ELSEVIER

Carbohydrate Polymers 43 (2000) 183–193

Carbohydrate Polymers

www.elsevier.com/locate/carbpol

Production of heat-stable, butyrogenic resistant starch

D. Schmiedl, M. Bäuerlein, H. Bengs, G. Jacobasch*

German Institute of Human Nutrition, Department of Food Chemistry and Preventative Nutrition, Arthur-Scheunert-Allec 114-116, D-14558 Bergholz-Rehbrucke, Germany

Received 24 March 1999; received in revised form 21 September 1999; accepted 27 September 1999

Abstract

Butyrate formed by glucose fermentation in the large intestine is both a substrate of the energy metabolism and an important signal metabolite for colonocyte growth. Butyrogenic preparations with a content of 34–94% of resistant starch type III (RS) were produced from 1,4-α-D-glucans with polymer chainlengths of 10–35 glucose units, depending on the retrogradation conditions. The procedure was optimized with respect to various parameters like temperature, polyglucan concentration in the gel, polymer chainlength distribution and fermentability. High concentrations and relatively high recrystallization temperatures (about 25°C) favor the formation of heat-stable RS with T_o and T_p values >110°C. The fermentation of these RS led to in vitro short-chain fatty acids levels (acetate, propionate, butyrate) of 2000–2500 μmol/g faeces dry weight with butyrate contents of 30–60 mol%. Readily fermentable heat-stable RS, which can be used in various "functional foods", can be produced from poly-1,4-α-D-glucan of optimal chainlength.

Keywords: Heat-stable; Butyrogenic resistant starch; Glucan length; short chain fatty acids

1. Introduction

High luminal butyrate levels in the large intestine have a protective effect on the intestinal membrane structure and barrier function (Jacobasch, Schmiedl & Schmehl, 1997; Kleessen, Stoof, Proll, Schmiedl, Noack & Blaut, 1997).

Butyrate is formed by bacterial fermentation from glucose. Given present-day eating habits, a possible means of obtaining adequate amounts of the substrate is to use resistant starches (RS) type III. They arise during recrystallization (retrogradation) of gelatinized starch with the formation of microcrystalline filaments, the so-called double helices from amylose chains and from the short A chains of amylopectin. These double helices can aggregate. During this process a partially crystalline network forms. The reformed partial crystallinity prevents the enzymatic hydrolysis of the starch polymers, i.e. the starch polymers are resistant to degradation by α-amylase (Englyst, Kingman & Cummings, 1992).

Russel, Berry and Greenwell (1989) reported that RS can be formed by the retrogradation of gelatinized wax corn starch debranched by pullulanase. The retrogradation of gelated, enzymatically debranched waxy maize starch gave rise to resistant linear α-glucans with a low degree of polymerization (DP ≤ 40). The resistant starch content of the retrograded product was 12%. In contrast, branched α-glucans of linterized waxy maize starch also formed only small amounts of RS after gelatinization.

Cairns, Sun, Morris and Ring (1995) described the effect of various retrogradation conditions on the properties and content of α-amylase-resistant structures in amylose gels and films. The retrogradation of long-chain amylose from pea starch under the conditions of excess water gave rise to RS contents of between 30 and 50%, with the DP of the resistant structures amounting to between 60 and 70 glucose units.

Further, Pomeranz and Sievert (1990) showed that by repeated autoclaving and subsequent cooling of an amylose-rich corn starch (Hylon VII), the RS content can be increased from 20 to about 35%.

Much higher resistant amounts were achieved by retrogradation of a debranched maltodextrin from potato starch (Kettlitz, Anger, Moorthamer & Stoof, 1994). Maltodextrins are starch hydrolysis products with a wide distribution of maltooligomeric and polymeric α-D-glucans. A maltodextrin can be characterized in greater detail using the method of anion exchange chromatography (Schmiedl, 1995). Partially debranched maltodextrins of potato starch are composed of a chromatographically separable and a non-separable polymer fraction. Both fractions are polymer

* Corresponding author.

0144-8617/00/$ - see front matter
PII: S0144-8617(00)00147-8

mixtures. The aim of debranching the maltodextrin was to release linear, low molecular, recrystallizable polymer chains. Thus recrystallization at 25°C of a 30% maltodextrin gel gave rise to an RS content of up to 65%, when the maltodextrin had been previously debranched with an isoamylase (Kettlitz et al., 1994).

Chromatographic investigations demonstrated that about 22% of this resistant starch product consisted of oligomers (up to DP 10) and higher contents of low molecular polymers with DPs of between 10 and 35 glucose units. These low molecular polymers represent a particularly suitable starting product for the production of readily fermentable RS with a high butyrate yield. Intensive studies of a largely linear maltodextrin preparation (Mdx01) showed that oligosaccharides present in high concentrations adversely affect the formation of resistant structures. A reduction in the oligosaccharide content led to a pronounced acceleration of the formation of resistant structures, during which process RS contents of up to 56% were achieved within 24 h (Schmiedl, 1995).

Using the enzyme amylosucrase it is possible to produce poly-1,4-α-D-glucans which contain large amounts of linear polymer chains of optimum length (Büttcher, Welsh, Willmitzer & Kossmann, 1997).

The aim of the study presented here was to clarify the effect of various process parameters including retrogradation temperature and glucan content of the gel and the influence of polymer chainlength distribution of the poly-1,4-α-D-glucan preparations studied on the resistant starch content and the heat stability of α-amylase-resistant structures. In addition, the fermentability of the α-amylase-resistant structures isolated was investigated with human intestinal microflora and compared with that of Novelose (National Starch).

2. Materials and methods

2.1. Materials

Starch UV test: Boehringer-Mannheim, Germany. Pancreatin from porcine pancreas 350 FIP Units/g (activated) protease, 6000 FIP Units/g lipase, 7500 FIP Units/g amylase (E.Merck, Darmstadt, Germany). Amyloglucosidase solution (*Aspergillus niger*) 6100 Units/ml (Sigma Chemical & Co., St Luis, USA).

2.1.1. Enzyme solution I

0.138 ml amyloglucosidase solution were diluted to 6 ml with deionized water and mixed.

2.1.2. Enzyme solution II

12 g of porcine pancreatin were suspended in 80 ml deionized water for 10 min at a temperature of 37°C (shaking water bath). The suspension was centrifuged for 10 min at 1500 g. 54 ml of the supernatant were transferred into a glass flask with a stopper, and the remainder was discarded.

2.1.3. Enzyme solution III

Preparation was carried out immediately before use according to Englyst et al. (1992). 4 ml deionized water and 6 ml enzyme solution I were added to 54 ml enzyme solution II and mixed.

