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Resistant starch derived from processed *ragi* (finger millet, *Eleusine coracana*) flour: structural characterization

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Abstract

From five cycle autoclaved finger millet (*ragi, Eleusine coracana*) flour, resistant starch (RS) was isolated by sequential enzymatic digestion and purified by GPC and SE–HPLC methods. Its molecular weight was $\sim 1.4 \times 10^6$ Da. Permethylation followed by GC–MS analysis revealed the RS to be a linear α-1,4-D-glucan, probably derived from a retrograded amylose fraction of *ragi* starch. X-ray diffraction data showed the *ragi* RS to have both B and V-type diffraction patterns. The enthalpy value of ~ 0.7 mcal mg^{-1} showed *ragi* RS to be thermally stable. The undigested material recovered from the ileum of rat intestine fed with processed *ragi* flour exhibited a close similarity in some of its properties to that of RS isolated by an in vitro method.

Keywords: Resistant starch; Cereal; Ragi; Processing; Structure

1. Introduction

The long-held belief that dietary starch, when consumed in the form of processed foods, is completely digested and utilized in the small intestine for energy release has been challenged and debated (Stephen, Haddad, & Phillips, 1983). In support of this, the dietary fibre (DF) content of processed foods was rather higher than the corresponding material before processing, indicating that some 'man-made' DF is being introduced during processing. It is now recognized that as much as 30% of the total apparent DF in wheat bread is undigestible starch (Cummings & Englyst, 1987a; Englyst & Macfarlane, 1986). This fraction of starch, which escapes digestion in the gastrointestinal (GI) tract but later gets fermented in the colon, is generally referred to as resistant starch (RS). The beneficial effects of RS fermentation in the large intestine are so numerous that the present trend in functional foods is to introduce RS in varying proportions (Cummings & Englyst, 1987b; Shetty & Kurpad, 1986).

Ragi (finger millet, *Eleusine coracana*) is a minor millet consumed by the economically weaker section of the population, especially by South Indian rural folk. It is rich in DF and calcium, and is used after various processing treatments (Malleshi & Hadimani, 1993). Malted *ragi* is a very useful material for the preparation of beverages and health foods, such as infant food and enteral food formulations (Meera, 1997). In continuation of studies on food-derived carbohydrates, we recently investigated the content and nature of RS in processed rice (Mangala, Malleshi, Mahadevamma, & Tharanathan, 1997a; Mangala & Tharanathan, 1997) and also the influence of non-starch constituents, viz., protein and lipids, as well as amylopectin, on RS formation (Mangala, Udayasankar, & Tharanathan, 1997b). In this communication, we describe the structural features of the RS derived from five cycle autoclaved *ragi* flour.

2. Materials and methods

2.1. Materials

A certified variety of *ragi* (finger millet, *E. coracana*, indaf variety), procured from local suppliers, was sun-dried and ground in a plate mill (60 mesh). All the chemicals used were of analytical reagent grade. Triton

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X-100, glucoamylase (E.C.3.2.1.3), pancreatic α-amylase (E.C.3.2.1.1), protease (E.C.3.2.24), D-glucose oxidase (E.C.1.1.3.4), pepsin (E.C.3.4.23.1), β-amylase (E.C.3.2.1.2) and peroxidase (E.C.1.11.1.7) were from Sigma Chemical Co., USA. Termamyl was from Novo, Denmark. Sepharose CL-2B and dextrans (T-10, T-20, T-40, T-70 and T-500) were from Pharmacia Fine Chemicals, Sweden. HPLC columns (μ-Bondagel E-linear and E-1000) were from Waters Associates, USA.

