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Resistant starch formation in bread as influenced by choice of ingredients or baking conditions

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The formation of resistant starch (RS) in bread products was evaluated *in vitro* in relation to the processing conditions. The impact of the particular baking conditions applied to pumpernickel bread was investigated as well as the presence of malt and sourdough acids, commonly present in such bread. Also studied was the potential effect of including wholemeal barley from a high-amylose genotype. In some bread, the rate of hydrolysis of the potentially available starch fraction was evaluated by an *in vitro* procedure. A low-temperature, long-time baked product (20 h at 120°C) contained significantly higher amounts of RS (5.4%, starch basis) than a corresponding ordinary baked bread (40 min at 200°C) (3.0%, starch basis). Addition of lactic acid increased RS recovery further (6.6% starch basis), whereas malt had no impact on RS yield. The highest level of RS was noted in a long-time baked bread based on high-amylose barley flour (7.7%, starch basis). In contrast to all other products, this bread also displayed a lowered rate of amylolysis of the non-RS fraction (hydrolysis rate index = 68). It is concluded that exchanging ordinary baking conditions for pumpernickel baking, particularly in the presence of certain organic acids, may substantially increase the RS content. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

Until recently, starch was regarded as a completely digestible carbohydrate. This assumption was based on the observation that no starch remnants could be detected in faeces after a meal. However, it is now well established that important amounts of starch escape digestion and absorption in the human small intestine (Cummings *et al.*, 1990; Cummings & Englyst, 1991). Such starch is commonly referred to as resistant starch (RS). Like most dietary fibre components, RS may be fermented by the colonic micro-flora. The metabolites formed include, for example, the short-chain fatty acids (SCFAs) acetate, butyrate and propionate (Cummings, 1981) with potential beneficial health effects. From a nutritional point of view, the digestibility of starch is, thus, of great importance. Besides its role in providing substrate to the faecal bacteria, hence increasing the bacterial mass and faecal bulk (Shetty & Kurpad, 1986), it has been purposed that RS is particularly prone to generating butyric acid upon fermentation (Scheppach *et al.*, 1988). Owing to the role of butyric acid as the major energy substrate for the colonocytes, RS might have a protective effect against colonic diseases (Bingham, 1990; Jenkins *et al.*, 1986). Moreover, it has been suggested that a higher RS content, commonly associated with *lente* starchy foods, improves long-term glycaemic and lipid metabolism through the SCFA produced during its fermentation (Jenkins *et al.*, 1987*a*; Muir *et al.*, 1993).

Three major forms of RS have been identified in the human diet: (1) physically trapped starch (e.g. in whole or partly ground grains); (2) resistant starch granules (e.g. raw potatoes); and (3) retrograded starch (e.g. heat-treated and cooled products) (Englyst & Cummings, 1987; Englyst & Kingman, 1990). The extent to which the different forms of RS are present in foods will depend on a number of factors including the type of processing and also the botanical source of the starch.

Cereal products are the most important sources of starch in the diet. Thus, the estimated annual intake of bread in European countries ranges from 46 to 100 kg per person (U. Eklund, Swedish Bread Institute, personal communication). Consequently, RS from bread can be expected to constitute a considerable part of the total quantity that escapes digestion in the small intestine. Attempts to modify RS intake in a mixed diet should thus focus on optimizing the RS content of bread.

According to *in vitro* determinations, common flour-based breads contain limited quantities of RS, i.e. below

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2% (starch basis) (Björck *et al.*, 1986; Englyst *et al.*, 1992; Holm & Björck, 1992; Liljeberg & Björck, 1994; Liljeberg *et al.*, 1992; Siljeström & Asp, 1985). Similarly, the RS recovered in ileostomy effluent in subjects given white bread was approximately 2.5% (starch basis) (Englyst & Cummings, 1985). In contrast, a much higher content (10% starch basis) was detected in the ileal effluent following ingestion of a pumpernickel bread (Jenkins *et al.*, 1987*b*), suggesting recovery of botanically encapsulated starch in cereal grains. Moreover, we previously reported a high level of RS in a commercial sourdough fermented pumpernickel bread, baked with mostly wholemeal rye flour. Approximately 8% (starch basis) was thus also resistant when employing an *in vitro* method which mainly recovers the retrograded amylose fraction (Liljeberg & Björck, 1994).