2.1.4. Other used materials

0.1 M acetate buffer pH 5.2.
0.1 M buffer according to Sörensen pH 6.5.
Helium.
Sterile, sealable cryo tubes.
Hermetically sealable sample crucibles, 70 μl, silver.
Poly-1,4-α-D-glucan preparations: “A”, “B”, “C”.

2.2. Methods

2.2.1. Preparation of poly-1,4-α-D-glucans

Three poly-1,4-α-D-glucan preparations were obtained by in vitro synthesis with amylosucrase according to the method described by Büttcher et al. (1997) in aqueous solution with different sucrose concentrations.

The biotransformations were started by adding an enzyme extract containing the amylosucrase of *Neisseria polysaccharea* followed by incubation at 37°C over several hours. During the incubation a white poly-1,4-α-D-glucan precipitates. When the conversion of sucrose to polyglucans had reached 90% the reaction was stopped by heating to 95°C. After centrifugation the pellet was washed twice with cold tap water and dried.

2.2.2. Preparation of resistant starch

The 10 and 30% (w/w) aqueous polyglucan suspensions of preparations A, B and C, respectively were completely dissolved by boiling briefly (3 min) and then autoclaving (120°C) for 20 min. The solutions were cooled down to 25°C (water bath) and 4°C (Cryostat) and retrograded for 24 h at these temperatures by leaving them to stand. The preparations were frozen overnight at −21°C and then lyophilized.

The samples were stored in closed containers at room temperature until required for further investigations.

2.2.3. Investigation of the RS content

Resistant starch was determined by using the methods of Englyst et al. (1992). 200 mg of RS-containing product were mixed with 15 ml of buffer (pH 5.2) and 5 ml of enzyme solution III and incubated for 120 min at 37°C (shaking water bath). After that the reaction mixture was cooled to room temperature and four times the amount (v/v) of ethanol were added. The closed container was allowed to stand for 1 h at room temperature and then it was centrifuged for 10 min (1500 g). The supernatant was removed

with a pipette. The pellet was washed once with absolute ethanol and then twice with 80% ethanol (v/v). Following lyophilization the starch content of the pellet was determined using the Boehringer starch UV test.

2.2.4. High performance anion exchange chromatography

10 mg (dry weight) of polyglucan were dissolved in 1 ml of 0.2 M sodium hydroxide solution and analyzed by HPAE chromatography (high performance anion exchange chromatography) (Fa. DIONEX) on a Carbopak PA1 9×250. The optical activity of chiral molecules was detected by a CHIRALYSER (manufactured by IBZ Messtechnik GmbH, Germany)).

The following solutions were used for the mobile phase.

Solution A: 150 mM sodium hydroxide solution
Solution B: 150 mM sodium hydroxide solution in 1 M acetate solution.

Before the solutions were prepared the ultrapure water was gassed with helium for 30 min. Similarly, all the other solutions were continuously gassed with helium. The flow rate of the mobile phase was 0.8 ml/min. HPLC pump 420, HPLC gradient former, HPLC autosampler 465 and DATA System 450TM2DAD manufactured by Kontron Instruments.

Gradient program: 0 min, $B = 16\%$; 5 min, $B = 23\%$; 20 min, $B = 31\%$; 45 min, $B = 35\%$; 60 min, $B = 40\%$; 65 min, $B = 45\%$; 80 min, $B = 100\%$; 82 min, $B = 100\%$; 85 min, $B = 16\%$; 105 min, $B = 16\%$. Detector setting: range 25.6 m°, average: 10.

2.2.5. DSC measurements of the retrograded products

The heat stability of products containing resistant starch was characterized using dynamic differential scanning calorimetry (DSC) in a DSC 120 system manufactured by Seiko Instr. The melting behavior of indium was used for the calibration (*onset temperature*, $T_o = 158.8$°C; peak temperature, $T_p = 159.6$°C; conversion enthalpy, $\Delta H = 20.7$ mJ/mg). 5 mg (dry weight) of the sample were weighed into silver containers and four times the amount of water (20 mg) was added (glucan/water = 1/5). A silver vessel containing water was used as the reference.

The DSC studies were carried out in a range of 10–200°C with a heating rate of 4°C/min. The T_o, T_p and (ΔH were determined using Seiko software. Duplicate determinations were carried out.

2.2.6. In vitro fermentation of RS

1 ml of a 5% faecal suspension (15 g of freshly taken human faeces, 50 ml of Sörensen buffer, pH 6.5) was mixed and homogenized with 10 mg (dry weight) of RS in sealed cryo tubes gassed with nitrogen; the 10 mg of RS had been isolated from the hydrolysis batch by Englyst et al. (1992). Fermentation was carried out at 37°C. Samples were taken at hourly intervals and immediately frozen at −21°C.

2.2.7. Determination of SCFA by means of gas chromatography

The concentration of short-chain fatty acids (SCFA) was measured in a faecal suspension by gas chromatography on a capillary column (Carbowax 20M) using a temperature program. The GC system was an HP 5890 Series II Station with an HP 7673 GC/SFC injector, HP GC autosampler controller, detector FID; software—HP Chemstation. Helium was used as the mobile phase.

200 mg of the faecal suspension were suspended and homogenized in four times the amount of water. A dilute faecal suspension was obtained. Part of this faecal suspension was used for the determination of the dry mass. 500 mg of the faecal suspension were centrifuged.

100 μl of the supernatant were mixed with 25 μg of isobutyrate (internal standard), 280 μl of 0.36 M of perchloric acid and 270 μl of 1 M sodium hydroxide solution. The sealed container with a pierced lid was placed in liquid nitrogen and freeze-dried. The dried sample was mixed with 100 μl of 5 M formic acid and 400 μl of acetone and shaken on a Vortex. The organic phase that formed was decanted into the vials of an autosampler, which were immediately sealed. 1 μl from each of these was injected into the gas chromatograph.

Acetic acid propionic acid, butyric acid, isobutyric acid, valeric acid and isovaleric acid from Supelco were used as external standards.

3. Results and discussion

3.1. Effect of the retrogradation conditions on the RS content

Three poly-1,4-α-D-glucan preparations (preparation: "A", "B", "C"), which had been obtained by in vitro synthesis with amylosucrase were investigated. Figs. 1–3 show the chromatograms of these poly-1,4-α-D-glucan preparations. The polyglucan preparations studied demonstrated a varying polymer chain distribution in the chromatograms. Admittedly, the polyglucan preparations "A", "B" and "C" investigated contained polymers capable of retrogradation with a DP of between 10 and 35 glucose units, but their relative composition varied. Preparation "A" had a relatively higher polymer content with DP 10–20 (Gaussian distribution of the peaks shifted to the left), whereas preparation "B" demonstrated a relatively higher polymer content with DP 20–35 (Gaussian distribution of the peaks shifted to the right). Preparation "C" displayed no Gaussian distribution in the chromatographic investigations.