2.2. Methods

Total sugar, reducing sugar and D-glucose were determined by the modified phenol–H_2SO_4 (Rao & Pattabiraman, 1989), Nelson–Somogyi (Nelson, 1944) and D-glucose oxidase (Dahlqvist, 1964) methods, respectively, using D-glucose or maltose as reference compounds. The approximate molecular weight (MW) of the RS was determined by GPC on a pre-calibrated (with dextrans of known MW) Sepharose CL-2B column (1.7×92 cm) eluted (18 ml h^{-1}) with water. Size exclusion–high performance liquid chromatography (SE–HPLC) was performed on a Shimadzu HIC-6A ion chromatograph equipped with a Shimadzu RID-6A refractive index (RI) detector, SCL-6A system controller, CR-2A chromatopac integrator, and E-linear and E-1000 μ-Bondagel columns (ss, 30 cm×3.9 mm, i.d.) connected in series with a guard column. Elution was done with water (0.2 ml min^{-1}) at 40°C. The V_o was measured using *Sesbanium* mosaic virus (MW, ~6×10^6). The RS solubilized in 85% aq. DMSO by heating at 95°C for 5 min, was centrifuged (2000 rpm for 5 min), and 1 μl of the clear supernatant solution was injected into the SE–HPLC column. X-ray diffraction patterns were obtained using a EG-7G solid state Germanium-liquid N_2 cooled detector Scintag XDS-2000 instrument equipped with θ-θ goniometer, and operating at 30 kV and 25 mA with Co.kα radiation at λ = 1.54184 nm. Diffractograms of the samples saturated with distilled water overnight in a desiccator were obtained from 4 to 40°, 2θ. Differential scanning calorimetric (DSC) analysis was carried out with a Rheometric Scientific instrument, UK, equipped with thermal software version 5.40. Samples (5–10 mg) with a moisture content of 50–80% (w/v) were heated (10°C min^{-1}) in aluminium cups and their thermal characteristic values recorded using indium as a reference standard.

^{13}C-NMR spectra of RS and amylose in DMSO.d_6 were recorded at a probe temperature of 80°C in a Brucker AMX-400 spectrometer at 100 MHz. The spectra were obtained from 8000 scans in the pulsed FT mode using tetramethylsilane as the external standard. Gas liquid chromatographic (GLC) analysis as alditol acetates of hydrolysed RS was done in a Packard 437 gas chromatograph fitted with FID and OV-225, 3% on Chromosorb W (100-200), ss column (1/8″×8 ft) at an isothermal temperature of 190°C. N_2 was the carrier gas and inositol was the internal standard used (Ramesh & Tharanathan, 1998).

RS was isolated from five cycle autoclaved rice by a sequential enzymatic method (Sievert & Pomeranz, 1989). The crude RS was solubilized in 2 N KOH, neutralized, centrifuged and the clear supernatant dialysed and lyophilized. To the dry residue, 30% $HClO_4$ (at 0°C) was slowly added with stirring for 30 min and centrifuged; the dialysed supernatant was subjected to GPC and SE–HPLC.

The purified RS (50 mg) was solubilized in aq. DMSO (85%, 2 ml) by heating in a boiling water bath for 10 min, and the resulting solution was cooled and centrifuged. The clear supernatant was made up to 10 ml with NaOAc buffer (0.1 M, pH 4.8) and was incubated at 37°C for 24 h with β-amylase (1500 units). The enzyme was heat-inactivated and the percentage β-amylolysis was calculated (Atwell, Hoseney, & Lineback, 1980). The β-limit dextrin (β-LD) obtained was subjected to a second β-amylolysis.

The RS (5 mg) was methylated using dimsyl anion and CH_3I (Hakomori, 1964), and the products purified by passing through SEP-PAK C18 cartridges. After depolymerization with formic acid-H_2SO_4, the partially methylated alditol acetates were prepared (using NaB^2H_4) in ^2H_2O) and analysed by GC–MS. The latter was carried out in a high performance quadrupole Shimadzu MS-QP-5000 instrument combined with GC-17A, and using a SP-2380 fused silica capillary column (30 m, i.d. 0.32 mm, film thickness 0.02 μ, Supelco, USA). The operating parameters were temperature programme mode, 150–200°C (2°C min^{-1}), ionizing voltage of 70 eV, mass range 40–400 amu and 4 ms $scan^{-1}$. Solvent used was chloroform and the carrier gas was helium.

The RS from the ileum of rat intestine was isolated as follows: male albino rats, weighing 198 ± 2 g and classified into three groups by random block design, each comprising of six rats, were housed individually in wire mesh cages. Five cycle autoclaved *ragi* samples were fed ad libitum to animals in groups one and two, whereas the group three animals were fed with the control corn starch diet. Daily food intake and the final gain in body weight were recorded. On day five the animals were sacrificed and the ileum portion of the small intestine was cut open and the undigested food particles were removed by squeezing and the content and nature of RS were determined.

