated with constant stirring at 40 °C overnight. After completed incubation, RS and nonstarch polysaccharides were precipitated with 95% ethanol (v/v) and filtrated through P2 crucibles. In the filtrates, glucose was measured at 450 nm using GLOX reagent and expressed as potentially available starch. The RS, comprising RS₁ + RS₂ + RS₃, was determined as total starch in dried (105 °C overnight) and milled filter residues.

Dietary Fiber Analysis. In the raw material the insoluble and soluble dietary fibers were assayed and corrected for ash and protein, following the enzymic gravimetric method of Asp et al. (1983).

Statistical Analysis. The results are expressed as mean \pm SD. Potentially available starch, RS, and the *in vitro* rate of starch hydrolysis were statistically evaluated by one-way ANOVA using the SPSS/PC+ program (SPSS Inc., Chicago IL). Comparisons between means were performed by Duncan's test. For the potentially available starch and RS, a value of $P < 0.001$ was considered to be significant. The corresponding value for the *in vitro* rate of starch hydrolysis was $P < 0.05$.

RESULTS AND DISCUSSION

Main Indigestible CHOs (RS and DF) and Potentially Available Starch. The contents of amylose, total starch, and DF in different pea samples are listed in Table 1.

The present study on hydrothermally treated green-seeded pea genotypes, evaluated as intact or milled seeds, indicates that the amylose/amylopectin ratio significantly changed the characteristics of starch and

its accessibility to the amylolytic enzymes. In recent times, several authors have studied different products based on high-amylose plant varieties or corresponding pure starches focusing on the retrogradation and/or RS formation [corn: Berry (1986), Sievert and Pomeranz (1989), and Granfeldt et al. (1993); barley: Björck et al. (1990), Szczodrak and Pomeranz (1991), and Xue et al. (1996); rice: Eggum et al. (1993)]. Significant positive correlations between the amylose content and the amount of RS have been found in these studies.

In Table 2, the potentially available starch and RS in different samples are shown and compared with the corresponding data for the white bread reference product. In all pea products prepared from either intact or milled dry seeds, significantly higher amounts of RS were present as compared with the white bread (0.8% RS, total starch basis) ($P < 0.001$). Three groups of pea samples could be differentiated. The 23-Am autoclaved purée or intact seeds yielded the lowest RS content (6.2–7.6%). With higher amylose/amylopectin ratio, the RS yield increased as observed in the case of 33-Am autoclaved purée (11% RS). The most pronounced RS formation was found in the 65-Am samples (31.2–33.4% RS, total starch basis).

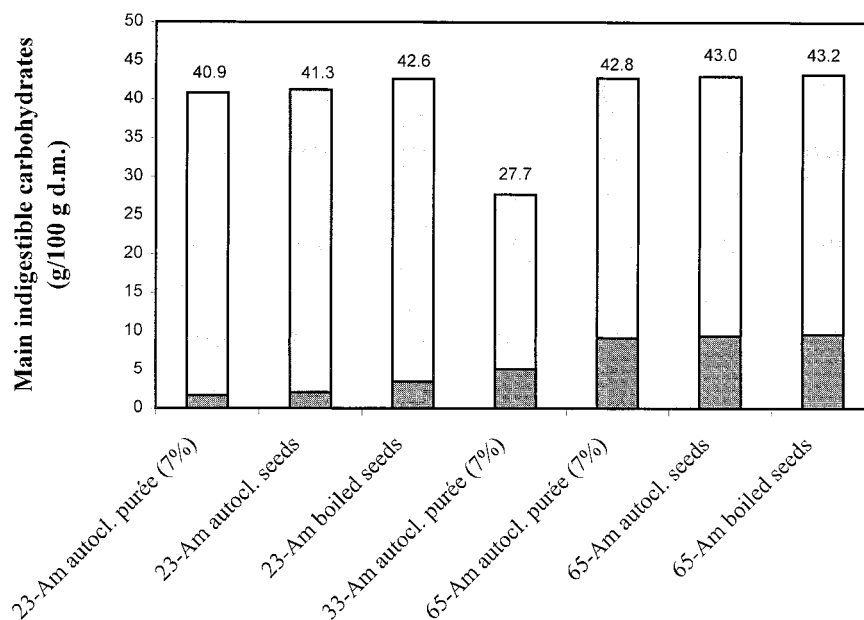
As judged from the comparatively high RS levels also in the 23-Am products, it could be concluded that pea starch appears to be more prone to retrograde and thus generate RS₃ at an amylose level comparable with that in cereal starches (i.e., wheat, ordinary corn, barley, and rice) (Berry, 1986; Björck et al., 1990; Eggum et al., 1993). In peas, it was previously shown (Colonna and Mercier, 1984; Lloyd et al., 1996) that a mutation at the *r* locus (such as in 65-Am peas) gave amylopectin with a wider range of molecular sizes as compared to the wild type (33-Am peas). In contrast, a mutation at the *rb* locus (such as in 23-Am peas) did not affect the amylopectin fraction. Accordingly, it is tempting to speculate that in the 65-Am samples studied, some outer chains and branches of amylopectin may be able to retrograde and thus contribute to the overall RS (retrograded starch) content in these particular products. Thus, some other mechanisms besides amylose retrogradation may have contributed to the unexpectedly high RS formation also in the 23-Am pea products. It should be noted, however, that the RS level was considerably lower in the autoclaved 23-Am purée than in a commercial autoclaved pea based infant purée (18% *in vitro* undigested starch) described previously (Björck and Siljeström, 1992). The 23-Am genotype therefore offers a potential means to reduce the RS level in this type of pea-based product.