Another extensively discussed nutritional parameter relates to the rate of starch hydrolysis and glycaemic response. Starch in many cereal products is rapidly digested and absorbed, resulting in unfavourable high blood glucose and insulin responses after a meal (Jenkins *et al.*, 1988*b*). This is particularly true with flour-based bread products (Holm & Björck, 1992; Jenkins *et al.*, 1988*a*; Liljeberg & Björck, 1994; Liljeberg *et al.*, 1992). Several food factors have been suggested to affect the rate of small intestinal starch uptake, e.g. the nature of the starch, food form, dietary fibre and the type or extent of food processing (Wolever, 1990). Many food factors which increase RS formation also reduce the rate of digestion and absorption in the small intestine (Jenkins *et al.*, 1987*b*). However, RS formation in relation to post-prandial glycaemia has not been fully elucidated.

The purpose of the present study was to see if the formation of RS in bread products could be affected by altering the processing conditions. Also studied was the potential relation between enzyme resistance and the rate of enzymic hydrolysis of the bulk of starch. The baking processes were either normal or performed at the low-temperature, long-time baking conditions commonly applied for pumpernickel bread. The impact of pumpernickel constituents such as malt and organic acids, formed at sourdough fermentation, was also investigated. In addition, the possibility of increasing RS yield by enclosure of high-amylose barley was evaluated. All bread products were based on milled ingredients. The *in vitro* RS content in the products was measured by quantifying total starch remnants in an enzymic–gravimetric dietary fibre residue. Although this procedure fails to determine enzyme resistance due to entrapment of starch, it does include RS in the form of retrograded amylose, known to be the major form of RS in flour-based bread. In some bread, the rate of hydrolysis of the potentially available starch fraction was evaluated with a method based on chewing the test product prior to incubation with pepsin, and subsequent incubation with amylase in a dialysis tubing (Granfeldt *et al.*, 1992). This method has been shown to predict glycaemia with good accuracy for a number of cereal and legume foods.

MATERIALS AND METHODS

Bread products

Ordinary bread, baked for 40 min at 200°C (WMB-ordinary), was made from commercial Swedish wholemeal rye flour and white wheat flour (Kungsörnen, Sweden), in a ratio of 70:30 (flour basis). Bread products baked with an extended baking process, 20 h at 120°C, were prepared from wholemeal rye flour (WMB) or wholemeal high-amylose barley flour (HAB), mixed with white wheat flour (70:30). The high-amylose barley genotype (Glacier, 42% amylose) was obtained from Montana State University, Bozeman, USA (Granfeldt *et al.*, 1994). Also, long-time baked breads were prepared from various ingredients. Two products were added with lactic acid (WMB-la) or acetic acid (WMB-aa), on a similar molar basis. Furthermore, malt was included in one product (WMB-m). Finally, long-time baked breads were baked with the addition of malt in combination with lactic acid (WMB-m-la) or acetic acid (WMB-m-aa). A standardized white wheat bread was baked in a home baking machine and used as a reference (Liljeberg & Björck, 1994).

Recipes

Ordinary baking

WMB-ordinary. Two breads were baked from 450 g wholemeal rye flour, 200 g white wheat flour, 400 g water (20°C), 70 g baker's yeast and 5 g NaCl. The dough was divided into two pieces and put into aluminium pouches, followed by proofing for 2 h at room temperature. Baking was performed at 200°C for 40 min.

Long-time baking

WMB (basic recipe). Two breads were baked from 450 g wholemeal rye flour, 200 g white wheat flour, 450 g water (20°C), 70 g yeast and 5 g NaCl. The dough was divided into two pieces and put into aluminium pouches covered by a double layer of aluminium foil, and followed by proofing for 2 h at room temperature. Baking was performed at 120°C for 20 h.

WMB-la and WMB-aa. The breads were baked from the basic recipe with the addition of 10.5 g lactic acid (90 wt%) and 7.0 g acetic acid (100 wt%), respectively.

WMB-m. Thirty grammes of Bavarian malt extract (Ireks GmbH, Kulmbach, Germany) was added to the basic recipe. The breads were proofed and baked as described above.

WMB-m-la and WMB-m-aa. The breads were baked from the basic recipe with the addition of malt (30 g) in combination with lactic acid (10.5 g), for WMB-m-la, or acetic acid (7.0 g), for WMB-m-aa.

