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Characterization of Resistant Starch Type III from Banana (*Musa acuminata*)

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Banana starch (*Musa acuminata* var. *Nandigobe*) was evaluated for its use in generating resistant starch (RS) type III. Structural, physicochemical, and biological properties of these products were analyzed. The investigated process includes debranching of the native starch and retrogradation under different storage temperatures and starch concentrations. After enzymatic debranching, a high amount of low-molecular-weight polymers with a degree of polymerization between 10 and 35 glucose units beside a higher molecular weight fraction were found. The resulting products comprised RS contents of about 50%. After heat-moisture treatment, the RS yield increased up to 84%. Peak temperatures of about 145 °C found in DSC measurements pointed to a high thermal stability of the RS products. In vitro fermentations of the RS products, carried out with intestinal microflora of healthy humans, resulted in a molar ratio of acetate:propionate:butyrate of about 49:17:34. The established method allowed the production of a high-quality RS with prebiotic properties for health preventing applications.

KEYWORDS: Resistant starch; *Musa acuminata*; retrogradation; heat-moisture treatment; DSC; in vitro fermentation

INTRODUCTION

For the past twenty years, it has been known that part of ingested starch is not digested in the small intestine of healthy humans. This part is defined as resistant starch (RS) (*1*).

In several studies, the physiological importance of RS has been investigated mainly with regard to glycemic index, cholesterol lowering capability, and colonic effects (*2*−*14*). The most important effect is based on the high fermentation rate of retrograded RS III to short-chain fatty acids (SCFA) with a high proportion of butyrate by action of the intestinal microflora, e.g., by *Eubacteria* (*15*). Butyrate is known to influence different metabolic processes. As a substrate for colonocytes, it determines the rate of the ATP production, and as a signal metabolite, it activates proliferation and differentiation (*16*).

Reasons for the resistance against α-amylase degradation in the small intestine are manifold and caused by structural characteristics of the starch. Four forms of RS are distinguished: RS type I is defined as physically inaccessible starch for instance in grains; type II is granular starch in raw potato and bananas; type III is retrograded starch, arising after hydrothermal treatment of starch; and type IV is considered to be a chemically modified starch (*17*).

The generation of RS after hydrothermal treatment is mainly due to increased interactions between starch polymers. Berry (*18*) showed that, after debranching of the starch, the linear fragments formed can contribute to a high RS content. The length of the linear chains influences the retrogradation significantly (*19*). Chain lengths of α-polyglucans with a degree of polymerization (DP) of about 20 are optimal for a high RS type III output (*20*). If the α-1,6-linked side chains are long enough, it is possible to produce RS type III from amylopectin rich starches. Banana starch contains such long outer amylopectin chains (*21*). Therefore, it seems to be an excellent source for the production of RS type III. Other factors, influencing the RS formation, are the processing temperature, starch concentration, storage conditions, and presence of lipids or low molecular substances such as sugars (*19*, *20*, *22*, *23*). These parameters were optimized in the present work.

The worldwide production of bananas in 2001 was 66.5 million t (*24*). In tropical countries it presents an important economic factor. Bananas belongs to the family *Musaceae*, genus *Musa*. By now, more than 700 varieties are known, 100 of which are cultivated. From the two wild ancestors, *Musa acuminata* COLLA and *Musa balbisiana* COLLA, descend the wide spread fruit banana *Musa sapientum* and the starch banana *Musa paradisiaca*, also called plantain (*25*). Plantains are characterized by a higher starch concentration, which is degraded to a relative small extent to monosaccharides. The cooking banana seems more suitable for the RS III production. It is descended from *Musa acuminata* and botanically different from the plantain. Cooking bananas and plantains are used as a staple food in Africa. The cooking bananas are mainly produced in Uganda. Physicochemical investigations of banana starch (*21*,

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26−*35*) supplied evidence that bananas are a good source for the generation of RS III as a supplement for a wide range of foods. Corresponding to our objective, the selection of the banana variety concentrated on cooking bananas with a high amount of amylopectin and the largest amylopectin side chains.

Until now, banana starch was used only as a source of RS II in several physiological studies (*36*−*38*). However, RS type II is poorly degraded to SCFA by the intestinal microflora.

The aim of the present study was to develop of RS type III from banana with a high yield of RS, a high thermal stability, necessary for the application in food industry, and a good fermentability with a high proportion of the physiological important SCFA butyrate.