The digestibility of starch varies between leguminous species and also within individual species. As recently reported by Periago et al. (1997), the choice of cooking method could be of significant importance for the extent of starch digestion within individual species. The RS values found in the 65-Am pea products in the present study (31.2–33.4%, total starch basis) are in accordance with the data obtained by García-Domingo et al. (1997), who reported 38% RS in canned peas and 33% in frozen peas (*P. sativum* L.). In two wrinkled pea cultivars (Citrina and Warindo), Periago et al. (1996) found less than 2 or 3% RS, respectively (wet weight basis) after cooking and canning. On the basis of the total starch content in the products studied, this would correspond to 20 or 26% RS, respectively. In general, when heat treatments (cooking versus canning) are compared, a

Table 2. Potentially Available Starch and RS Contents in Pea Products, Expressed as Relative (Total Starch Basis) or Absolute (dmb) Values^a

sample	form	potentially available starch \pm SD (g/100 g of total starch)	RS \pm SD (g/100 g of total starch)	potentially available starch \pm SD (g/100 g of dm)	RS \pm SD (g/100 g of dm)
23-Am	autoclaved purée (7%) ^b	101.02 \pm 1.19 ^{ef}	6.17 \pm 0.24 ^b	27.08 \pm 0.32	1.65 \pm 0.06
23-Am	autoclaved purée (10%) ^c	104.23 \pm 0.75 ^f	7.23 \pm 0.60 ^b	27.93 \pm 0.20	1.94 \pm 0.16
23-Am	autoclaved seeds	90.91 \pm 2.68 ^{cd}	7.64 \pm 0.24 ^b	24.36 \pm 0.72	2.05 \pm 0.07
23-Am	boiled seeds	76.13 \pm 5.32 ^b	12.85 \pm 5.28 ^c	20.40 \pm 1.43	3.44 \pm 1.42
33-Am	autoclaved purée (7%) ^b	89.36 \pm 0.41 ^c	11.03 \pm 0.14 ^{bc}	41.26 \pm 0.15	5.10 \pm 0.06
65-Am	autoclaved purée (7%) ^b	75.84 \pm 0.57 ^b	31.79 \pm 0.25 ^d	21.84 \pm 0.16	9.16 \pm 0.07
65-Am	autoclaved purée (10%) ^c	76.31 \pm 0.71 ^b	31.15 \pm 1.57 ^d	21.98 \pm 0.20	8.97 \pm 0.45
65-Am	autoclaved seeds	71.75 \pm 3.26 ^{ab}	32.63 \pm 3.35 ^d	20.67 \pm 0.94	9.40 \pm 0.97
65-Am	boiled seeds	67.42 \pm 5.53 ^a	33.40 \pm 4.22 ^d	19.42 \pm 1.59	9.62 \pm 1.21
wheat bread	crumb	96.71 \pm 2.46 ^{de}	0.76 \pm 0.05 ^a	75.42 \pm 1.92	0.59 \pm 0.04

^a Values with different superscript letters in the same column are significantly different ($P < 0.001$). ^b The final concentration of starch in water reached 7% (w/w). ^c The final concentration of starch in water reached 10% (w/w).

**Figure 1.** Main indigestible CHOs in pea products (dmb), expressed as a sum of DF and RS.

slightly higher amount of starch remained undigested after cooking (Periago et al., 1996).

In the present study, the choice of heat treatment or the extent of botanical disruption of peas influenced the formation of RS only in the case of 23-Am samples. Consequently, significantly higher amounts of RS were recovered in the boiled intact seeds compared with the autoclaved purées or autoclaved seeds, respectively ($P < 0.001$). It is suggested that boiling (as a less rough process than autoclaving) retained a part of pea starch within the cell integrity and thus rendered it less accessible to enzymic action in the case of 23-Am sample. Thus, in the RS fraction of boiled 23-Am seeds, a part of RS may be present as physically entrapped starch (RS₁) and possibly also in the form of incompletely gelatinized starch granules (RS₂). No such indications of a higher RS content following boiling as opposed to autoclaving were noted in the 65-Am material. This might be due to the fact that the 65-Am genotype had a significantly lower DF content, which may reflect a thinner and more vulnerable cell wall structure.