HAB

Two breads were baked from 450 g wholemeal high-amylose barley flour, 200 g white wheat flour, 500 g water (20°C), 70 g yeast and 5 g NaCl. The products were proofed and baked as described in the basic recipe.

Before cutting into slices, all breads were stored at room temperature overnight. The crust was removed and two slices were wrapped in aluminium foil, put into plastic bags and stored in a freezer until utilized.

Chemical analysis

A portion from each bread was air dried and milled (Cyclotec, Tecator, Sweden) (< 0.8 mm) prior to analysis. The bread products were analysed for potentially available starch (Holm *et al.*, 1986), and *in vitro* RS was measured by quantifying total starch remnants in an enzymatic–gravimetric dietary fibre residue (Siljeström *et al.*, 1989).

***In vitro* starch hydrolysis**

Some of the bread products were tested *in vitro* to determine the rate of release of starch hydrolysis products following incubation with salivary α-amylase, pepsin and pancreatic α-amylase (Granfeldt *et al.*, 1992). Subjects rinsed their mouths with tap water and subsequently chewed the bread products for 15 s (approximately 15 times). They were told not to eat within 1 h prior to the experiment. All bread portions contained 1 g of starch and were given in randomized order to six subjects. The products were then expectorated into a beaker containing 50 mg of pepsin (2000 FIP-U/g, Merck, Darmstadt, Germany) in 6 ml of 0.05 M Na,K-phosphate buffer (containing 0.4 g/litre NaCl) adjusted to pH 1.5 with 2 M HCl. Finally, the subjects rinsed their mouths with 5 ml of Na,K-phosphate buffer (pH 6.9) for 60 s and expectorated the rinsing solution into the beaker. The contents were stirred and pH adjusted to 1.5. Each sample was incubated at 37°C for 30 min with gentle mixing three times during incubation. The pH was re-adjusted to 6.9 with NaOH before incubation with porcine pancreatin α-amylase (A 6255 Sigma Chemical, St Louis, Missouri, USA). The enzyme (110 Sigma units) was dissolved in 10 ml of buffer, and 1 ml of this solution was added to the beaker. The sample was brought to volume (30 ml) with phosphate buffer, and transferred to the dialysis tubing (13 cm strips, Spectra Por No. 2, width 45 mm, molecular weight cut-off 12 000–14 000). Each bag was incubated at 37°C for 3 h in a beaker with phosphate buffer (800 ml). The beaker was placed in a stirred water bath. Every 30 min, aliquots (2 ml) from the dialysate were removed for analysis of reducing sugar content by the 3,5-dinitro salicylic acid (DNS) method (Hostettler *et al.*, 1951). A standard curve was prepared using maltose. In addition, a hydrolysis rate index (HI) was calculated as 100 times the area under the curve (0–180 min) for the product divided by the corresponding area obtained with white wheat bread (WWB) chewed by the same person (Liljeberg & Björck, 1994). This procedure has been shown to predict glycaemic responses in healthy subjects with good accuracy according to the following formula; $GI = 0.862 \cdot HI + 8.198$ (Granfeldt, 1994), where GI represent the glycaemic index.

Statistical methods

One-way analysis of variance design followed by the Duncan procedure for multiple comparison were used to determine significant differences in RS content. The results concerning the rate of starch hydrolysis are expressed as means ± SEM, and the statistical significance of differences were assessed by the Wilcoxon matched-pair signed-ranks tests. The SPSS/PC+ advanced statistics program (version 2.0, SPSS, Chicago, Illinois, USA) was used. A value of $P < 0.05$ was considered significant.

RESULTS AND DISCUSSION

All flour-based low-temperature, long-time baked products (20 h at 120°C) contained higher amounts of RS ($P < 0.05$) than a corresponding ordinary baked bread (40 min at 200°C) (Table 1). The main RS fraction in the long-time baked products required solubilization in alkali to render it available to analytical amylases, indicating the presence of retrograded starch. The bread baked for 20 h (WMB) contained 5.4% RS (starch basis), which is almost twice the amount (3.0%, starch basis) analysed in the ordinary baked bread (WMB-ordinary). An even more pronounced RS formation was noted ($P < 0.05$) in the bread baked with added lactic acid (WMB-la, pH 4.3), 6.6% starch basis, or lactic acid in combination with malt (WMB-m-la), 6.7% starch basis, showing that lactic acid might further promote retrogradation of starch. In contrast, addition of acetic acid on an equivalent molar basis (WMB-aa, pH 4.6) did not exert this effect, nor did malt (WMB-m) or malt in combination with acetic acid (WMB-m-aa).