3. Results and discussion

In consonance with the earlier reports (Mangala et al., 1997a) repeated autoclaving of aqueous *ragi* flour suspension gave a relatively higher RS content (native 0.008% and five cycle autoclaved 0.12%). In comparison

with rice (Mangala et al., 1997a) or wheat (Bjorck et al., 1986), the content of RS in processed *ragi* was considerably less. The MW of the HPLC-purified RS (see Fig. 1) eluting at 23.89 min was $\sim 1.4 \times 10^6$ Da, a value slightly less than that of *ragi* amylose (eluting at 22.64, $\sim 1.6 \times 10^6$ Da, data not shown), which is in accordance with its origin from the amylose fraction of starch. Results of β-amylolysis (~93%) as well as permethylation and GC–MS analyses indicated *ragi* RS to be essentially linear. A major peak of 2,3,6-*tri*-*O*-methyl D-glucose and a minor peak of 2,3,4,6-*tetra*-O-methyl D-glucose were identified (Fig. 2). The latter is derived from the non-reducing end of the RS molecule. Their mass fragmentation data were in conformity with the assigned substitution pattern (Jansson, Kenne, Leidgren, Lindberg, & Lonngren, 1976). Almost comparable ^{13}C-NMR spectra were obtained for both *ragi* amylose and RS (Fig. 3), suggesting that the latter is indeed derived from the former. The presence of a doublet in the region 100–102 ppm has been assigned to C-1 of the B-type crystalline polymorph of RS (Gidley & Bociek, 1988). Rice amylose, used as a reference compound, did not show this doublet. Probably, due to its low level of crystallinity, the *ragi* RS did not give sharp signals. Nevertheless, all the spectral frequencies were identified by making use of their chemical shift values.

Like other cereal starches (Zobel, 1964) the native *ragi* starch showed an A-type X-ray diffraction pattern (2θ angles at 15.2, 15.4, 17.3, 18.4, 18.6, 23.5 and 24.20°); its RS showed major peaks at 17.2 and 17.6° along with several minor peaks at 15.2, 15.5, 18.4, 20.5, 22.4 and 22.6°, characteristic of B and V-type starches. Unlike the native starch, however, which gave well defined peaks (Fig. 4), the RS showed rather fewer sharp signals, probably due to its poor crystallinity and poor water of hydration.

To understand the influence of flour components on starch properties, the thermal behaviour of starch was evaluated by differential scanning calorimetry (DSC).

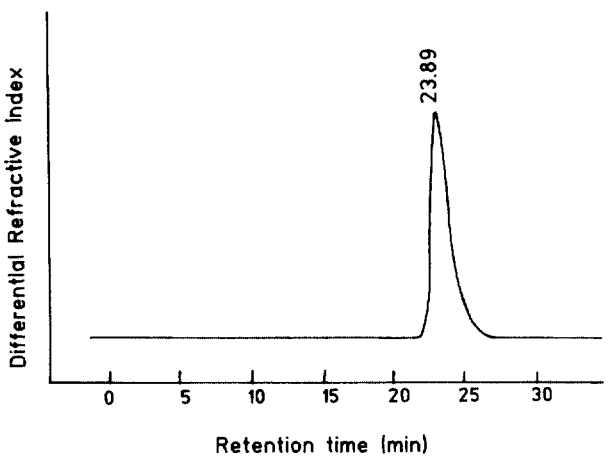

Fig. 1. SE-HPLC of *ragi* RS.

From the results presented in Table 1 and Fig. 5, it may be argued that the various constituents of *ragi* flour, such as protein, lipids, non-starch polysaccharides, do have a lowering effect on the gelatinization temperature and enthalpy values of starch. The reduction in enthalpy of the flour (as also indicated by decreased T_o and T_c values) is attributable to delayed starch gelatinization caused by the presence of protein, lipids and other simple sugars in the *ragi* flour. Simple sugars such as glucose, maltose and sucrose have been shown to alter the gelatinization endotherm of starches (Eerlingen, Van Den Broeck, Delcour, Slade, & Levine, 1994). In addition to inhibiting the starch gelatinization endotherms, the various non-starch components would also dilute the amount of starch per se in the sample. It is also possible that the solubility of the contaminating substance may be an important factor affecting gelatinization temperature in a limited water system (Normand & Marshall, 1989). Depending upon the substance, the interaction and, as a result, T_o and T_c may either increase or decrease.