When the CHOs in peas that are able to escape digestion in the small intestine are considered, the RS fraction should not be neglected. Consequently, in mixed diets, RS and DF constitute the main substrates for microbial fermentation in healthy individuals (Mortensen and Clausen, 1996).

In peas, some oligosaccharides (raffinose = 5–6% dmb) also resist digestion in the small intestine (Johansen et al., 1996) and thus to some extent increase the fermentable mass from pea products. García-Domingo et al. (1997) studied the *in vitro* indigestible fraction in canned and frozen peas. The total amounts of 39.8 and 36%, respectively, consisted of nonstarch polysaccharides (NSP), RS, resistant protein, Klason lignin, and oligosaccharides. Only minor amounts of the latter were reported (García-Domingo et al., 1997).

In Figure 1, the sum of total DF and RS for the pea products is displayed. Regardless of the amylose content, both the 23-Am and 65-Am products contained considerable amounts of indigestible CHOs, which exceeded even 40% (dmb). The corresponding value in the 33-Am purée was significantly lower (27.7% dmb). As shown in the present study, low amylose itself does thus not provide an assurance for a low content of indigestible CHOs. The high amounts of indigestible CHOs in 23-Am and 65-Am samples suggest that these particular pea products can provide the large bowel with important amounts of fermentable substrates, leading to a production of short-chain fatty acids (SCFAs) and gases (CO₂, H₂, and CH₄) (Mortensen and Clausen, 1996). At present, the physiological significance of SCFAs, especially butyric acid, is highly acknowledged

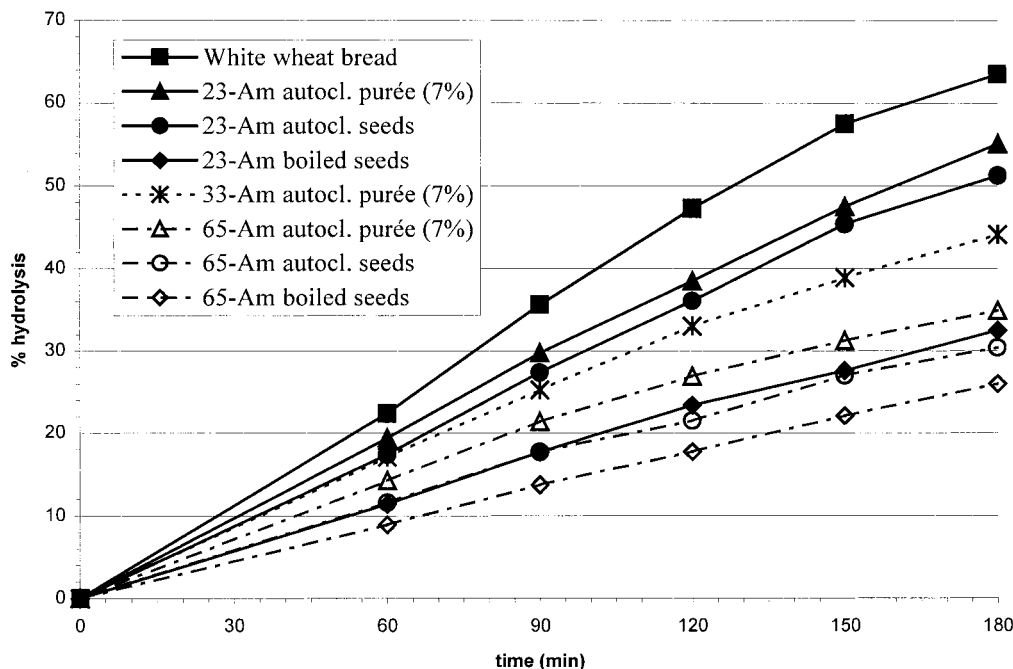


Figure 2. Rate of starch amylolysis in processed peas as compared to the reference white wheat bread.