MATERIALS AND METHODS

Materials. Banana starch was obtained from Uganda. Bananas were a clone (*Nandigobe*) of the *Musa* AAA-EA (triploid, acuminata cultivar) group from the upland type of East Africa which originate from *Musa acuminata*.

Starch Characterization. The starch was analyzed for its contents of moisture, crude protein, crude fat, starch, ash, and true amylose. The shape and size were analyzed microscopically.

Table 1. Composition of Banana Starch *Musa acuminata* Var. *Nandigobe* (% in Dry Matter)[a]

moisture (%)	amylose (%)	fat (%)	protein (%)	ash (%)
14.3 ± 0.83	8.47 ± 0.19	0.18 ± 0.01	0.44 ± 0.02	0.04 ± 0.004

[a] Data are means ± SD; $n = 3$.

Debranching. An aqueous starch gel (20% w/w) was gelatinized under stirring, autoclaved for 30 min at 121 °C, and suspended with 0.1 M acetate buffer pH 5.2 to obtain a 10% (w/w) gel. After cooling, Promozym (Pullulanase) obtained from Novo Nordisk (Bagsvaerd, Denmark) was added to achieve a substrate:enzyme ratio of 20:1. The suspension was stirred for 24 h at 50 °C. After that the starch was washed twice with water and freeze-dried.

Retrogradation. The raw or debranched starches were suspended in water (10% and 20% w/w) and autoclaved for 30 min at 121 °C. The samples were cooled and stored at 4 or 25 °C for 24 h and then freeze-dried. In addition, the retrograded product was analyzed after heat-moisture treatment. A 80% w/w starch paste was autoclaved for 60 min at 121 °C, cooled for 2 h at room temperature, frozen for 60 min at −20 °C, and freeze-dried.

RS contents. The RS content of the samples was measured by a modified method from Englyst et al. (*17*).

High-Performance Anion Exchanger Chromatography (HPAEC). Chromatographic analysis of polymer chain distribution were performed by HPAEC (Dionex, Idstein, Germany) with a CarboPac PA 1 ion exchange column (9 × 250 mm) (Dionex). The samples were analyzed by pulsed amperometric detector (PAD) (Dionex). The pulse potentials and durations were: $E_1 = 0.1$ mV and 300 ms, $E_2 = 0.6$ mV and 120 ms, and $E_3 = -0.6$ mV and 300 ms. Eluent A contained of 150 mM NaOH and eluent B of 150 mM NaOH with 1 M NaOAc. A 16−100% gradient over 105 min was used. The flow rate was 0.8 mL/min. A 10 mg sample was solved in 0.1 mL water and 0.1 mL 2 M NaOH and diluted with 0.8 mL water, and 60 μL of the sample volume was loaded onto the column. Maltoheptaose was used as an internal standard.

Differential Scanning Calorimetry (DSC). DSC experiments were performed with a Seiko DSC-120 (Seiko Instruments, Neu Isenburg, Germany). Indium was used for calibration. The samples were weighed into silver pans, and water was added to obtain a dry matter:water ratio of 1:5 (w/w). The samples were sealed and heated from 10 to 220 °C (4 °C/min). A pan filled with water was used as reference. The onset, peak, and conclusion temperatures T_o, T_c, and T_p as well as conversion enthalpie ΔH were calculated by integration using Seiko software (SSC 5200 H, Seiko).

In Vitro Fermentation of RS With Human Feces Flora. The used method was similar to that of Barry et al. (*39*). Feces of five healthy persons were freshly taken and mixed with Sörensen buffer (pH 6.5) while gassed with nitrogen to obtain a 5% fecal suspension. A 10 mg sample of isolated RS was mixed with 1 mL of fecal suspension in cryo tubes and incubated at 37 °C in a shaking water bath (180 strokes/min). Samples were taken in intervals and immediately frozen at −21 °C.