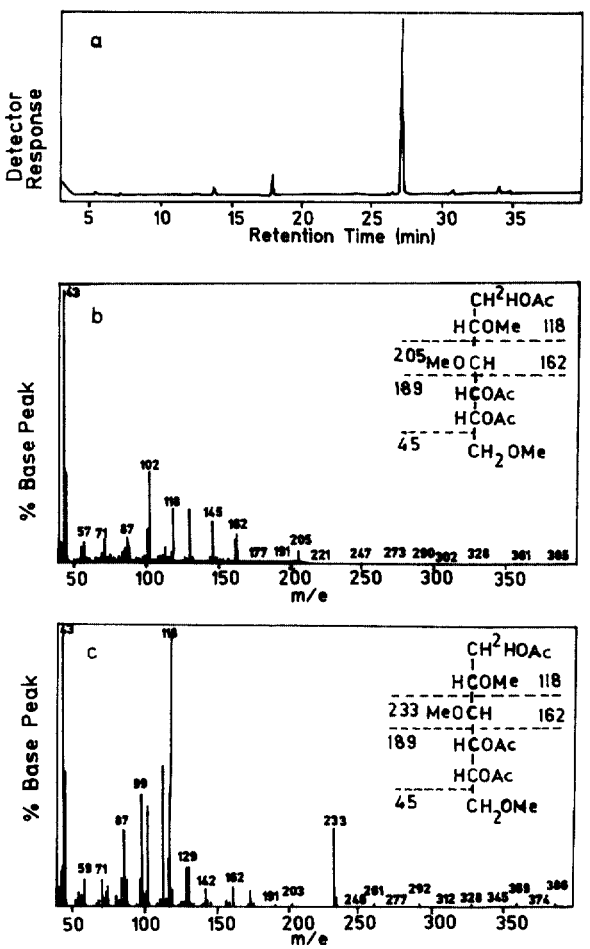

Fig. 2. GC–MS of permethylated alditol acetates derived from *ragi* RS. (a) GC profile; (b and c). MS and fragmentation patterns of 2,3,4,6-Me_4-Glc (T_R 17.9 min) and 2,3,6-Me_3-Glc (T_R 27.2 min), respectively.

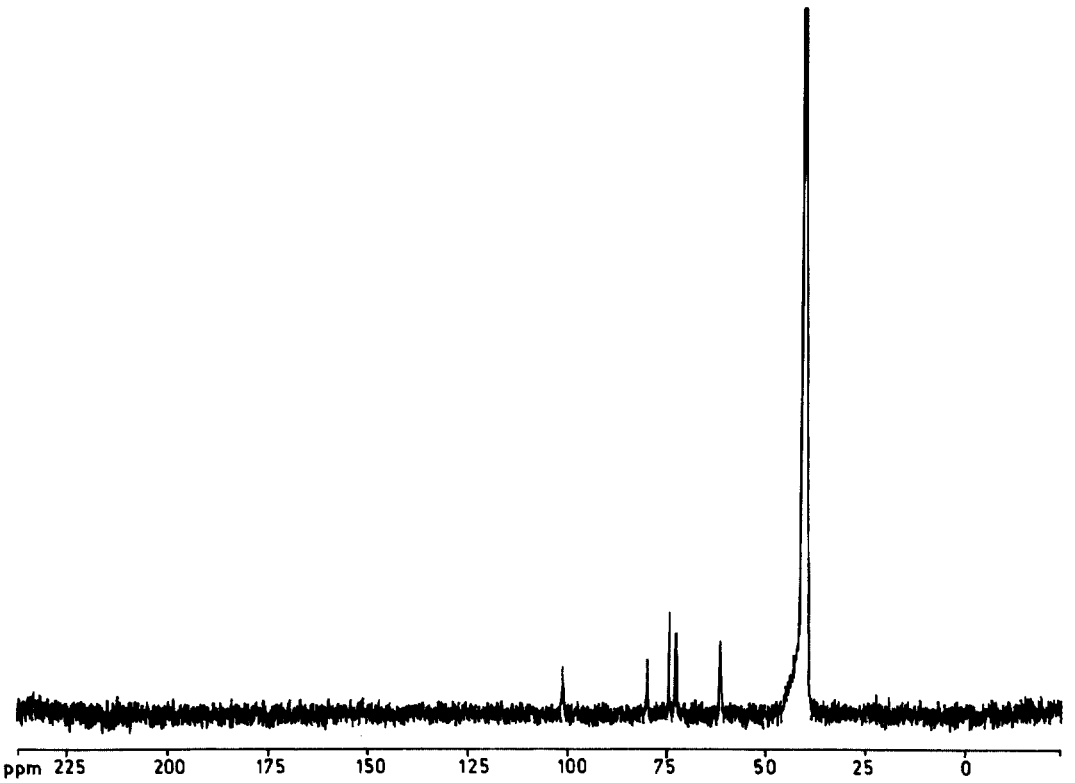

Fig. 3. ^{13}C-NMR spectrum of *ragi* RS.