In order to obtain RS products with a high proportion of resistant structures and high physiological efficacy, retrogradation studies were carried out with these preparations, the retrogradation conditions (solids content, temperature) being deliberately varied.

10 and 30% (w/w) gels of the polyglucans A, B and C,

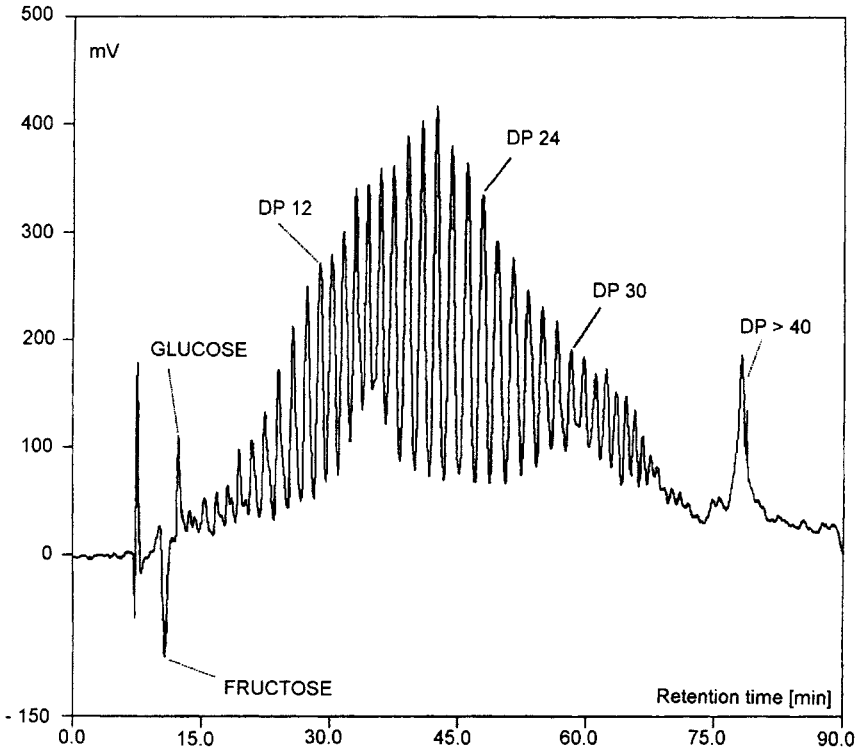

Fig. 1. Chromatogram of polyglucan "A". Column: Carbopak PA 1, 9 × 250; Detector: Chiralyser.

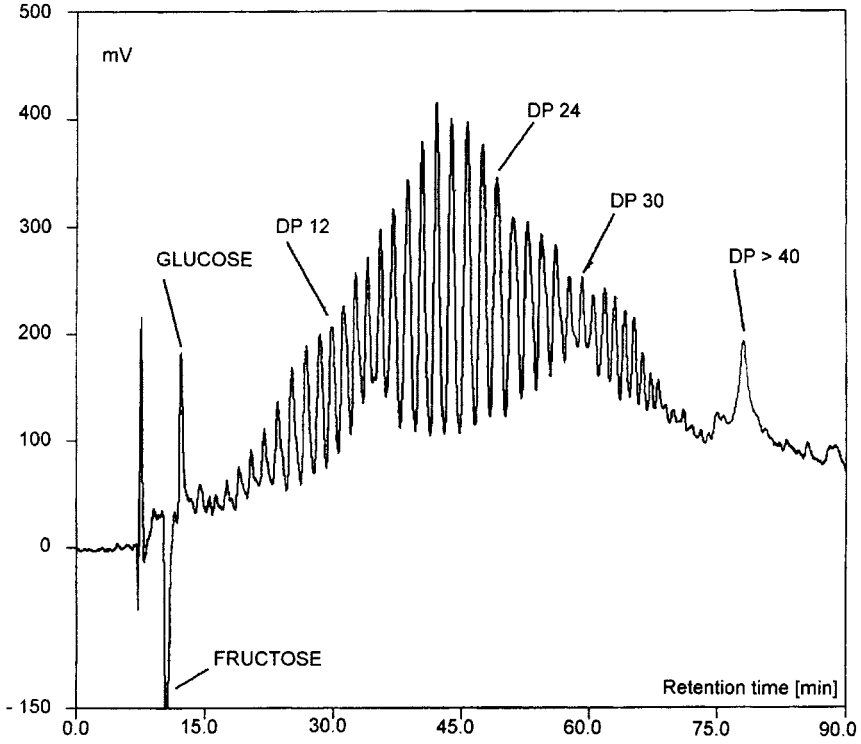

Fig. 2. Chromatogram of polyglucan "B". Column: Carbopak PA 1, 9 × 250; Detector; Chiralyser.

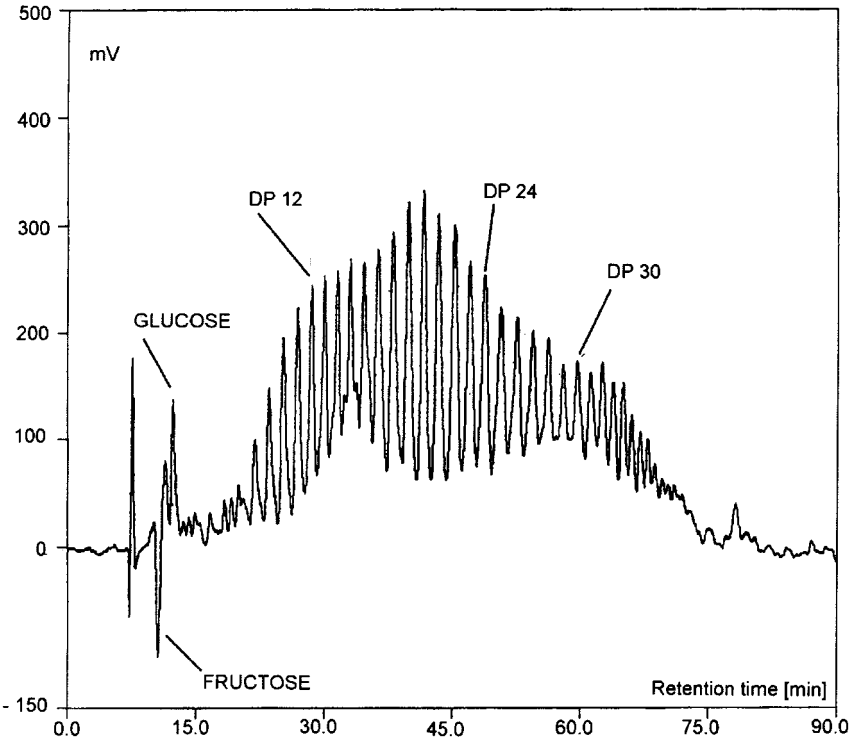

Fig. 3. Chromatogram of polyglucan "C". Column: Carbopak PA 1, 9 × 250; Detector; Chiralyser.

respectively were retrograded at 25 and 4°C for up to 24 h and then lyophilized. Figs. 4–6 illustrate the temporal course of the formation of RS in 30% gels of the preparations A, B and C at 25 and 4°C.