The high content of RS formed during long-time baking is in agreement with the amount (8.1%, starch basis) previously found in a commercial sourdough fermented pumpernickel bread baked with an extended baking process (20 h) (Liljeberg & Björck, 1994). The high RS levels reported in this and the presently described low-temperature, long-time baked breads are indeed noteworthy in that the RS method used involved grinding and gelatinization, i.e. removing two potential sources of RS. Thus, the mechanism for RS formation is probably retrogradation of amylose. However, linearization of amylopectin has been reported to significantly increase RS formation during wet-autoclaving (Berry, 1986). Hence, it cannot be ruled out that such linearization of starch occurs during the long low-temperature baking process due to the prolonged activity of intrinsic amylases in the dough. Moreover, the linearization might be more prominent in the presence of certain organic acids, thus explaining the increased

Table 1. Ingredients, pH and content of starch in the different bread products

Products	Ingredients	pH	Starch	
			Available (dry wt%)	Resistant (RS)[1](%)
Ordinary baking (40 min, 200°C)				
WMB-ordinary	Wholemeal rye flour, white wheat flour (70:30)	5.8	65.9	3.0 ± 0.2^{a}
Long-time baking (20 h, 120°C)				
WMB	Wholemeal rye flour, white wheat flour (70:30)[2]	5.1	61.6	5.4 ± 0.1^{bd}
WMB-la	Lactic acid	4.3	63.2	6.6 ± 0.5^{e}
WMB-aa	Acetic acid	4.6	61.9	5.7 ± 0.2^{cd}
WMB-m	Malt extract	5.0	60.8	5.1 ± 0.2^{b}
WMB-m-la	Malt, lactic acid	4.3	61.6	6.7 ± 0.3^{e}
WMB-m-aa	Malt, acetic acid	4.6	61.6	5.2 ± 2^{bd}
HAB	Wholemeal high-amylose barley flour, white wheat flour (70:30)	5.1	59.8	7.7 ± 0.3^{f}

[1]Values are given on starch basis (mean ± SD, $n = 3$). Values not sharing the same letters as superscripts are significantly different ($P < 0.05$).
[2]All long-time baked breads (except for HAB) included wholemeal rye flour, white wheat flour (70:30).

RS formation in the bread product baked with added lactic acid (WMB-la) in the present study. Recently, the effect of lactic acid in relation to RS formation could not be shown in bread baked under ordinary conditions, demonstrating an impact of the baking process *per se* (Liljeberg *et al.*, 1995).

When comparing all the long-time baked bread products, the highest RS level (7.7% starch basis) was detected in the bread prepared from high-amylose barley flour, $P < 0.05$ (Table 1). This shows that formation of RS was strongly related to the amylose content. Similarly, higher amounts of RS have been shown in barley porridge (Granfeldt *et al.*, 1994) or Arepa corn bread (Granfeldt *et al.*, 1993) made from high-amylose genotypes, compared with corresponding products prepared from flours with ordinary amylose levels. In the case of the Arepa bread, RS was measured *in vitro* as in the present study, and *in vivo* using antibiotic-treated rats (Granfeldt *et al.*, 1993). From that work it was concluded that the RS analysed with the two different methods were in good agreement. Consequently, although the RS method has limitations, as it includes milling and heat-treatment in the analytical procedure, it is likely that the substantial increase in RS during long-time baking conditions or with high-amylose barley flour in the present study also applies *in vivo*.

Attempts to increase the RS content in bread products have been made by adding defined starch fractions to white bread. Thus, according to Eerlingen *et al.* (1994), 7.7–8.4% *in vitro* RS (dry weight basis) was found in bread products with added high-amylose or extruded retrograded high-amylose starch fractions. However, addition of isolated starch fractions to bread may be less advantagous than optimization of RS content by use of modified composite raw materials or processing conditions, in that addition of pure starch will reduce the nutrient density of the bread.