Determination of SCFA by Gas Chromatography. Fecal samples were diluted with water (1:4), part of which was used to determine the

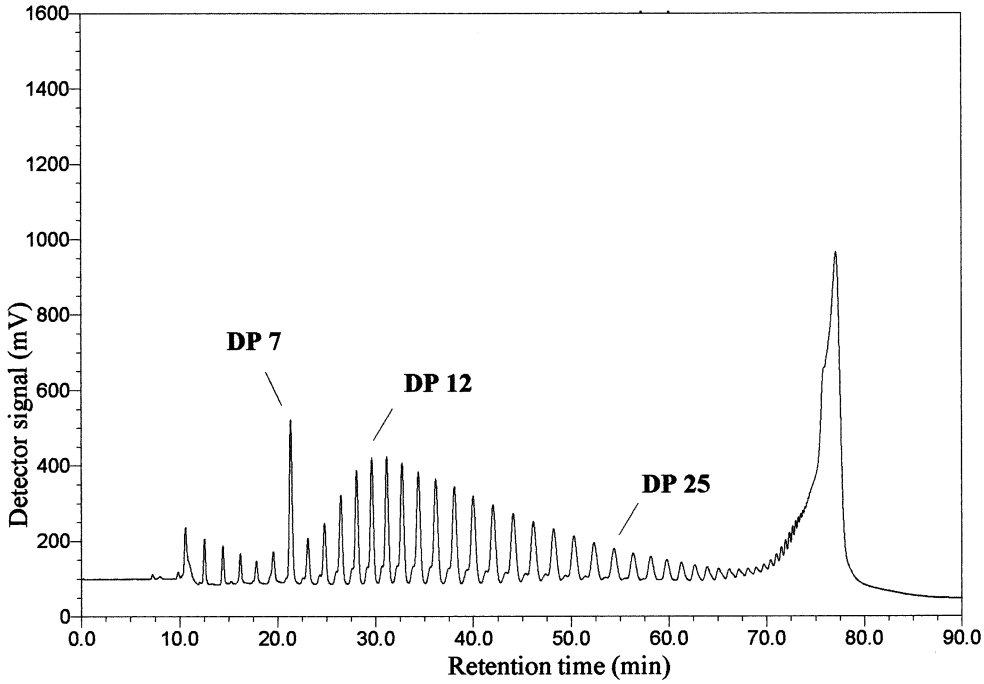

Figure 1. HPAEC chromatogramm of debranched retrograded (24 h, 25 °C, 20% w/w starch gel) RS product; DP degree of polymerization; DP 7 internal standard.

Table 2. Resistant Starch Content of the Banana Starch Products after Different Treatments[a]

sample	retrogradation condition: storage temperature (°C)	retrogradation condition: starch concentration in gel (%)	yield of resistant starch (%)
native starch	–	–	95.1 a ± 1.26
native starch	25	10	5.9 b ± 0.14
	25	20	6.5 b ± 0.37
debranched starch	4	10	47.54 cd ± 1.91
	4	20	49.88 cd ± 1.54
	25	10	45.7 c ± 0.79
	25	20	50.6 d ± 1.67
HMT after retrogradation	25	20	84.0 e ± 0.66

[a] Data are means ± SD; $n = 3$; numbers in the column followed by a letter in common were not significantly different ($p < 0.05$).

dry matter, and 600 mg was centrifugated (4000 g, 5 min). A 100 μL sample of the supernatant was mixed with 270 μL of 1 M NaOH, 280 μL of 0,36 M $HClO_4$, and 50 μL of 12 mM isobutyric acid as internal standard. The sample was mixed and freeze-dried. The dried residue was dissolved in 200 μL of 5 M formic acid and 800 μL of water. After centrifugation (4000*g*, 5 min) the sample was filled into vials closed with caps, and 1 μL of the sample was injected into a gas chromatograph (HP 5890+, Hewlett-Packard, Waldbronn, Germany) equipped with a HP-FFAP column (30 m × 53 mm, film thickness 1 μm) and a flame ionization detector. A temperature program was used in the range of 85−160 °C, and helium was used as carrier gas at a flow rate of 20 mL/min and a split ratio 1:1. For calibration fatty acid standards were used (acetic, propionic, butyric, isobutyric, valeric, and isovaleric acid, Supelco, Bellefonte, PA).

Statistical Analyses. The data represent a mean of at least as three replicates. Significant differences were tested using Students t-Test with statistical program SPSS 8.0.

RESULTS AND DISCUSSION

Composition, Shape, and Size of Banana Starch. The chemical properties differ within the banana varieties and the stage of ripeness. The composition of the starch (**Table 1**) with 0.18% fat, 0.44% protein, and 0.04% ash and the irregular round shape and size of 10−50 μm of the starch granules correspond with the results of Kayisu (*21*). Amylose contents of 9−12% in plantains, 13−17% in cooking bananas, and 20% in fruit bananas were described (*27*, *29*, *31*, *32*).