The results clearly show firstly that the rate of RS formation is very rapid in all three mixtures, because 50 and 65% of resistant structure are achieved after only 2 h retrogradation.

Secondly, it can be seen that the formation of α-amylase-resistant structures is virtually completed after just 2 h in the gels of preparations A and C and an extension of the retrogradation time up to 22 h does not lead to a marked increase in the RS content. On the contrary, the proportion of α-amylase-resistant structures rises with time in sample B from about 65 to approximately 90%.

Investigations into heat stability using dynamic DSC and into the content of resistant structures were performed in the retrograded, lyophilized products. Table 1 shows the retrogradation conditions and the content of resistant starch.

From the information given in Table 1 and in Figs. 4–6 it can be concluded that RS products with a high RS content can be obtained by recrystallization of the poly-1,4-α-D-glucans. The RS content that can be obtained depends on both the retrogradation conditions and the polyglucan characteristic.

Whereas the retrogradation of polyglucan "A" within 24 h at temperatures of 4 and 25°C and with glucan concentrations of 10 and 30% increased only to an average RS content of about 65%, the formation of resistant structures in the gel of "B" was substantially affected by these

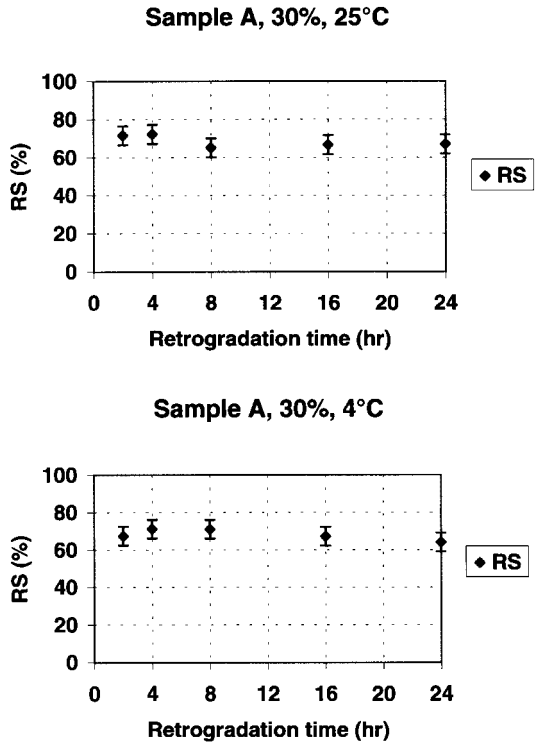

Fig. 4. Time dependence of the formation of RS in 30% gels of polyglucan "A" at a storage temperature of 25°C (above) and 4°C (below) up to a maximum storage time of 24 h.

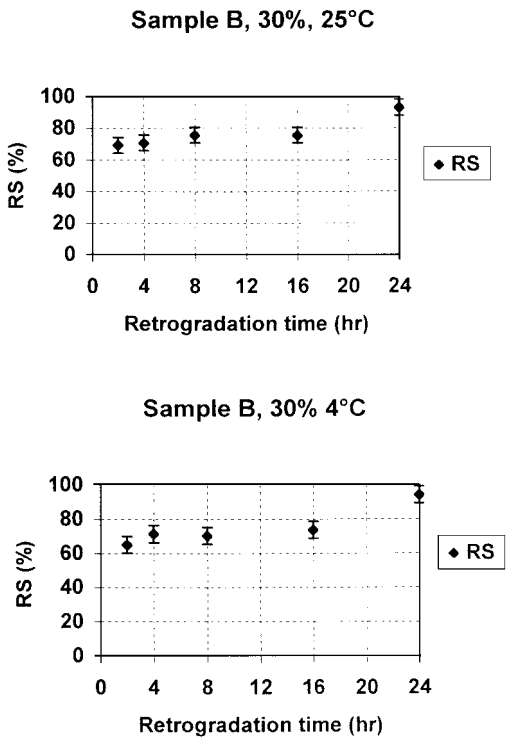

Fig. 5. Time dependence of the formation of RS in 30% gels of polyglucan "B" at a storage temperature of 25°C (above) and 4°C (below) up to a maximum storage time of 24 h.

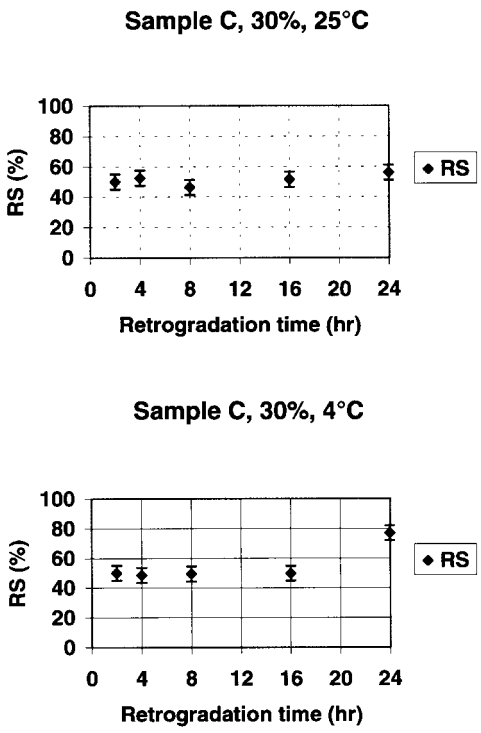

Fig. 6. Time dependence of the formation of RS in 30% gels of polyglucan "C" at a storage temperature of 25°C (above) and 4°C (below) up to a maximum storage time of 24 h.

retrogradation conditions. Much higher RS contents (87%) occurred in 10% gels at a temperature of 25°C than at 4°C (70%). In a glucan gel of 30%, 94% RS was found.