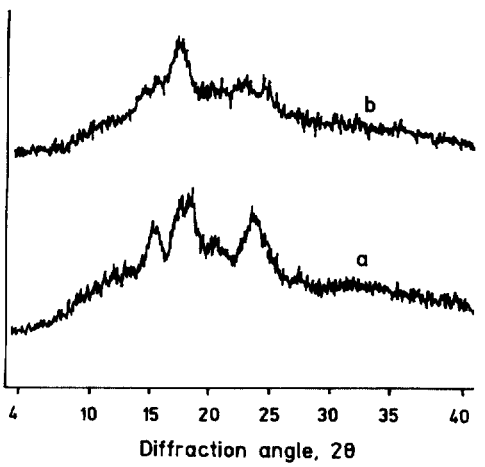

Fig. 4. X-ray diffraction pattern of (a) native *ragi* starch and (b) its RS.

Table 1
Differential scanning calorimetric characteristics of flour and starch of *ragi*

	T_o	T_p	T_c	$\Delta T(T_c-T_o)$	ΔH (mcal mg^{-1})
Flour	41.6	52.5	60.8	19.2	7.86
Starch	59.6	65.3	72.3	12.7	9.86

T_o = Onset temperature; T_p = peak temperature; T_c = completion temperature; ΔT = differential temperature (all °C); ΔH = enthalpy.

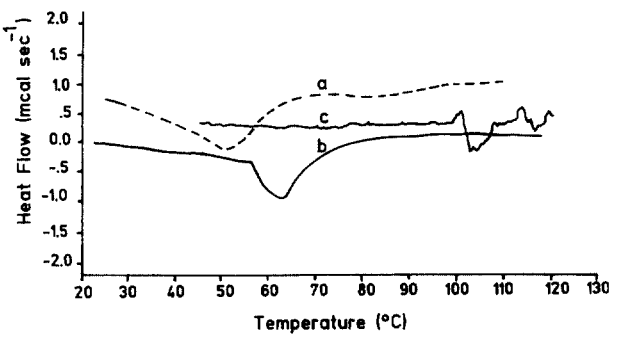

Fig. 5. DSC thermogram of *ragi* (a) flour, (b) starch and (c) RS.

From the thermogram of *ragi* RS (see Fig. 5) it is clear that the endothermic peak starts at around 100°C and reaches a maximum of 104°C, as also reported for a few other cereal resistant starches (Gidley et al., 1995). Its enthalpy was low (7.86 mcal mg^{-1}), a value much lower than that of rice RS (9.86 mcal mg^{-1}). Broad endothermic transitions have been reported for amylo-maize starches (Gruchala & Pomeranz, 1993; Sievert & Wursch, 1993).

In order to find out whether the RS derived from the in vivo system is significantly different from that of in vitro isolated material, the undigested starch from the ileum of rat intestine was recovered and some of its properties were studied. From the GPC profile it was found that both the RS fractions have almost

comparable MW values, which establishes that the RS is indeed an enzyme-resistant fraction of dietary starch, whether isolated by the in vitro or in vivo method. The λ_{max} and the blue value index of in vivo-isolated *ragi* RS were 576.6 and 23 nm, respectively.

References

Atwell, W. A., Hoseney, R. C., & Lineback, D. R. (1980). Debranching of wheat amylopectin. *Cereal Chemistry, 57*, 12–16.

Bjorck, I., Nyman, M., Pedersen, B., Siljestrom, M., Asp, N. G., & Eggum, B. D. (1986). On the digestibility of starch in wheat-studies in vitro and in vivo. *Journal of Cereal Science, 4*, 1–11.

Cummings, J. H., & Englyst, H. N. (1987a) Digestion of polysaccharides of potato in the small intestine of man. *American Journal of Clinical Nutrition, 45*, 423–431.

Cummings, J. H., & Englyst, H. N. (1987b) Fermentation in the human large intestine of the available substrates. *American Journal of Clinical Nutrition, 45*, 1243–1255.

Dahlqvist, A. (1964). Method for assay of intestinal disaccharidases. *Analytical Biochemistry, 7*, 18–25.