Starch from eight different banana varieties which were grown in Uganda were investigated for its amylopectin part to find those with long s-chains, optimal for RS production and a high amylopectin content. The underlying consideration was that through enzymatical debranching by pullulanase chains near the optimal chain, lengths in the range between 12 and 35 can be liberated. The best requirements for the realization of the aim provided the variety *Nandigobe*. *Nandigobe* (variety of *Musa acuminata*) is a clone with a low amylose content of 8.6% (**Table 1**).

Polymer Chain Distribution. The native starch contained no low-molecular-weight polymers (data not shown). Such polymers occurred only after enzymatical debranching. In the debranched sample, low-molecular-weight peaks were dominant, α-1,4-glucans with DP 6−30 with a high amount of DP 6−22 (**Figure 1**). The peak at a retention time of 75 min consisted of amylose, a small amount of nondebranched amylopectin, and long amylopectin side chains with a DP of approximately 65, which has been reported for banana starch (*21*). After debranching, the product was washed twice with cold water to remove buffer and liberated mono- and oligosaccharides, which impede the retrogradation. The in vitro optimal chain length for effective retrogradation of concentrated starch gels (*20*) can be approached by debranching.

Steps to Produce RS III in High Yield and Quality. The RS contents of native and modified banana starch are presented in **Table 2**. Raw banana has a high RS type II content of approximately 95%. High RS II contents in bananas have been reported in the literature (*37*, *38*). After autoclaving and cooling, RS contents of 6% are formed, due to the small amylose amount.

Debranching, autoclaving, and cooling led to RS contents up to 51%. The generation of low-molecular-weight polymers by debranching promoted the retrogradation. This corresponds to synthesized linear α-glucans with a degree of polymerization of about 20−35, resulting in a RS content of 94% (*20*). A higher starch concentration in the gel from 10% to 20% raised the RS content significantly. Berry (*18*) estimated a RS content of up to 46.8% in purified potato amylopectin after Pullulanase treatment, drying, and heat processing. The source of starch, debranching, and retrogradation conditions and the method used

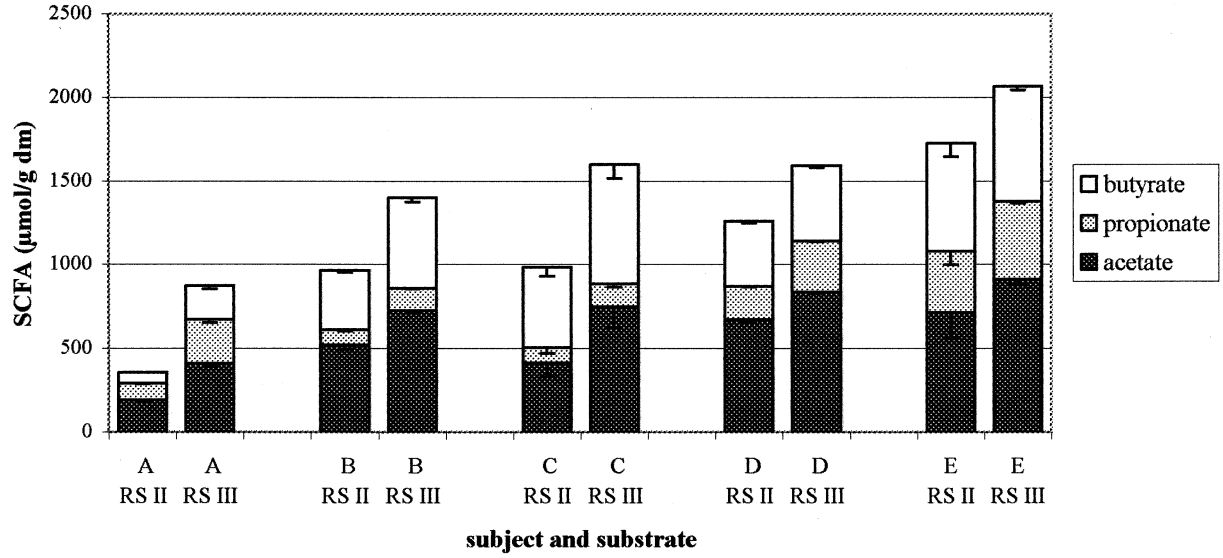

Figure 2. Short-chain fatty acids obtained after 8 h in vitro fermentation with RS type II and III as a substrate and human intestinal feces flora of five subjects (A–E).