Whereas the retrogradation of polyglucan "C" at 4°C with an increase in the glucan concentration to 30% led to an increase in the RS content from 38 to 77%, an average RS content of 59% was obtained by retrogradation of 10% gels at a temperature of 25°C. It was not always found that an increase in the glucan concentration increases the RS content of the product. Generally an extremely high α-amylase-resistant starch proportion of up to 94% can be obtained by retrogradation of hot, aqueous poly-1,4-α-D-glucan gels in a temperature interval of 4–25°C and with a glucan concentration of up to 30% in the gel. Despite similarities in the polymer distributions of the polyglucans A, B, C (Figs. 1–3) structural differences are assumed to be responsible for their varying retrogradation behavior.

It can be concluded that RS rich products of high quality

Table 1
Effect of retrogradation conditions (storage temperature, gel concentration) on the resistant starch content

Sample	Retrogradation condition		RS (%)
	Storage temperature (°C)	Gel concentration (%)	RS ± SD, $n = 5$
A	− 70	–	34 ± 8
	4	10	67 ± 9
		30	64 ± 9
	25	10	63 ± 7
		30	67 ± 2
B	− 70	–	78 ± 4
	4	10	70 ± 2
		30	94 ± 2
	25	10	87 ± 1
		30	93 ± 1
C	4	10	38 ± 4
		30	77 ± 3
	25	10	59 ± 3
		30	56 ± 6

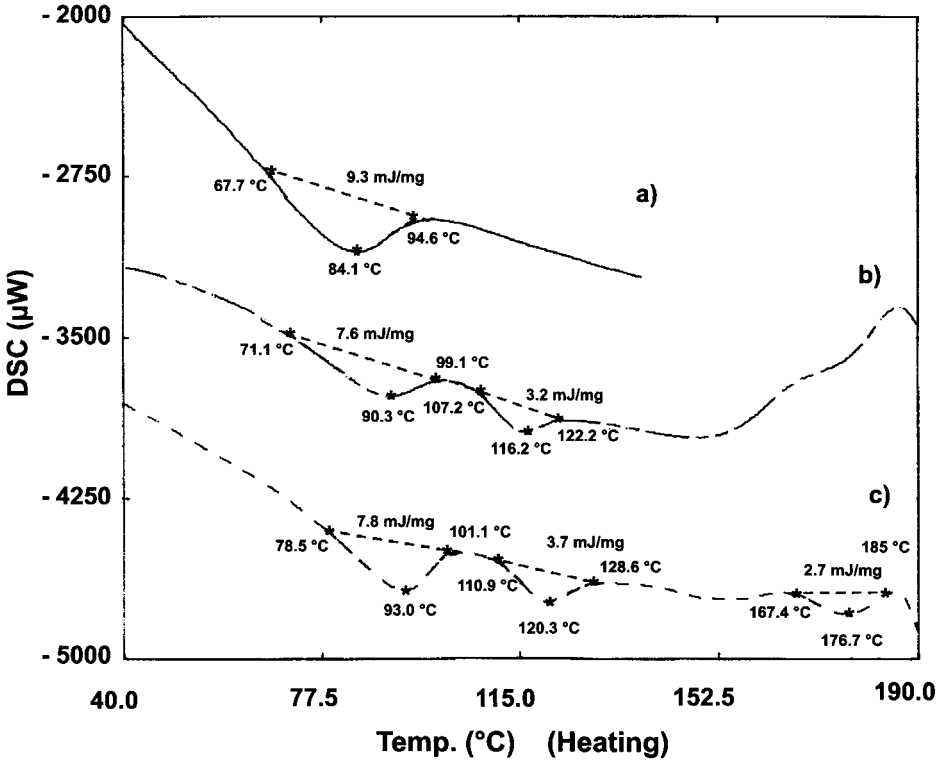

Fig. 7. DSC thermograms of lyophilized polyglucan "A" (sample (dry weight)/water = 1/5, (w/w)): (a) original; (b) after 2 h; and (c) 24 h retrogradation at 25°C.

can be obtained by standardized, reproducible synthesis of glucans with defined chainlengths as the starting material. The consumption of an almost completely α-amylase-resistant product is nutritionally advantageous because it is associated with a very low energy uptake.

3.2. Effect of retrogradation on the heat stability of the end products

DSC measurements made on granular starches and starch polymers is excess water usually produce endotherms with distinct peaks. These endothermic peaks are characterized more closely by the parameters T_o, T_p, T_c, and ΔH. The onset temperature T_o indicates the start of thermal conversion. The value for T_p shows the temperature at which maximum thermal conversion of the crystalline material takes place, whereas T_c represents the temperature at which the conversion process is completed. The conversion enthalpy ΔH is determined by calculating the peak area. It represents the total energy needed for the transformation. Results of DSC measurements were used to characterize the thermal stability of RS products.

Figs. 7–9 show endotherms which were recorded by means of DSC of the original preparations (index a) and of retrograded samples (index: b): retrogradation time 2 h, (c) retrogradation time 24 h. The retrogradation conditions (30% gel (w/w)) and the temperature (25°C) are identical for the three preparations. The enthalpy values given are based on the dry weight.

From the endotherms which are given index (a), it becomes clear that a partially crystalline portion is present in the starting material itself. The thermal stability however is very low. Heating for a short time and subsequent autoclaving bring about complete dissolution of 10 and 30% (w/w) aqueous suspensions, respectively. It can therefore be assumed that at time $t = 0$ of the retrogradation process only a very small to insignificant part of the sample is present in crystalline form. The endotherms with the indices (b) and (c) reflect the conversion behavior of retrograded (2 h, 24 h) samples.