Table 3. HI and Predicted GI for Different Pea Products

sample	HI \pm SD	predicted GI
white bread	100 ^e	100
23-Am autoclaved purée (7%) ^b	84.3 \pm 8.34 ^d	81
23-Am autoclaved seeds	83.7 \pm 13.32 ^d	80
23-Am boiled seeds	53.0 \pm 10.02 ^b	54
33-Am autoclaved purée (7%) ^b	70.8 \pm 6.46 ^c	69
65-Am autoclaved purée (7%) ^b	58.6 \pm 9.15 ^b	59
65-Am autoclaved seeds	50.9 \pm 5.79 ^{ab}	52
65-Am boiled seeds	41.7 \pm 5.05 ^a	44

^a Values with different superscript letters in the same column are significantly different ($P < 0.05$). ^b The final concentration of starch in water reached 7% (w/w).

for the welfare and health of the human large intestine (ProFibre, 1998). As RS is among the substances providing the highest percentage of butyric acid following in vitro fermentation (Hill, 1995; Silvester et al., 1995), the 65-Am pea products might be of particular interest in relation to colonic health.

In Vitro Rate of Starch Hydrolysis. The average rates of starch hydrolysis following the enzyme incubation of the processed 23-Am, 33-Am, and 65-Am products are shown in Figure 2. Considering the ratios between the area under the hydrolysis curve of a particular pea product and of the reference white wheat bread, the calculated HI and predicted GI for different pea products are given in Table 3.

As judged from the HIs, starch in all pea products was hydrolyzed at a significantly slower rate ($P < 0.05$) than starch in the white wheat reference bread. The hydrolysis rate was highest in the purée (HI = 84) made from the 23-Am genotype. A significantly lower ($P < 0.05$) HI was shown with the 33-Am purée (HI = 71), whereas the highest proportion of amylose in 65-Am purée resulted in the lowest HI (59). A similar tendency between amylose content and reduced rate of starch hydrolysis was observed also for the intact autoclaved or boiled seeds (Figure 2).

In the case of 23-Am products, the HI for the boiled seeds was significantly lower than the corresponding value for autoclaved seeds. However, the HI values for

autoclaved and boiled 65-Am seeds were not statistically different. Consequently, the rate of starch amylolysis was determined not only by the amylose/amylopectin ratio but also by the method of heat treatment, and the botanical structure of the seed coats and cell walls of studied material appeared to be important.

Most studies regarding in vitro starch digestion are usually performed on various types of beans or other legumes that have been processed differently, whereas the genetic background is seldom specified. Low HI values of legumes as compared to the reference product (white wheat bread) were recently documented by Velasco et al. (1997) and by Garcia-Alonso et al. (1998). Concerning the HI values in processed peas (*P. sativum* L.), there are no comparable data available in the literature.

The predicted GI values for most of the pea products (GI = 44–69) were lower than those for the majority of breakfast cereals, flour-based breads, or potato products and comparable with those of other legumes or pasta products (i.e., spaghetti) (Granfeldt et al., 1992; Foster-Powell and Brand Miller, 1995; Björck et al., 1996). Instead, the GI values predicted for the autoclaved 23-Am products (~80) were significantly higher, indicating that the choice of pea genotype may affect glycemic responses. In the literature the GI for boiled green peas (*P. sativum*) varies in a range from 55 to 77 (Foster-Powell and Brand Miller, 1995). However, no information about the raw material or cooking process is given, but probably ordinary peas with ~33% amylose (starch basis) were used in these studies.

Conclusions. In the present in vitro study the correlation between the amylose/amylopectin ratio and the RS formation or the rate of starch hydrolysis in different pea genotypes was shown. Besides the influence of the starch composition, starch accessibility was also affected by the choice of thermal treatment and/or the botanical structure of the peas studied. It is expected that during industrial canning of whole pea seeds the effect of botanical integrity might be lost, as there were no significant differences evident between the auto-

claved purées and the corresponding autoclaved intact seeds. From a nutritional point of view, various pea lines can ensure a wide range of products regarding the desired course of starch digestion. Related to other starchy foods—such as cereals and tubers—pea (*P. sativum* L.) is ranked in the group having low-GI features. However, as predicted with the in vitro study, the presently described pea products covered a broad GI interval (44–81). Therefore, discrepancies in GI data may arise when the composition and handling of the particular pea are not clearly defined.

In addition, both the 65-Am and 23-Am pea products represent an abundant source of DF, which, together with RS, could be expected to provide substrate for the colonic microflora. It is concluded that in a further work it would be interesting to extend the investigation to the in vivo situation. The molar ratios between individual SCFAs formed by fermentation of pea substrates differing in their genetic characteristics should be emphasized.

ABBREVIATIONS USED

23-Am, genotype with 23.3% amylose; 33-Am, genotype with 32.7% amylose; 65-Am, genotype with 65.1% amylose; ANOVA, analysis of variance; CHO, carbohydrates; DF, dietary fiber; dmb, dry matter basis; GLOX, glucose oxidase–peroxidase reagent; GI, glycemic index; HI, hydrolysis index; MWCO, molecular weight cutoff; NSP, nonstarch polysaccharides; RS, resistant starch; SCFAs, short-chain fatty acids; SD, standard deviation.

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