Table 3. Thermal Stability of the BananaStarch Products (DSC Measurement)

sample[a]	T_o	T_p (°C)	T_c	ΔH (mJ/mg)
A	69.5	73.3	78.7	10.0
B	112.0	147.7	166.3	20.8
C	118.2	144.7	168.8	15.1
D	87.4	103.3	111.1	4.4
	139.3	162.5	177.6	5.3

[a] Sample A: native starch. Sample B: debranched; retrograded at 25 °C for 24 h; starch concentration in gel 10%. Sample C: debranched; retrograded at 25 °C for 24 h; starch concentration in gel 20%. Sample D: HMT treatment.

Table 4. Molar Ratios of Short-Chain FattyAcids Obtained after 8 h in Vitro Fermentation of RS Type II and III from Banana and Human Feces Flora of Five Subjects (A–E)

substrate	subject	acetate	propionate	butyrate
RS II	A	54	27	19
RS III	A	47	30	23
RS II	B	54	9	37
RS III	B	52	9	39
RS II	C	42	9	49
RS III	C	47	9	44
RS II	D	53	16	31
RS III	D	53	19	28
RS II	E	41	21	38
RS III	E	44	23	33

for RS analysis determine the difference to the present results. A storage temperature of 4 °C resulted in similar RS contents as 25 °C. A heat-moisture treatment after the retrogradation yielded RS amounts of 84%. This is the highest RS type III yield found using native starch sources. The obtained product offers good sensory properties and a palatable mouth feeling. It could provide a wide range of RS substitutes in foods.

Thermal Stability of the RS Products. The peak temperature of the gelatinization of raw banana starch was 73 °C (**Table 3**). Similar results were found by Ling et al. (*31*). After debranching and retrogradation, the peak temperatures increase up to 145−147 °C. A higher starch concentration in the gel used during the retrogradation enhanced the onset temperature of the RS product. The RS III preparations showed a substantially higher thermostability than the RS type II of the banana starch. The HMT treated starch reveals two endothermes with peak temperatures, namely, 103 and 162 °C. A high conversion enthalpy, which was higher than 20 mJ/mg, was a characteristic of the RS III preparations. As a result of retrogradation, the enthalpy values were doubled, but after HMT, only a slight increase of the transition enthalpy was observed. HMT enhances the stability of resistant structures. The results point to a high-temperature stability of the prepared products as a precondition for a wide range of processing possibilities (cooking, baking) without a loss of resistant structures.

In Vitro Fermentations. As an example for physiological properties of the developed RS type III product, in vitro fermentations using isolated RS and human intestinal feces flora were performed. In contrast to other dietary fibers such as pectin, cellulose, or starch-free bran, RS is a good source for butyrate production (*40*). The produced RS III from banana was fermented with a higher fermentation rate than the native RS type II. The SCFA concentrations varied after 8 h fermentation time using RS II as a fermentation substrate between 370 and 1800 μmol/g dry matter and using RS III as a substrate between 890 and 2100 μmol/g dry matter (**Figure 2**). Likewise, Englyst and Cummings (*37*) described a lower utilization of raw banana starch. Some people seem to miss the ability to ferment special sources of starch. Obviously, granular amylopectin-rich starches are a better substrate for fermentation than amylose-rich starch granules. On the other hand, molar ratios of the SCFA were similar in both RS sources. We found the following ratios between acetate, propionate, and butyrate: 49:17:34 (**Table 4**). Botham et al. (*41*) found after pea starch fermentation similar molar ratios of SCFA. The highest molar butyrate portion (60%) was obtained from an in vitro synthesized α-1,4-glucan (*20*).

These data demonstrate that RS type III from banana starch is fermented to a high portion of butyrate. Bananas fulfill many requirements, especially for functional food production. They contain high potassium and magnesium contents and well-digestible carbohydrates. Until now, cooking bananas have been mostly locally traded and not exported. As the work shows, bananas with a low amylose content are a good source for functional food production.

Abbreviations Used. RS, resistant starch; SCFA, short-chain fatty acids; DP, degree of polymerization, HPAEC, high-performance anion exchanger chromatography; DSC, differential scanning calorimetry; HMT, heat-moisture treatment.

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