A common feature of these endotherms is that even when the measurement is carried out with an excess of water (sample/water = 1/5) at least two peaks occur. Another feature common to all the samples is that as the retrogradation time is extended from 2 to 24 h the total enthalpy ($\Delta H_{total} = \sum \Delta H_i$; sum of area of all the peaks) increases. ΔH_{total} of the retrograded samples A, B and C show the following trend after 24 h retrogradation:

$$\Delta H_{total\ B} > \Delta H_{total\ A} > \Delta H_{total\ C}\ (28.7\ mJ/mg > 14.2\ mJ/mg > 10.3\ mJ/mg).$$

This trend correlates very closely to that for the RS content

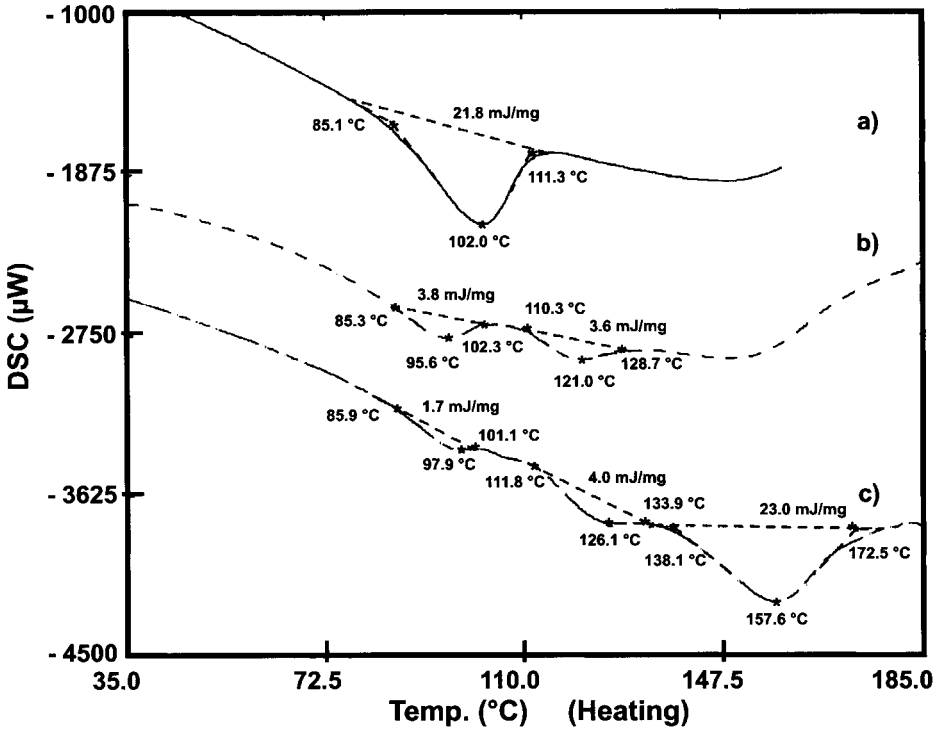

Fig. 8. DSC thermograms of lyophilized polyglucan "B" (sample (dry weight)/water = 1/5, (w/w)): (a) original; (b) after 2 h; and (c) 24 h retrogradation at 25°C.

Table 2
Effect of retrogradation conditions (storage temperature, gel concentration) on DSC parameters (T_o, T_p, T_c and ΔH)

Sample	Retrogradation conditions		DSC parameter			
	Storage temperature (°C)	Gel concentration (%)	T_0 (°C)	T_p (°C)	T_c (°C)	ΔH (mJ/mg)
A	−70	–	67.7	84.1	94.6	9.3
		10	78.1	93.4	103.9	13.2
	4		72.6	94.3	102.3	7.9
		30	111.2	120.3	128.9	4.8
			137.7	148.8	172.3	12.4
		10	84.9	98.4	107.0	12.6
	25		78.5	93.0	101.1	7.8
		30	110.9	120.3	128.6	3.7
			167.4	176.7	ca. 185	2.7
B	−70	–	85.1	102.0	111.3	21.8
		10	81.8	96.7	108.0	16.3
	4		86.2	98.2	103.7	1.8
		30	109.3	124.5	136.8	13.3
			142.7	ca. 150	165.6	2.9
		10	88.6	101.0	108.0	15.0
	25		85.9	97.9	101.1	1.7
		30	111.8	126.1	133.9	4.0
			138.1	157.6	172.5	23.0
C	−70	–	68.5	84.9	97.3	7.2
		10	73.8	91.0	101.7	13.7
	4	30	70.1	90.7	98.4	6.0
			107.6	117.4	123.4	3.2
			123.7	128.0	133.7	0.4
		10	83.7	97.7	105.2	12.2
	25	30	75.8	91.7	101.4	9.3
			112.0	119.9	125.0	1.0

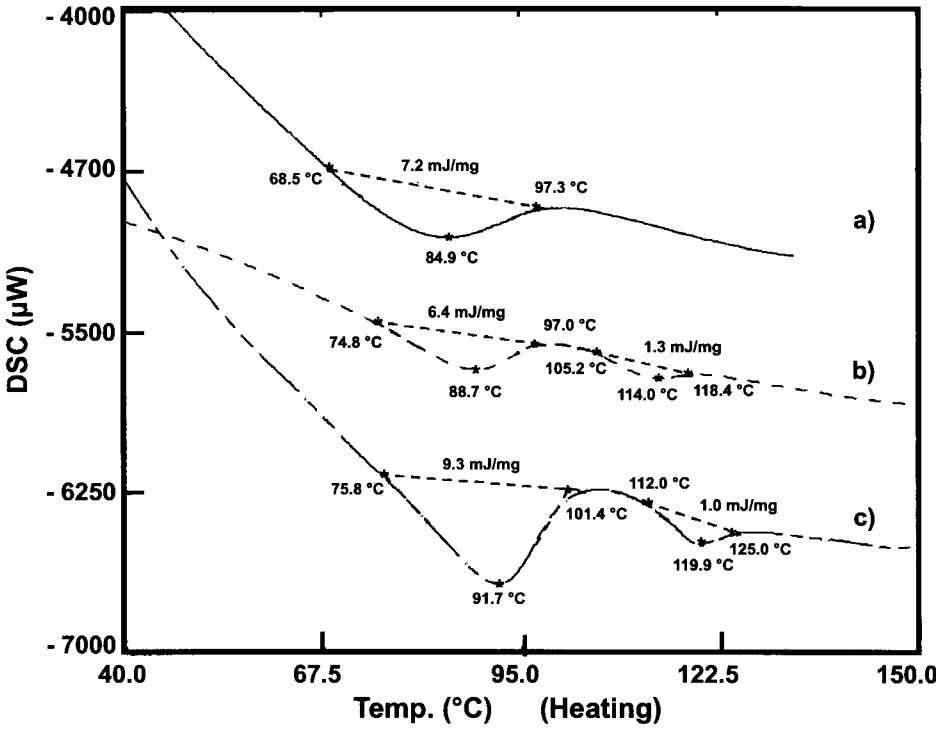

Fig. 9. DSC thermograms of lyophilized polyglucan "C" (sample (dry weight)/water = 1/5, w/w): (a) original; (b) after 2 h; and (c) 24 h retrogradation at 25°C.

of the samples A, B and C (see Table 1):

$[RS_B] > [RS_A] > [RS_C]$ (93% > 67% > 56%)

To discern a trend for partial crystallinity from these findings would be purely speculative however, as the DSC reaction enthalpy ΔH is influenced not only by the crystalline proportion of the sample but also by the length of crystalline areas. Nevertheless, X-ray crystallography investigations may help to clarify this matter. They are at present in progress.