The rate of hydrolysis of the potentially available starch fraction remained unaffected in most long-time baked breads (WMB, WMB-la, WMB-aa) (Fig. 1 and Table 2), and HI remained similar to that of the white reference bread (WWB) (Table 3). However, as an associated feature, the high RS content in the long-time baked bread made from high-amylose barley flour was accompanied by a reduced rate of hydrolysis of the bulk of starch (HI = 68) ($P < 0.05$). The fact that the rate of starch hydrolysis remained high in all the other bread products suggests that the mechanism for RS formation may differ, and that the formation of RS may or may not affect the availability of the non-RS fraction. Based on previous correlations, the HI in the case of the high-amylose bread (HAB) corresponds to a GI of 67 using the following equation for calculation; $GI = 0.862 \cdot HI + 8.198$ (Granfeldt, 1994). A lowering of the rate of starch hydrolysis is in agreement with results with high-amylose Arepa corn bread (Granfeldt *et al.*, 1995), which also had higher RS content and displayed lowered HI, compared with the corresponding product based on a genotype with an ordinary amylose level. This high-amylose Arepa product also reduced glycaemia in healthy subjects.

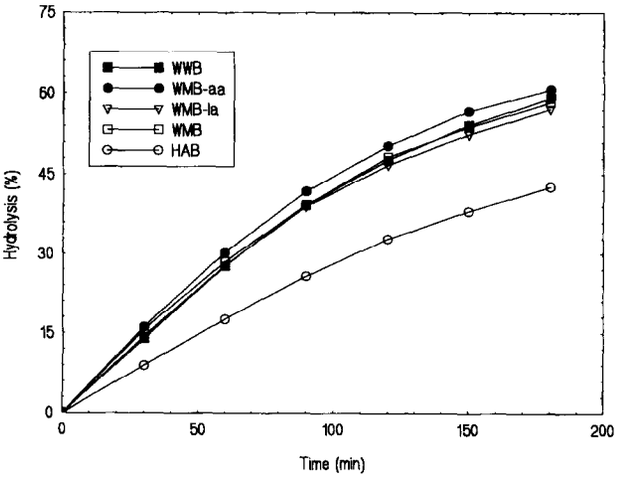

Fig. 1. The rate of *in vitro* starch hydrolysis of the potentially available starch fraction. Values are means, $n = 6$. Error terms are given in Table 2.

Table 2. The percentage of starch hydrolysed within 0–180 min[1]

	30 min	60 min	90 min	120 min	150 min	180 min
Long-time baked breads						
WMB	15.6 ± 0.5^{cd}	28.6 ± 0.7^{bc}	39.3 ± 0.8^{bc}	48.3 ± 0.6^{bc}	53.8 ± 0.5^{b}	58.4 ± 0.5^{bc}
WMB-la	14.4 ± 0.4^{bc}	27.8 ± 0.7^{b}	39.0 ± 0.8^{b}	46.8 ± 0.9^{b}	52.5 ± 0.8^{b}	57.2 ± 0.7^{b}
WMB-aa	16.1 ± 0.4^{d}	30.3 ± 0.8^{c}	41.9 ± 0.9^{c}	50.4 ± 1.1^{c}	56.8 ± 1.1^{c}	60.7 ± 1.1^{c}
HAB	9.0 ± 0.3^{a}	17.6 ± 0.7^{a}	25.8 ± 0.9^{a}	32.9 ± 1.0^{a}	38.1 ± 1.0^{a}	42.7 ± 1.0^{a}
Reference bread						
WWB	14.0 ± 0.2^{b}	27.7 ± 0.3^{b}	39.2 ± 0.4^{b}	47.7 ± 0.5^{b}	54.2 ± 0.5^{b}	59.2 ± 0.5^{bc}

[1]Vaules are means ± SEM, $n = 6$. Values not sharing the same letters as superscripts are significantly different ($P < 0.05$).

Table 3. Hydrolysis rate index (HI)[1]

	HI (%)
Long-time baked breads	
WMB	101.2 ± 2.2^{abd}
WMB-la	98.5 ± 2.1^{ad}
WMB-aa	106.4 ± 3.3^{b}
HAB	68.2 ± 2.1^{c}
Reference bread	
WWB	100^{a}

[1]Values are means ± SEM, $n = 6$. Values not sharing the same letters as superscripts are significantly different ($P < 0.05$).

Results from the present study show that an extended baking process favours formation of RS in bread products. In addition, lactic acid might further promote retrogradation of starch as judged from the increased RS recoveries obtained *in vitro*. Sourdough baking is an old traditionally used process, and its effect on dough rising, bread taste, flavour, texture and shelf-life is well established (Lönner, 1988). The information concerning sourdough fermentation, i.e. lactic acid, in relation to RS formation is, however, new. The highest level of RS was noted in a long-time baked bread based on high-amylose flour. This bread also lowered the rate of amylolysis of the potentially available starch fraction.