Eerlingen, R. C., Van Den Broeck, I., Delcour, J. A., Slade, L., & Levine, H. (1994). Enzyme-resistant starch. Influence of sugars on resistant starch formation. *Cereal Chemistry, 71*, 472–476.

Englyst, H. N., & Macfarlane, G. T. (1986). Break down of resistant and readily digestible starch by human gut bacteria. *Journal of Science of Food and Agriculture, 37*, 699–706.

Gidley, J. J., & Bociek, S. M. (1988). ^{13}C-CP/MAS NMR studies of amylose inclusion complexes, cyclodextrins and the amorphous phase of starch granules: relationship between glycosidic linkage conformation and solid state ^{13}C chemical shifts. *Journal of the American Chemical Society, 110*, 3820–3829.

Gidley, M. J., Cooke, D., Darke, A. H., Hoffman, R. A., Russel, A. L., & Greenwell, P. (1995). Molecular order and structure in enzyme-resistant retrograded starch. *Carbohydrate Polymers, 28*, 23–31.

Gruchala, L., & Pomeranz, Y. (1993). Enzyme-resistant starch: studies using differential scanning calorimetry. *Cereal Chemistry, 70*, 163–170.

Hakomori, S. I. (1964). A rapid permethylation of glycolipid and polysaccharide catalyzed by methyl sulfinyl carbanion in dimethyl sulfoxide. *Journal of Biochemistry (Tokyo), 55*, 205–208.

Jansson, P. E., Kenne, L., Leidgren, H., Lindberg, B., & Lonngren, J. (1976). *Chemical Communications, University of Stockholm, 8*, 1.

Malleshi, N. G., & Hadimani, N. A. (1993) Nutritional and technological characteristics of small millets and preparation of value-added products from them. In K. W. Riley, S. C. Gupta, A. Seetharam, & J. N. Mushonga (Eds.), *Advances in Small Millets* (pp. 271–288). Oxford: Oxford IBH Pub. Co., Delhi.

Mangala, S. L., Malleshi, N. G., Mahadevamma, & Tharanathan, R. N. (1997a). Resistant starch from differently processed rice and ragi (finger millet). *Zeitschrift für Lebensmittel-Untersuchung und-Forschung A* (Accepted).

Mangala, S. L., Udayasankar, K., & Tharanathan, R. N. (1997b). Resistant starch from processed cereals—the influence of amylopectin and other non-carbohydrate constituents in its formation. *Food Chemistry* (in press).

Mangala, S. L., & Tharanathan, R. N. (1997). Structural studies of resistant starch derived from processed (autoclaved) rice. *Zeitschrift für Lebensmittel-Untersuchung und-Forschung A* (Accepted).

Meera, C. (1997). Development of enteral foods based on malted cereals. Ph.D. thesis, University of Mysore, Mysore.

Nelson, N. (1944). A photometric adaptation of the Somogyi method for the determination of glucose. *Journal of Biological Chemistry, 153*, 375–380.

Normand, F. L., & Marshall, W. E. (1989). Differential scanning calorimetry of whole grain milled rice and milled rice flour. *Cereal Chemistry, 66*, 317–320.

Ramesh, H. P., & Tharanathan, R. N. (1998). Structural characteristics of a mixed linkage β-D-glucan from sorghum (*Sorghum bicolor*). *Carbohydrate Research, 308*, 239–243.

Rao, P., & Pattabiraman, T. N. (1989). Reevaluation of the phenol–sulfuric acid reaction for the estimation of hexoses and pentoses. *Analytical Biochemistry, 181*, 18–22.

Shetty, P. S., & Kurpad, A. (1986). Increasing starch intake in the human diet increases fecal bulking. *American Journal of Clinical Nutrition, 43*, 210–212.

Sievert, D., & Pomeranz, Y. (1989). Enzyme-resistant starch. Characterisation and evaluation by enzymatic, thermoanalytical and microscopic methods. *Cereal Chemistry, 66*, 342–347.

Sievert, D., & Wursch, P. (1993). Thermal behaviour of potato amylose and enzyme-resistant starch from maize. *Cereal Chemistry, 70*, 333–338.

Stephen, A. M., Haddad, A. C., & Phillips, S. F. (1983). Passage of carbohydrate into the colon. Direct measurement in humans. *Gastroenterology, 85*, 589–595.

Zobel, H. F. (1964). X-ray analysis of starch granules. *Methods in Carbohydrate Chemistry, 4*, 109–113.