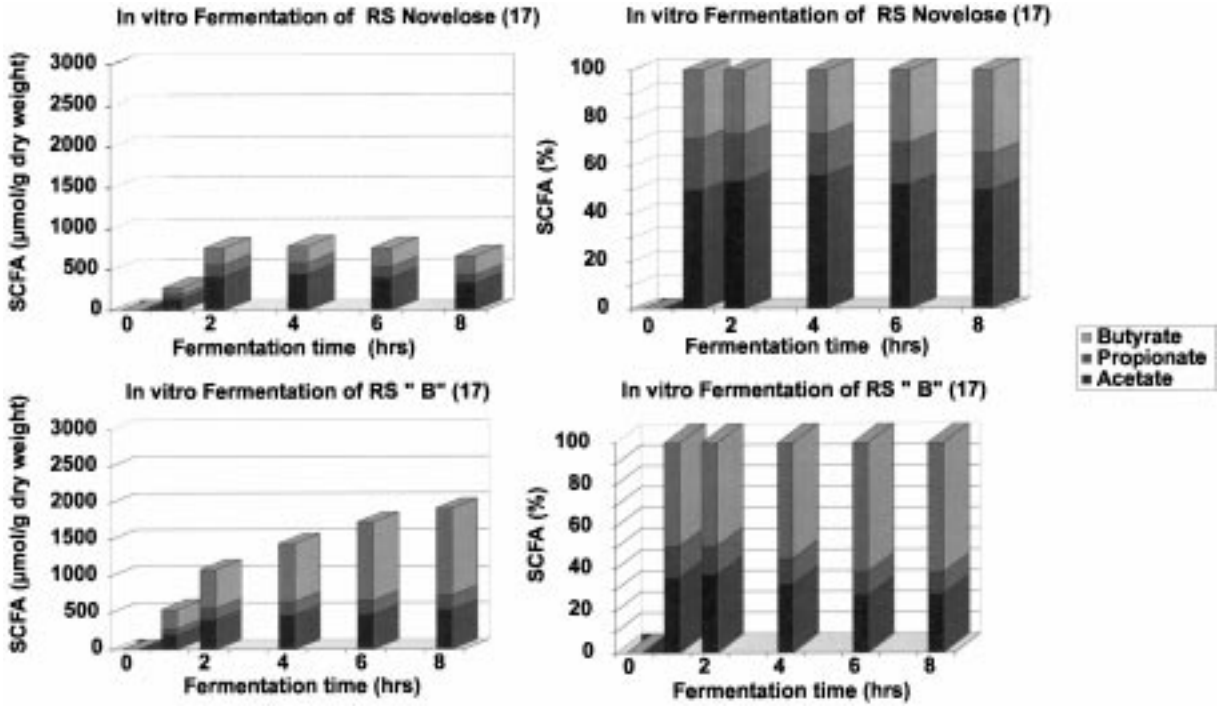

Fig. 10. Formation rate and SCFA spectrum of the short-chain fatty acids acetic, propionic and butyric acid as a result of fermentation of RS, isolated from Novelose (above) and polyglucan "B" (below), with fresh human flora from the large intestine of subject 17.

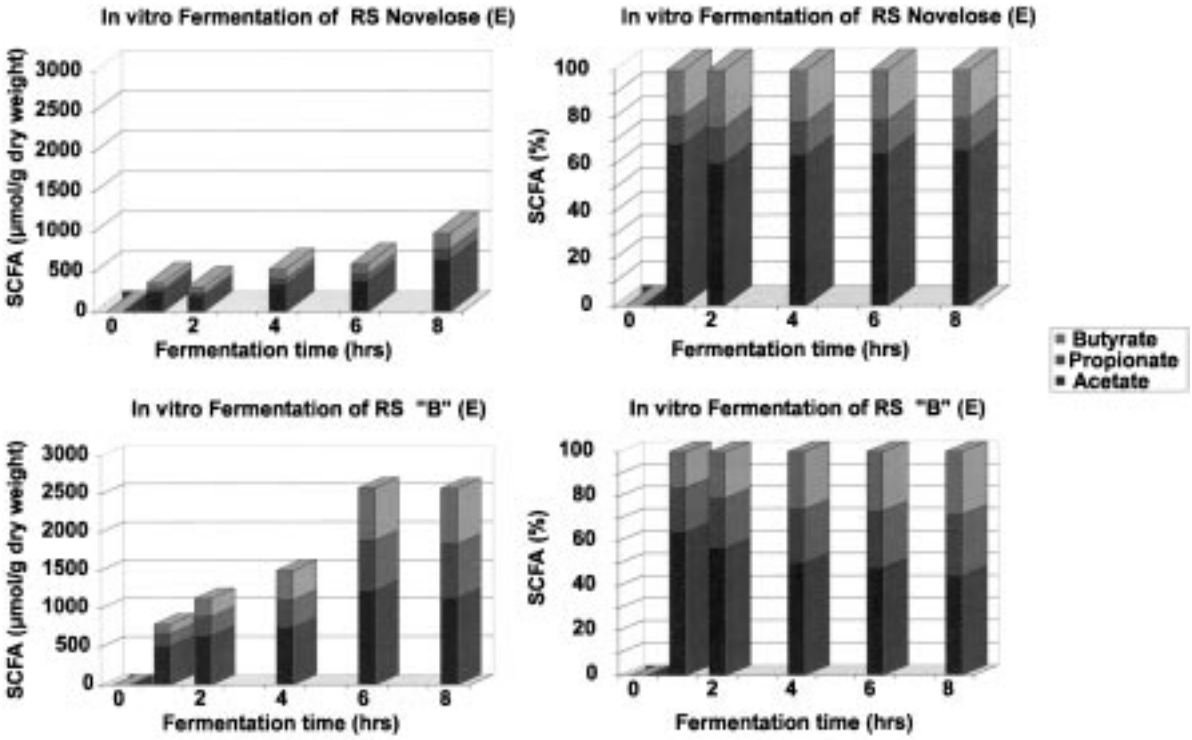

Fig. 11. Formation rate and SCFA spectrum of the short-chain fatty acids acetic, propionic and butyric acid as a result of fermentation of RS, isolated from Novelose (above) and polyglucan "B" (below), with fresh human flora from the large intestine of subject E.