It is concluded that the RS content of flour-based bread products could be considerably increased by altering the baking process. Consequently, by changing from conventional baking to low-temperature, long-time baking, the RS content increased from 3 to about 8%, starch basis, the highest yields being obtained when lactic acid or high-amylose barley was included. In light of the quantitative importance of bread, such differences will even affect total RS intake on a mixed diet. Based on a EU-mean bread intake (175 g/day), a switch from conventional bread products to pumpernickel-type breads may increase total RS intake from the recently estimated 4 g/day (Dysseler & Hoffem, 1994) to approximately 7 g/day.

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REFERENCES

Berry, C. S. (1986). Resistant starch: formation and measurement of starch that survives exhaustive digestion with amylolytic enzymes during the determination of dietary fibre. *J. Cereal Sci.*, **4**, 301–314.

Bingham, S. A. (1990). Mechanisms and experimental and epidemiological evidence relating dietary fibre (non-starch polysaccharides) and starch to protection against bowel cancer. *Proc. Nutr. Soc.*, **49**, 153–171.

Björck, I., Nyman, M., Pedersen, B., Siljeström, M., Asp, N.-G. & Eggum, B. (1986). On the digestibility of starch in wheat bread—studies *in vitro* and *in vivo*. *J. Cereal Sci.*, **4**, 1–11.

Cummings, J. H. (1981). Short chain fatty acids in the human colon. *Gut*, **22**, 763–779.

Cummings, J. H., Banwell, J. G., Segal, I., Coleman, N., Englyst, H. N. & Macfarlane, G. T. (1990). The amount and composition of large bowel contents in man. *Gastroenterology*, **98**, A408.

Cummings, J. H. & Englyst, H. N. (1991). Measurement of starch fermentation in the human large intestine. *Physiol. Pharmacol.*, **69**, 121–129.

Dysseler, P. & Hoffem, D. (1994). Estimation of resistant starch intake in Europe. In *Proceedings of the Concluding Plenary Meeting of EURESTA. European FLAIR-concerted Action No. 11 (COST 911)*, pp. 84–85.

Eerlingen, R. C., Van Haesendonck, I. P., De Paepe, G. & Delcour, J. A. (1994). Enzyme-resistant starch. III. The quality of straight-dough bread containing varying levels of enzyme-resistant starch. *Cereal Chem.*, **71**, 165–170.

Englyst, H. N. & Cummings, J. H. (1985). Digestion of the polysaccharides of some cereal foods in the human small intestine. *Am. J. Clin. Nutr.*, **42**, 778–787.

Englyst, H. N. & Cummings, J. H. (1987). Digestion of polysaccharides of potato in the small intestine of man. *Am. J. Clin. Nutr.*, **44**, 423–431.

Englyst, H. N. & Kingman, S. M. (1990). Dietary fibre and resistant starch. A nutritional classification of plant polysaccharides. In: *Dietary Fibre: Chemistry, Physiology and Health Effects*, eds D. Kritchevsky, C. Bonfield & J. A. Andersson. Plenum Press, New York, pp. 49–65.

Englyst, H. N., Kingman, S. M. & Cummings, J. H. (1992). Classification and measurement of nutritionally important starch fractions. *Eur. J. Clin. Nutr.*, **46**(Suppl. 2), S33–S50.

Granfeldt, Y., Björck, I., Drews, A. & Tovar, J. (1992). An *in vitro* procedure based on chewing to predict metabolic response to starch in cereal and legume products. *Eur. J. Clin. Nutr.*, **46**, 649–660.

Granfeldt, Y., Drews, A. & Björck, I. (1993). Starch bioavailability in Arepas made from ordinary or high amylose corn: concentration and gastrointestinal fate of resistant starch in rats. *J. Nutr.*, **123**, 1676–1684.

Granfeldt, Y. (1994). Food factors affecting metabolic responses to cereal products. Ph.D. thesis, University of Lund, Sweden.

Granfeldt, Y., Drews, A. & Björck, I. (1995). Arepas made from high amylose corn flour produce favorably low glucose and insulin responses in healthy humans. *J. Nutr.*, **125**, 459–465.

Granfeldt, Y., Liljeberg, H., Drews, A., Newman, R. & Björck, I. (1994). Glucose and insulin responses to barley products. Influence of food structure and amylose–amylopectin ratio. *Am. J. Clin. Nutr.*, **59**, 1075–1082.

Holm, J. & Björck, I. (1992). Bioavailability of starch in various wheat bread products—evaluation of metabolic responses in healthy subjects and rate and extent of *in vitro* starch digestion. *Am. J. Clin. Nutr.*, **55**, 420–429.

Holm, J., Björck, I., Drews, A. & Asp, N.-G. (1986). A rapid method for the analysis of starch. *Starch/Stärke*, **38**, 294–297.

Hostettler, F., Borel, E. & Devel, H. (1951). Uber die reduktion der 3,5-dinitrosalicylsäure durch zucker. *Helv. Chim. Acta*, **34**, 2132–2139.

Jenkins, D. J. A., Cuff, D., Wolever, T. M. S., Knowland, D., Thompson, L., Cohan, Z. & Prokipch, V. K. (1987*a*). Digestibility of carbohydrate foods in an ileostomate: relationship to dietary fiber, *in vitro* digestibility, and glycemic response. *Am. J. Clin. Nutr.*, **82**, 709–717.

Jenkins, D., Jenkins, A., Wolever, T., Collier, G., Rao, A. & Thompson, L. (1987*b*). Starchy foods and fiber: reduced rate of digestion and improved carbohydrate metabolism. *Scand. J. Gastroenterol.*, **22**, 132–141.

Jenkins, D. J. A., Jenkins, A. L., Wolever, T. M. S., Rao, A. V. & Thompson, L. U. (1986). Fiber and starchy foods: gut function and implication in disease. *Am. J. Gastroenterol.*, **81**, 920–930.

Jenkins, D. J. A., Wesson, V., Wolever, T. M. S., Jenkins, A. L., Kalmusky, S. G., Csima, A., Josse, R. G. & Wong, G. S. (1988*a*). Wholemeal versus wholegrain breads: proportion of whole or cracked grain and the glycaemic response. *Br. Med. J.*, **297**, 958–960.

Jenkins, D. J. A., Wolever, T. M. S. & Jenkins, A. L. (1988*b*). Starchy foods and glycemic index. *Diabetes Care*, **11**, 149–159.

Liljeberg, H. & Björck, I. (1994). Bioavailability of starch in bread products. Postprandial glucose and insulin responses in healthy subjects and *in vitro* resistant starch content. *Eur. J. Clin. Nutr.*, **48**, 151–163.

Liljeberg, H., Granfeldt, Y. & Björck, I. (1992). Metabolic responses to starch in bread containing intact kernels versus milled flour. *Eur. J. Clin. Nutr.*, **46**, 561–575.

Liljeberg, H. G. M., Lönner, C. H. & Björck, I. M. E. (1995). Sourdough fermentation or addition of organic acids or corresponding salts to bread improves nutritional properties of starch in healthy humans. *J. Nutr.*, **125**, 1503–1511.

Lönner, C. (1988). Starter cultures for rye sour doughs. Ph.D. thesis, University of Lund, Sweden.

Muir, J. G., Young, G. P., O'Dea, K., Cameron-Smith, D., Brown, I. L., Brown, I. L. & Collier, G. R. (1993). Resistant starch—the neglected 'dietary fiber'? Implication for health. *Diet. Fiber Bibliogr. Rev.*, **1**, 33–47.

Scheppach, W., Fabian, C., Sachs, M. & Kasper, H. (1988). Effect of starch malabsorption on faecal SCFA excretion in man. *Scand. J. Gastroenterol.*, **23**, 755–759.

Shetty, P. S. & Kurpad, A. V. (1986). Increasing starch intake in the human diet increases fecal bulking. *Am. J. Clin. Nutr.*, **43**, 210–212.

Siljeström, M. & Asp, N.-G. (1985). Resistant starch formation during baking—effect of baking time and temperature and variations in the recipe. *Lebensm. Unters. Forsch.*, **181**, 4–8.

Siljeström, M., Eliasson, A.-C. & Björck, I. (1989). Characterization of resistant starch from autoclaved wheat starch. *Starch/Stärke*, **41**, 147–151.

Wolever, T. M. S. (1990). The glycemic index. *World Rev. Nutr. Diabet.*, **62**, 120–185.