Table 2 gives an overview of the DSC parameters that can be achieved as a function of the retrogradation conditions. From the results of the calorimetric studies presented in Table 2, it can be postulated that the retrogradation conditions affect not only the α-amylase resistant content but also the thermal stability of the retrograded product. In particular, an increase in the recrystallization temperature led to a rise in the heat stability of the end products. Compared with the DSC values after recrystallization at 4°C, the DSC parameters T_o and T_p were shifted to higher values by retrogradation of the glucans at 25°C. Furthermore, an increase in the concentration from 10 to 30% in the gel brought always an increase in the heat stability of the retrograded products. The endotherms recorded using DSC showed more than one peak. These additional peaks are characterized by T_o and T_p values of $\geq$110°C.

The increased heat stability is of particular interest as RS products of this type can probably also be used in bakery and extrusion products. These findings demonstrate that it is possible to produce relatively heat-stable RS products, which contain very large amounts of low molecular linear polymers. The increase in heat stability opens up outlets for the use of the newly developed RS preparation, which were hitherto not considered possible. It can be subjected to short cooking and baking times.

3.3. In vitro fermentation of α-amylase resistant starch

Starch polysaccharides and cell wall polysaccharides, which are resistant to breakdown by host enzymes, reach the large intestine and serve the bacteria there as a substrate for the generation of energy. During the fermentation of indigestible polysaccharides, volatile carboxylic acids, the so-called SCFA, are formed in addition to gases (hydrogen, methane and carbon dioxide). The main representatives of these are acetic acid, propionic acid and butyric acid. It is well known that the chemical and physical structure of the polysaccharides, which can be used as substrates, affect the rate and extent of fermentation, as well as the profile of the short-chain fatty acids formed. Only a few species of bacteria, whose growth is promoted by glucose, are capable of forming butyrate. A continuous carbohydrate intake into the large intestine therefore ensures a high luminal butyrate level and the associated trophic effects on the structure of the mucous membrane of the large intestine. Our own studies show that the resistant starches of types II and III, which are included amongst the ballast materials, demonstrate varying fermentation behavior (Jacobasch et al., 1997). Responsible for this is the chemical and physical structure, particularly the polymer chain distribution in the resistant part. Fermentation studies in isolated α-amylase resistant starches substantiate these findings.

Fig. 10 illustrates the fermentation behavior of Novelose resistant starch, a RS product based on retrograded amylomaize starch (National Starch & Chemical, USA). Contrasted with this RS product is the fermentation behavior of isolated resistant starch of the retrograded polyglucan preparation "B". In each case the fermentation of the

resistant starches was carried out in parallel in duplicate samples of fresh human faecal flora taken from the same subject. The fermentability of the RS products used differs in terms of both the formation rates of SCFA and their SCFA spectra. As a result of in vitro fermentation of the RS of polyglucan "B", markedly higher SCFA and butyrate levels were obtained in comparable fermentation periods. Whereas about 2000 μmol SCFA/g dry weight with a molar butyrate content of about 60% were formed by 8 h fermentation of the RS isolated from polyglucan "B", only about 750 μmol SCFA/g dry weight were obtained by fermentation of Novelose. At 35%, the butyrate content was also much lower.

As a result of the bacterial fermentation of resistant starch of the poly-1,4-α-D-glucan, more butyrate is produced in vitro than by that of Novelose. The total quantity of SCFA and also the molar content of butyrate differ for subjects whose bacterial flora are not identical. This applies to many subjects because there are marked individual differences in eating habits. The results given in Figs. 10 and 11 demonstrate this by means of two examples. The microflora of each of the two subjects however ferment RS preparation "B" better than Novelose.

Irrespective of individual differences, it can be clearly shown that the fermentation of resistant starches with high contents of low molecular polymers compared with conventional RS preparations based on retrograded amylose-rich starch gives rise to significantly higher butyrate levels.

These results in Figs. 10 and 11 document the effect which can be achieved with the newly developed RS preparations at a specific microflora level.

The effectiveness of butyrate formation with high quality RS preparations, such as those presented here for the first time becomes all the greater, the more butyrate formers in the intestinal microflora dominate as a result of the intake of carbohydrates. The results presented in this report show that it is possible to produce heat-stable, readily fermentable RS preparations with a yield of almost 100%, which are ideal for use in health-promoting high quality functional food products.

References

Büttcher, V., Welsh, T., Willmitzer, L. S., & Kossmann, J. (1997). Cloning and characterization of the gene for amylosucrase from *Neisseria polysaccharea*: production of linear α-1,4-glucan. *J. Bacteriol.*, *179*, 3324–3330.

Cairns, P., Sun, L., Morris, V. J., & Ring, S. G. (1995). Physicochemical studies using amylose as an in vitro model for resistant starch. *Journal of Cereal Science*, *21*, 37–47.

Englyst, H. N., Kingman, S. M., & Cummings, J. H. (1992). Classification and measurement of nutritionally important starch fractions. *European Journal of Clinical Nutrition*, *46* (2), S33–S50.

Jacobasch, G., Schmiedl, D., & Schmehl, K. (1997). Darmkrebsprävention durch resistente Stärke? Teil l: Diekolorektale Kanzerogenese: molekulare Ursachen, Manifestation polypöser und nichtpolypöser Karzinome, Veränderungen der Dickdarmkryptenstruktur bei Defekten des apc1-Gens. *Ernährungs-Umschau*, *44*, 318–326.

Jacobasch, G., Schmiedl, D., & Schmehl, K. (1997). Darmkrebsprävention durch resistente Stärke? Teil ll: Stoffwechselproduckte der intestinalen Mikroflora, deren Beeinflussung durch resistente Stärke (RS) sowie deren Wirkung auf die Dickdarmschleimhaut und die kolorektale Kanzerogenese. *Ernährungs-Umschau*, *44*, 369–373.

Kettlitz, B., Anger, H., Moorthamer, E. & Stoof, G. (1994). Patent specification: EP 0688872A1.

Kleessen, B., Stoof, G., Proll, J., Schmiedl, D., Noack, J., & Blaut, M. (1997). Feeding Resistant Starch Affects Fecal and Ceacal Mircoflora and Short-Chain Fatty Acids of Rats. *Journal of Animal Science*, *75*, 2453–2462.

Pomeranz, Y. & Sievert, D. (1990). Patent specification: WO 90/15147.

Russel, P. L., Berry, C. S., & Greenwell, P. (1989). Characterization of resistant starch from wheat and maize. *Journal of Cereal Science*, *9*, 1–15.

Schmiedl, D. (1995). *Zum Einfluß der Retrogradation von Stärken und Stärkehydrolyseprodukten auf die Bildung und Struktur resistenter Stärke*. Dissertationsschrift A, Fachbereich Lebensmittelwissenschaft und Biotechnologie der Technischen Universität Berlin, Bundesrepublik Deutschland.